

# ESA-SRB-AOTA 2019 ABSTRACTS

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## Sperm dysfunction: Making heads or tails of male infertility

**Brett Nixon**<sup>1,2</sup>

1. Priority Research Centre for Reproductive Science, The University of Newcastle, Callaghan, NSW, Australia

2. Pregnancy and Reproduction Program, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia

Infertility is a major worldwide health burden, with 1 in 6 Australian couples taking recourse to assisted reproductive technologies. While the reasons for this concerning level of infertility are undeniably complex, a male factor is implicated in approximately 50% of these cases. In a vast majority of infertile males patients, sufficient numbers of spermatozoa are produced to achieve fertilization. However, the functionality of these cells has become compromised, thus ranking defective sperm function among the largest single defined cause of human infertility. The major aim of my laboratory is to understand the underlying molecular mechanisms responsible for sperm dysfunction in order to improve the clinical assessment and management of male infertility patients. In work conducted over the past two decades, we have studied the underlying pathophysiology of sperm dysfunction using a range of contemporary proteomic, molecular and cell biology technologies. A focus for this work has been defective sperm-oocyte recognition, a relatively common aetiology associated with the spermatozoa of idiopathic male infertility patients. Here, I will present our research into the functional maturation of the spermatozoon that underpins sperm-egg recognition and the mechanisms by which this process becomes so severely compromised in cases of infertility. I will also discuss our investigation of promising therapeutic interventions to mitigate the growing health burden posed by male infertility.

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## Germ cell pluripotency control: developmental mechanisms underlying fertility and disease

**Cassy M Spiller**<sup>1</sup>, **Guillaume Burnet**<sup>1</sup>, **Chun-Wei Feng**<sup>1</sup>, **Peter Koopman**<sup>2</sup>, **Josephine Bowles**<sup>1,2</sup>

1. School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia

2. Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia

Pluripotency of male germ cells must be tightly balanced during embryonic development: sufficient numbers of pluripotent stem cells must be allocated for fertility, but unconstrained pluripotency gives rise to cancer precursors.

Nodal/Cripto is a classical developmental signaling pathway that also controls pluripotency in ES cells and is overexpressed in many cancers. Our studies in mice identified the Nodal co-receptor Cripto as controlling pluripotency of fetal male germ cells during the time in which they are particularly susceptible to malignant transformation. To explore the relationship between correct fetal germ cell pluripotency/differentiation and tumorigenesis we are investigating germ cell-specific, single and combined, Nodal/Cripto gain- and loss- of function transgenic mouse models.

When Nodal/Cripto signaling is suppressed in fetal germ cells, we find germline pluripotency is decreased and that Cripto can signal independently of Nodal and vice versa. In our mouse model of Cripto-overexpressing germ cells we find that spermatogonial stem cells are still specified, but these cells fail to undergo spermatogenesis and retain a stem cell like state. I will present our recent work in which we analyse the role of Cripto signaling in both fetal and neonatal germline development and discuss the implications for fertility and disease.

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## Characterising the impacts of cancer therapies on the female reproductive tract

**Amy L Winship**<sup>1</sup>

1. Department of Anatomy & Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

In recent years, attention has shifted from conventional cancer therapies to more personalised, targeted treatments with reduced off-target effects. This includes the introduction of immunotherapies and small molecule inhibitors, like PARP inhibitors to the clinic, but with no preclinical investigations or understanding of their impacts on the female reproductive tract, or fertility. The number and quality of primordial follicle oocytes in the ovary are non-renewable and the source of all mature ovulatory oocytes. They are therefore indispensable for female fertility. I've shown that the PARP inhibitor, olaparib, administered at clinically relevant levels, dramatically depletes the primordial follicle reserve in mice. I am currently investigating the impacts of two immunotherapies on the ovaries in mice. And importantly, while the oncofertility field has largely focused on the ovary, I am leading a study to uncover the impacts of radiotherapy and chemotherapy on the uterus *in vivo*, for the first time. Together, these studies could not only improve our understanding of the extent and mechanisms of cancer-therapy mediated damage, but also provide novel insights for better strategies to protect fertility.

## Paternal environment and male-female seminal fluid influence on the conception environment

**John E Schjenken<sup>1</sup>, DJ Sharkey<sup>2</sup>, LM Moldenhauer<sup>2</sup>, HY Chan<sup>2</sup>, PY Chin<sup>2</sup>, LY Sandeman<sup>2</sup>, H Shehadah<sup>2</sup>, T Fullston<sup>2</sup>, R Ogle<sup>3</sup>, MA Baker<sup>3</sup>, NO McPherson<sup>2</sup>, M Lane<sup>2</sup>, SA Robertson<sup>2</sup>**

1. Robinson Research Institute and Adelaide Medical School, University of Adelaide, Adelaide, SA. 5005, Priority Research Centre for Reproductive Science, The University of Newcastle, Callaghan, NSW, Australia

2. Robinson Research Institute and Adelaide Medical School, University of Adelaide, Adelaide, SA. 5005

3. Priority Research Centre for Reproductive Science and School of Environmental and Life Sciences, University of Newcastle, Callaghan, 2308

Parental experiences and exposures prior to conception shape fetal development and life-course trajectory, with both maternal and paternal factors contributing to influence childhood health. Genetic and epigenetic alterations to sperm are implicated as a key mechanism behind transmission of adverse impact. An additional pathway involves seminal plasma, which is a major factor influencing female immune adaptation to pregnancy. Since immune adaptation is established at conception, perturbations at this time can profoundly affect embryo implantation, placental development and fetal growth, ultimately impacting offspring phenotype. We have postulated that in addition to sperm integrity, altered capacity for seminal fluid signalling in males may form a novel pathway that contributes to paternal programming. To investigate this, we have utilised mouse models to define the impact of altered seminal fluid exposure at conception. These studies have characterised the capacity of different components of the ejaculate to activate the female tract immune response, including both sperm and seminal plasma which are both involved in the induction of peri-conception cytokines and miRNAs and expansion of regulatory T cells. In particular, we have assessed the impact of paternal obesity on seminal fluid composition and function. Our data demonstrate that induction of obesity through a high fat diet leads to altered seminal plasma composition and sperm quality, which perturbs the female cytokine and T cell response to insemination, in turn contributing to the observed altered fetal and placental development and offspring outcomes. Our ongoing studies aim to build understanding of the physiological significance of other paternal environmental exposures on male and female reproductive function. As events around the time of conception have a profound impact at conception with consequences over the course of pregnancy, defining the significance of seminal fluid function will increase our understanding of how infertility, miscarriage and disorders of pregnancy arise, and how the health of the child after birth has origins in both maternal and paternal environmental determinants.

## Determining embryo health with a light touch

**Kylie Dunning<sup>1,2</sup>**

1. Robinson Research Institute and Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia

2. ARC Centre of Excellence for Nanoscale BioPhotonics, University of Adelaide, Adelaide SA 5005, Australia

Most human embryos are mosaic for chromosome abnormalities: containing cells that are euploid and aneuploid. Currently, a cell biopsy from the trophectoderm lineage of the blastocyst embryo is used to diagnose aneuploidy. However, this does not provide a diagnosis of the proportion of aneuploid cells in the remaining trophectoderm cells or within the inner cell mass (cells that form the fetus). Hence, the development of a non-invasive tool to determine the proportion of aneuploid cells would be clinically valuable. Aneuploidy in human embryos leads to altered metabolism. Co-factors utilised in cellular metabolism are autofluorescent and can be used to predict the metabolic state of cells. We hypothesised that aneuploid cells within the preimplantation embryo could be non-invasively discerned by their autofluorescent spectra. Using primary human fibroblast cells with known aneuploidies and a mouse embryo model with differing ratios of euploid:aneuploid cells we investigated whether we could distinguish euploid from aneuploid using hyperspectral imaging. Hyperspectral imaging of 1:1 (euploid:aneuploid) chimeric embryos showed a distinct spectral profile compared to euploid embryos. Following unsupervised linear unmixing, the abundance of FAD in the inner cell mass of aneuploid blastocysts was significantly lower compared to euploid blastocysts. For human fibroblasts, we were able to clearly distinguish between euploid and aneuploid with different karyotypes. We demonstrate that hyperspectral imaging is able to distinguish cells based on their ploidy status making it a promising tool in assessing embryo mosaicism.

## Testosterone and women: the known knowns, the known unknowns, and the unknown unknowns.

**Susan Davis<sup>1</sup>**

1. Women's Health Research Program, School of Public Health and Preventive Medicine, Monash University, Level 1, 553 St Kilda Rd, Melbourne VIC Australia 3004

Testosterone is a critical hormone for women. *Known knowns* are that the ovaries and adrenals are the primary sources of circulating testosterone, that iatrogenic suppression, removal of both ovaries or both adrenals, or spontaneous ovarian/adrenal failure will result in low testosterone, and that exogenous testosterone treatment will improve sexual function in women presenting with low sexual desire.

*Known unknowns:* An age-related decline in testosterone levels, commencing in the reproductive years has been reported but these data are limited by either use of RIA, convenience sampling, small numbers or inclusion of women with factors that might interfere with androgen physiology. Lacking are normative values for reproductive-aged women, by menstrual cycle phase and

by age, for the androgens and pre-androgens, including the recently described adrenally-derived keto-androgens. Whether circulating levels of keto-androgens in postmenopausal women are similar to premenopausal women is not known. Despite women now living many years post menopause, normative data for sex steroid levels in elderly women are lacking. Without this knowledge, "androgen insufficiency" at any life phase cannot be fully described.

Large studies of premenopausal women, postmenopausal women and elderly women have provided the opportunity for measurement of sex steroids, using liquid chromatography tandem mass spectrometry, offering new insights into the physiology of androgens in women. These data sets will enable us to explore the associations between sex steroids and an array of clinical characteristics and health outcomes in women, moving some things from the unknown into the known realm. The *unknown unknowns* will remain.

*Contributors to this work include Ms Marina A Skiba, Ms Penelope J Robinson, Dr Rakibul M Islam, Professor Robin J Bell and Professor David Handelsman*

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### The Year in Diabetes in Pregnancy

#### Samantha Hocking<sup>1</sup>

1. *The Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, University of Sydney, Sydney, NSW, Australia*

2019 has revealed more evidence for the detrimental effects to offspring of in-utero exposure to maternal hyperglycemia. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Follow-up study has revealed that increasing maternal glycemia in pregnancy is associated with worsening childhood glucose metabolism persisting into early adolescence. Longitudinal data from the ESTER and AYLS cohort studies have shown that maternal pre-pregnancy overweight and GDM are associated with unhealthy body size and composition in offspring over 20 years later. The association between glycaemia in pregnancy and stillbirth was highlighted by a case-control study from the UK which found a four-fold greater risk of late stillbirth in women with raised fasting plasma glucose but not diagnosed with GDM. Observational studies have also suggested a link between maternal dysglycemia in pregnancy and autism and ADHD.

Advances in technology were also featured in the 2018/19 literature. Studies on the use of flash glucose monitoring, intermittent continuous glucose monitoring and closed-loop insulin delivery in pregnancy will be reviewed.

Precision medicine in diabetes in pregnancy was also topical in 2018/19 with many studies aiming to identify biomarkers predictive of future gestational diabetes, complications of diabetes in pregnancy and future risk of diabetes in women with prior GDM. Highlights from this literature will be presented.

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### Advanced technologies in the management of diabetes- what's new?

#### Jane Holmes-Walker<sup>1,2</sup>

1. *Westmead Hospital, Westmead, NSW, Australia*

2. *Sydney Medical School, Westmead Hospital, University of Sydney, Sydney, NSW, Australia*

While clinical trials demonstrate the effectiveness of innovations such as Hybrid Closed Loop and continuous glucose monitoring (both real time and flash glucose monitoring) translating clinical trial outcomes to the real world experience has not produced consistent outcomes. In Australia, increased access to continuous glucose monitoring has resulted in both consumer and endocrinologist input to diabetes care outcomes. Outcomes in turn will be influenced by consumer and endocrinologist engagement with technology and perceived benefits of the technology.

To provide appropriate support to consumers it is critical for Endocrinologists to have an understanding of how each technology works, its benefits and limitations. It is important that technology when introduced reduces treatment burden. High rates of discontinuation of CGM occur both in those using Hybrid closed loop (which depends on CGM) and in those whose diabetes therapy does not rely on CGM for management.

New innovations in insulin delivery will reduce insulin infusion set failures, will incorporate insulin with more rapid onset to action and will reduce alarm fatigue. Different mathematical models of hybrid closed loop may mean that different models suit different individuals and at this stage we have no head to head comparisons of closed loop devices. Increased time in target (3.9-10 mmol/L) will become the new clinical measure to monitor as complications outcomes will be tied to time in range instead of the imperfect glycosylated haemoglobin.

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### The long and short of growth hormone replacement therapy in New Zealand

#### Penny Hunt<sup>1</sup>

1. *Christchurch Hospital, Strowan, CANTERBURY, New Zealand*

Recombinant human growth hormone was developed in the 1980s, following which it has been used worldwide for the treatment of short stature. Over the last almost 30 years, there has been increasing experience with the use of GH therapy for adults with confirmed GH deficiency, predominantly in the setting of known pituitary/hypothalamic disease. Many countries now support funded adult GH replacement therapy, including more recently Australia. New Zealand introduced nationally funded treatment for GH deficient adults in 2010. This talk will address the NZ experience with adult GH therapy, aiming to provide a practical perspective on treatment criteria, monitoring and outcomes.

## TERT promoter mutation and its clinical implication in thyroid cancer

**Jae Hoon Chung**<sup>1</sup>

1. Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Considering the long-term survival in most patients with thyroid cancer, it is very important to distinguish patients who need aggressive treatment from those who do not. Clinicopathological prognostic factors have been used to predict their prognoses, but could not completely predict the final outcome of each patient.

Molecular marker-based risk stratification of thyroid cancer has been recently proposed to better estimate its prognostic risk. The BRAF mutation has drawn much attention since 2000. However, cancer-related mortality has been low although the BRAF mutation is common in thyroid cancer. In many studies, the BRAF mutation was reported to be associated with an increased cancer-specific mortality, but it was no longer significant after the adjustment of risk factors.

Telomere reverse transcription (TERT) activation, one of the hallmarks of cancer, enables unlimited proliferation and is driven by oncogenes. In 2013, two point mutations (C228T and C250T) in the TERT promoter have been found in 71% of melanomas and have also been identified in over 50 cancer types including thyroid cancer. These somatic mutations enhanced promoter activity which might immortalize proliferative cancer cells by maintaining telomere length.

The TERT promoter mutation was found in approximately 10% of papillary thyroid carcinoma, 17% of follicular thyroid carcinoma, and more than 40% of poorly differentiated or anaplastic thyroid carcinoma. It has been reported to be associated with large tumor size, old age, dedifferentiation, aggressive histology, advanced stages, distant metastasis, recurrence and mortality in thyroid cancer. Concomitant TERT and BRAF mutations diminished the survival rate. Inclusion of TERT promoter mutation analysis with conventional clinicopathological evaluation could lead to better prognostication and management for individual patients. Its prognostic strength highlights its potential use as a clinical biomarker in thyroid cancer.

## Transmission of a long non-coding RNA in sperm is a candidate mechanism for achieving genomic imprinting through the histone-protamine transition in therian mammals.

**Teruhito Ishihara**<sup>1</sup>, **Oliver W Griffith**<sup>1</sup>, **Shunsuke Suzuki**<sup>2</sup>, **Marilyn B Renfree**<sup>1</sup>

1. School of BioSciences, The University of Melbourne, Melbourne, VIC, Australia

2. Agricultural and Life Sciences, Shinshu University, Nagano, Japan

Genomic imprinting is an epigenetic phenomenon that leads to the expression of genes in a parent-of-origin-specific way. In higher vertebrates, it occurs only in therian mammals: marsupials and eutherians. Imprinted genes are usually regulated by differentially methylated regions (DMRs). In the maternally-imprinted gene, *Mesoderm Specific Transcript (MEST)*, there is no DMR in marsupials, but imprinting may instead depend on histone modification(s). In contrast, both mouse and human *MEST* genes have a distinct DMR. Despite this, inhibition of DNA methylation does not induce *Mest* expression from the maternal allele in the mouse, suggesting that there may be a conserved non-DMR mechanism, such as histone modification-based imprinting, that controls imprinting in therian mammals. Interestingly, in mature mouse spermatozoa, this gene locus has a distinct active histone mark in its DMR, although 99% of histones are replaced with protamines during spermatogenesis. To achieve histone modification-based imprinting, males need to transmit specific histone marks to the next generation. Therefore, males must overcome the histone-protamine transition because this process would remove specific histone imprinting during spermatogenesis. At the human *MEST* gene locus, there is an antisense lncRNA, *MESTIT1*, which is predominantly expressed in the testis and in mature spermatozoa. Accumulation of lncRNAs such as *MESTIT1* in spermatozoa could transmit information to the next generation via the histone-protamine transition to establish histone modification-based imprinting. To test this idea, we investigated whether there was a marsupial lncRNA in the *MEST* gene locus. We identified a novel lncRNA from adult testes of the tammar wallaby and showed that the transcript was present in mature sperm. These data suggest that this antisense transcript in sperm may be necessary to establish genomic imprinting of the *MEST* gene through the histone-protamine transition.

## Paternal exposure to a toxicant across multiple generations is detrimental to the reproductive health of male progeny

**Caitlin G Chambers**<sup>1,2,3</sup>, **Brett Nixon**<sup>1,2</sup>, **Shaun D Roman**<sup>1,2,3</sup>

1. Discipline of Biology, School of Environmental and Life Sciences, University of Newcastle, Callaghan, New South Wales, Australia

2. The Priority Research Centre for Reproductive Biology, University of Newcastle, Callaghan, New South Wales, Australia

3. The Priority Research Centre for Drug Development, University of Newcastle, Callaghan, New South Wales, Australia

Humans are chronically exposed to acrylamide in carbohydrate-rich foods cooked above 120°C. We previously demonstrated that chronic administration of acrylamide to male mice, at a human-relevant dose (1 µg/mL drinking water for 6 months (M)) increased DNA damage in spermatozoa [1]. Thus, there is a concern for the increased susceptibility of offspring to acrylamide-induced DNA damage. To explore this possibility, we utilised a shorter exposure regimen (3M at 1 µg/mL acrylamide) and extended the study to the F2 generation. In addition, we conducted exposure studies on both the F1 and F2 generations. We

demonstrated that the shorter exposure regimen increased DNA fragmentation in spermatozoa of acrylamide-exposed F0 males (128% of control), replicated in the F1 generation, where DNA fragmentation was elevated in spermatozoa from the exposed progeny of unexposed fathers (154% of control). We determined the unexposed F1 progeny of F0 exposed males had increased DNA fragmentation in their spermatozoa (136% of control), whilst their exposed F1 littermates had DNA fragmentation equal to the unexposed progeny of F0 controls. Following exposure to the F2 generation, we established grand-paternal exposure elevated DNA fragmentation in spermatozoa of F2 progeny (115-135% of control) irrespective of exposure to the F1 or F2. Furthermore, we demonstrated significant correlations between DNA damage in the fathers (F0 or F1) and that of their male progeny (F1 or F2). This study also revealed that grand-paternal acrylamide exposure significantly affected the male reproductive tract, with reduced testis to body weight ratio of F2 progeny, regardless of F1 or F2 exposure.

This study provided the first evidence that acrylamide results in transgenerational damage extending to the F2 generation. This study also demonstrates that paternal and grand-paternal acrylamide exposure has a detrimental effect on male offspring and their genetic potential with, or without, further exposure to acrylamide

1. Katen AL, Chambers CG, Nixon B, Roman SD. Chronic acrylamide exposure in male mice results in elevated DNA damage in the germline and heritable induction of CYP2E1 in the testes. *Biol Reprod.* 2016;95(4):Article 86, 1-15.

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### The impact of acute acrylamide exposure on the small non-protein-coding RNA profile of mouse spermatozoa

**Natalie A Trigg<sup>1</sup>, Shaun D Roman<sup>1,2</sup>, Andrew L Eamens<sup>1</sup>, Miguel J Xavier<sup>1</sup>, Brett Nixon<sup>1</sup>**

1. Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia

2. Priority Research Centre for Drug Development, School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, 2308, Australia

Mature spermatozoa harbour a diverse population of small non-protein-coding RNAs (sRNA), which are delivered to the oocyte upon fertilisation, and thereafter, influence embryonic development and the subsequent health of offspring. Moreover, the sperm sRNA profile has been demonstrated to be altered in response to a range of environmental insults, with significant post-fertilisation consequences. Here, we assessed the impact to the global sRNA profile of mouse sperm after exposure to the xenobiotic, acrylamide. In mice, acute acrylamide exposure is known to lead to paternal-mediated embryo loss owing to dysregulation of post-testicular sperm maturation in the epididymis, a critical developmental window during which the sperm sRNA profile is dramatically remodelled. Hence, we exposed adult male mice to acrylamide (25mg/kg bw/day; i.p.) for a 5-day period, timed to coincide with sperm epididymal transit. Following exposure, next-generation sequencing was employed to survey differences in the global sRNA profile of mature sperm. Our data revealed a subset of sRNAs that were significantly altered in spermatozoa following acrylamide exposure (i.e. sRNA species with either elevated or reduced abundance of  $\geq 2$ -fold compared to controls;  $p < 0.05$ ). Alterations were documented for each major sRNA class, including the microRNA (miRNA) class. Specifically, 5.7% of all detected miRNAs were altered in their abundance. Furthermore, bioinformatic analysis identified the predominant pathways regulated by these differentially abundant miRNAs, including the cell growth and survival pathways implicated in early embryo development. Accordingly, we have validated the differential abundance of candidate miRNAs using RT-qPCR and confirmed that these changes originate in the sperm during epididymal transit. Building on these data, we now aim to elucidate the mechanisms by which acrylamide exposure mediates such pronounced changes in the sperm epigenetic landscape and determine the downstream biological consequences of an altered sperm miRNA profile on early embryo gene expression and development.

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### A mouse model of human SRY to study the structure/function relationship of the sex determining gene

**Ella Thomson<sup>1,2</sup>, Liang Zhao<sup>1</sup>, Brittany Croft<sup>3</sup>, Enya Longmuss<sup>1</sup>, Ee Ting Ng<sup>1</sup>, Emanuele Pelosi<sup>1,2</sup>, Andrew Sinclair<sup>3</sup>, Peter Koopman<sup>1</sup>**

1. Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia

2. Centre for Clinical Research, University of Queensland, Brisbane, Queensland, Australia

3. Murdoch Children's Research Institute, Melbourne, Victoria, Australia

Sex determination, the process of developing testes or ovaries, is a conserved process amongst most mammals. This is largely controlled by the Y chromosome gene, Sry. This triggers male specific gene expression by activating Sox9, and concurrently blocking expression of female specific genes. Low levels of conservation outside the DNA binding domain HMG-Box, make analysis of human Sry in mouse or other species difficult. The N and C terminal domains of hSry have no known function, but mutations in these regions have been identified in patients with disorders of sex development (DSD), suggesting important roles for protein stability or cofactor recruitment. To understand the structure/function relationship of Sry in humans, we have generated a single copy transgenic mouse model expressing human Sry (hSry). This produces chromosomally female, but phenotypically male mice. By generating mutations within each of the domains using CRISPR delivered by electroporation we can assess the functionality of various patient mutations as well as domain deletions on Sry action during sex determination. Using this method we have shown that the C terminal domain is critical for function, and *in vivo* evidence suggests the N terminal domain is also required. This mouse will be a useful tool in which to model human sex determination, study SRY mutations of DSD patients, and will help to further our understanding of the structure/function relationship of human SRY.

## Genistein directly impacts penis development causing hypospadias and may contribute to erectile dysfunction

**Luke C Govers<sup>1</sup>, Deidre Mattiske<sup>1</sup>, Andrew Pask<sup>1</sup>**

1. School of BioSciences, The University of Melbourne, Melbourne, Victoria, Australia

Hypospadias is a congenital abnormality in males, characterised by the abnormal termination of the urethral opening on the penis. Hypospadias is one of the most common disorders of sexual development (DSD), occurring in 1 in every 125 live male births in Australia and the U.S.A. Alarming the incidence of hypospadias has doubled in recent decades. This increase has been largely attributed to environmental endocrine disruptors (EEDs), which disrupt the delicate balance of hormonal signals required for normal penis development. Several human epidemiological studies have linked vegetarian and vegan diets to a 3-4 fold increase risk of hypospadias. These diets are typically high in plant based oestrogenic compounds (phytoestrogens), specifically genistein. However, a direct link between genistein exposures and hypospadias has not been defined, and is a major gap in our current knowledge of maternal diet-linked congenital abnormalities.

To address this question we have used our established high throughput *ex vivo* organotypic culturing system alongside micro-CT scanning and RNA seq experiments to conclusively demonstrate that the phytoestrogen genistein directly induces hypospadias and other penile abnormalities. Additionally, through our transcriptome analysis we have uncovered a previously unknown association with phytoestrogens and erectile dysfunction, a condition which is suspected to have increased, similar to hypospadias, over the past few decades. These findings provide a significant new insight into the risks associated with maternal ingestion of phytoestrogens over the window of penis development.

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## Antenatal corticosteroid treatment alters placental and fetal cardiac hemodynamic function in a gestation stage-dependent manner in mice

**Emma N Panting<sup>1</sup>, Jessica R Ivy<sup>2</sup>, Adrian Thomson<sup>2</sup>, Carmel M Moran<sup>2,1</sup>, Karen E Chapman<sup>2,1</sup>, Caitlin S Wyrwoll<sup>1</sup>**

1. School of Human Sciences, The University of Western Australia, Perth, WA, Australia

2. Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland

Potent synthetic glucocorticoids like dexamethasone are administered to pregnant women at risk of delivering preterm to reduce neonatal morbidity and mortality. In experimental models, chronically high antenatal glucocorticoid exposure adversely affects placental efficiency and offspring cardiovascular function. However, placental and fetal vulnerability to synthetic glucocorticoid exposure may depend on gestational stage. We hypothesised that dexamethasone exposure prior to, or coincident with, the normal rise in fetal endogenous glucocorticoids will differentially impact placental and cardiovascular function. C57BL/6 mice were time-mated and the day of plug designated E0.5. Dams were injected IP with vehicle or dexamethasone (500µg/kg) at E13.5 or E16.5 (n=5-7/group). *In vivo* pulsed-wave Doppler ultrasound scanning was conducted 24h post-injection (E14.5 or E17.5, respectively) to measure umbilical artery (UA) blood flow and fetal cardiac function. Immediately after scanning, tissues were collected. Data were analysed by 2-way ANOVA with post-hoc Bonferroni's test. Dexamethasone treatment reduced fetal weight at E14.5 (p=0.002) and placental weight at E17.5 (p=0.009). UA pulsatility index and velocity time integral remained unchanged at E14.5 and E17.5. An interaction effect was observed at E17.5 in UA resistance index (p=0.027) and systolic/diastolic ratio (p=0.025). At E14.5, dexamethasone reduced fetal heart rate (p=0.004) and increased isovolumetric relaxation time (p=0.007), ejection time (p=0.003) and mitral deceleration index (p=0.037). In contrast, at E17.5, dexamethasone had no significant effect on fetal cardiac function, though a strong trend (p=0.052) for increased myocardial performance index was evident. Fetal, but not placental, tissues appear sensitive to precocious dexamethasone exposure at E14.5, with reduced fetal growth and altered cardiac parameters suggesting impaired cardiovascular function. However, at peak fetal endogenous glucocorticoid synthesis (E16.5-17.5) when the fetus is no longer naive to glucocorticoids, fetal growth and cardiac function were preserved following dexamethasone exposure. This preservation at E17.5 was despite impaired placental capacity, alluding to protective fetal mechanisms.

## Pomegranate polyphenol punicalagin: A potential novel prevention therapy for preterm birth.

**Caitlyn Nguyen-Ngo<sup>1,2</sup>, Ratana Lim<sup>1,2</sup>, Jane Willcox<sup>3</sup>, Martha Lappas<sup>1,2</sup>**

1. University of Melbourne, Heidelberg, VIC, Australia

2. Mercy Hospital for Women, Heidelberg, VIC, Australia

3. La Trobe University, Bundoora, VIC, Australia

**INTRODUCTION:** Spontaneous preterm birth (sPTB) is the leading cause of neonatal morbidity and mortality. Inflammation plays a key role in initiating and maintaining uterine contractions and rupture of fetal membranes. Therefore, blunting the inflammatory response presents a therapeutic opportunity. Polyphenols, bioactive components in plants, exhibit potent anti-inflammatory properties. While epidemiological studies have linked plant-based diets with reduced risk for sPTB, the contribution of polyphenols to these effects are unknown. Thus, this study aimed to investigate the effects of punicalagin on mediators involved in active labor using *in vitro* and *in vivo* models of sPTB.

**METHODS:** Primary human myometrial, amnion mesenchymal, amnion epithelial and decidual cells were treated with or without 10 $\mu$ M punicalagin in the presence of 1ng/ml IL-1B or 10ng/ml TNF (n=6-7 patients/group). Endpoint analysis was assessed by RT-qPCR, ELISA and gelatin zymography. Myometrial cell contractility was determined by collagen gel assay. C57BL/6 mice were injected with 1 mg/kg punicalagin on gestational day (GD)14.5 until GD16.5. On GD16.5, mice were injected with 15 $\mu$ g LPS, sacrificed after 6h, and tissues collected for assessment of intrauterine inflammation. Data was analysed by one-way ANOVA and  $P < 0.05$  was considered significant.

**RESULTS:** Punicalagin treatment significantly suppressed IL-1B/TNF-induced expression and secretion of pro-inflammatory cytokines and chemokines in primary human myometrial, amnion and decidual cells. In myometrial cells, punicalagin significantly decreased IL-1B/TNF-induced secretion of PGF2 $\alpha$ , expression of contraction associated proteins, and collagen gel contractility. In amnion and decidual cells, punicalagin significantly suppressed IL-1B/TNF-induced expression and activity of matrix metalloproteinases. *In vivo*, punicalagin significantly reduced intrauterine inflammation induced by LPS.

**CONCLUSION:** Using human and murine pre-clinical models of sPTB, we have demonstrated that the pomegranate polyphenol punicalagin suppressed the expression of mediators involved in myometrial contractions and rupture of fetal membranes. Punicalagin may represent a novel therapeutic to prevent sPTB and related sequelae.

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### High dose radiotherapy directly damages the uterus and influences pregnancy success

**Meaghan Griffiths<sup>1</sup>, Amy Winship<sup>1</sup>, Karla Hutt<sup>1</sup>**

1. Department of Anatomy and Developmental Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

As cancer survivor rates rise, understanding and preventing adverse, long-term impacts of cancer treatments, including those on fertility, have become increasingly important. Clinical data show radiotherapy stunts uteri growth and causes resistance to hormone replacement therapy in pre-pubertal girls. Female cancer survivors also experience lower clinical pregnancy rates, take a longer time to achieve pregnancy (despite normal ovarian and endocrine function), require a greater number of embryo transfers to achieve pregnancy and exhibit higher prevalence of pregnancy complications. Together, this suggests that cancer treatments damage the uterus, but this has been largely ignored by the oncofertility field. We aimed to determine the impact of radiotherapy on the uterus, independent of ovarian effects, and its ability to establish and maintain healthy pregnancy.

Adolescent (4-6-week-old) female mice exposed to whole body high dose (7 Gray) irradiation, or non-irradiated control were ovariectomised before hormonal stimulation to induce endometrial receptivity, or transfer of equal number of day 3 blastocysts from healthy, unexposed donor mice (n=6-8/group).

Within hours of irradiation, markers of DNA damage (yH2AX), cell death (CC3 and TUNEL), and the intrinsic apoptosis pathway (PUMA) localise to irradiated adolescent mouse uteri. Additionally, irradiated mice hormonally induced to become receptive had significantly decreased uteri weight ( $p < 0.05$ ) and myometrial area ( $p < 0.01$ ) versus controls, and reduced estrogen receptor alpha mRNA ( $p < 0.05$ ). Irradiated mice that received embryo transfers demonstrated a trend for decreased implantation site number ( $p < 0.1$ ), with pale, atrophic uteri suggesting vascular defects. To investigate this possibility further we are employing Doppler ultrasound to determine the effect of irradiation on uterine artery blood flow in pregnant and non-pregnant mice. Defining the extent of uterine damage caused by cancer treatments and the underlying mechanisms would be a breakthrough in understanding how cancer treatments compromise reproductive outcomes.

## 19

### AR-45 is a novel placental androgen receptor variant that regulates trophoblast growth and angiogenic pathways

**Ashley Meakin<sup>1</sup>, Zarqa Saif<sup>1</sup>, Vicki Clifton<sup>1</sup>**

1. Mater Medical Research Institute - University of Queensland, Woolloongabba, QLD, Australia

#### Background

Androgen concentrations rise throughout gestation in the maternal and fetal circulations and may drive sex-specific differences in fetal growth. These sex-steroids function through the androgen receptor (AR) to transcriptionally regulate genes primarily involved in growth pathways. Indeed, current epidemiological data shows male fetuses have greater birthweight outcomes but are at a greater risk of being born small for gestational age (SGA), when compared to female fetuses, especially in the presence of pregnancy complications such as maternal asthma. The mechanisms contributing to these male-specific outcomes remain unclear; however, recent findings from our group identified sex-specific expression of a novel placental AR variant, AR-45, that may ensure appropriate male growth in uncomplicated asthmatic pregnancies (i.e. the absence of an exacerbation throughout gestation). The current study therefore focuses on characterising the function of AR-45 in the human placenta and defining its role in regulating growth *in vitro*.

#### Methodology

AR-45 cellular localisation was measured in response to 0.1nM dihydrotestosterone (DHT), as was the expression of androgen-mediated downstream targets. AR-45-overexpressing trophoblast cell proliferation was also measured in response to DHT.

#### Results

*In vitro* DHT stimulation increased total AR-45 protein and *IGF-1R* and *IGFBP-5* mRNA. In AR-45-overexpressing cells, AR-45 was observed to localise to the nucleus upon 0.1nM DHT stimulation, and this was associated with a two-fold increase in the expression of *VEGF* mRNA. In contrast to endogenous protein studies, AR-45-overexpressing cells stimulated with 0.1nM DHT had reduced *IGF-1R* mRNA expression. Furthermore, proliferation rates of AR-45-overexpressing cells was reduced upon 0.1nM DHT stimulation, when compared to controls.

#### Conclusion

Our data shows AR-45 inhibits growth signalling but enhances the expression of angiogenic factors in an *in vitro* trophoblast model. This pathway specific function requires more investigation but may be a compensatory mechanism instituted by the placenta to ensure vasculogenesis and angiogenesis is appropriately regulated.

## 20

### Maternal overexpression of anti-Müllerian hormone causes post-implantation embryo loss in mice.

**Michael W Pankhurst<sup>1</sup>, Christine Neyt<sup>1</sup>, Rebecca L Kelley<sup>2</sup>, Karmilla Jaafar Amsak<sup>1</sup>, Jane E Girling<sup>1</sup>**

1. Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

2. School of Biosciences, University of Melbourne, Melbourne, Victoria, Australia

In females, anti-Müllerian hormone (AMH) is primarily produced in the ovary to regulate folliculogenesis. However, recent studies have reported the presence of AMH and the AMH type 2 receptor mRNA and protein in the placenta and uterus. We have observed high rates of foetal resorption in the uteri of AMH-overexpressing (*Thy1.2-AMH<sup>Tg0</sup>*) female mice, which might indicate that uncontrolled AMH signalling during pregnancy leads to miscarriage. However, we have also observed abnormal embryo development in the *Thy1.2-AMH<sup>Tg0</sup>* mice, which could arise from defects during oogenesis and folliculogenesis. The aim of this study was to determine whether embryos derived from wild-type donor females could be carried to term after embryo-transfer into *Thy1.2-AMH<sup>Tg0</sup>* females. Embryos from wild-type dams were chosen because the oocytes and preimplantation embryos would be exposed to normal AMH levels in the in the ovary and oviduct. Wild-type females were mated with wild-type studs and the embryos were flushed from the uteri and were vitrified at 3.5 days-post coitus. A total of 10 embryos were then transferred into wild-type control or *Thy1.2-AMH<sup>Tg0</sup>* recipient females on day 3.5 of pseudopregnancy. The transfer of embryos into *Thy1.2-AMH<sup>Tg0</sup>* females did not yield a single livebirth compared to livebirth rates of 50% in wild-type control recipients. These findings suggest that elevated levels of AMH during pregnancy can lead to miscarriage and that these effects are independent of the actions of AMH on oogenesis. Research is ongoing to determine the role of endogenous AMH in the uterus and placenta.

## 21

### Evolving mechanisms of incretin action. Where and how

**Daniel Drucker<sup>1</sup>**

1. Mt. Sinai Hospital University of Toronto, Toronto, ONTARIO, Canada

Enteroendocrine hormones, exemplified by GLP-1 control multiple facets of food ingestion, gut motility, nutrient assimilation and energy storage, supporting the development of gut hormone therapies for the treatment of metabolic disorders such as diabetes and obesity. Surprisingly, although the physiological and pharmacological actions of GLP-1 have been extensively studied, the precise mechanisms and cellular sites linking GLP-1 action to the control of metabolism remain controversial. Here we summarize controversies in the field, providing an overview of key concepts linking the secretion of GLP-1 to the control of cardiometabolic actions in distant target tissues.

## 22

### Digital Medicine in Thyroidology: A New Era of Managing Thyroid Disease

**Jae Hoon Moon<sup>1</sup>**

1. Seoul National University College of Medicine, Bundang-gu, Seongnam-si, GYEONGGI-DO, South Korea

Digital medicine has the capacity to affect all aspects of medicine, including disease prediction, prevention, diagnosis, treatment, and post-treatment management. In the field of thyroidology, researchers are also investigating potential applications of digital technology for the thyroid disease. Recent studies using artificial intelligence (AI)/machine learning (ML) have reported reasonable performance for the classification of thyroid nodules based on ultrasonographic (US) images. AI/ML-based methods have also shown good diagnostic accuracy for distinguishing between benign and malignant thyroid lesions based on cytopathologic findings. Assistance from AI/ML methods could overcome the limitations of conventional thyroid US and fine-needle aspiration cytology. Some web-based solutions for thyroid disease care have been developed. A web-based database for thyroid cancer care is expected to serve as a clinical platform to facilitate better thyroid cancer care and as a research platform providing comprehensive disease-specific big data. A web-based application for detecting thyrotoxicosis using biosignals from wearable devices could aid in the management and early detection of thyroid dysfunction. In the thyroidology field, research involving the range of digital medicine technologies and their clinical applications is expected to be even more active in the future.

## 23

### Immune-checkpoint inhibitors and thyroid diseases

**Hidefumi Inaba<sup>1</sup>**

1. The First Department of Medicine (Department of Diabetes, Endocrinology and Metabolism), Wakayama Medical University, Wakayama, Japan

Immune-checkpoint inhibitors (ICIs) are monoclonal antibodies to cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1), and its ligand (PD-L1). ICIs are promising agent for treatment of malignant diseases.



However, ICIs may induce immune-related adverse events (irAEs) in several organs, including skin, gastrointestinal tracts, liver, lung, nervous systems, and endocrine organs.

The Endocrine irAEs include hypopituitarism, primary adrenal insufficiency, thyroid dysfunction, hypoparathyroidism, and type 1 diabetes.

The incidence of thyroid irAEs following treatment with anti-PD-1 antibodies is 5-10%, and higher than that observed with anti-CTLA-4 antibodies. Thyroid irAEs classified to thyrotoxicosis (suppressed levels of serum TSH, and elevated levels of serum FT4) and hypothyroidism (increased levels of serum TSH, and low levels of serum FT4). Low T3 syndrome, often seen in patients with advanced malignancies, should be considered separately.

Thyrotoxicosis develops 2-6 weeks after the administration of ICIs in most cases, and is often followed by hypothyroidism. The activation of thyroid autoimmunity seems to be associated with development of thyroid irAEs, and positivity of thyroid autoantibodies (TgAb and/or TPOAb) prior to ICIs therapy may predict thyroid irAEs.

As symptoms such as fatigue can also be observed in patients with malignancies, precise diagnosis of thyroid irAEs are therefore based on clinical manifestation, laboratory testing, ultrasound examination, and thyroid scintigrams. Thyrotoxicosis may be relieved with beta-blockers, and hypothyroidism may be treated with L-T4. The use of ICIs in patients with thyroid irAEs should be withheld until they became stable.

Positive correlation of thyroid irAEs and clinical antitumor effectiveness has been reported, and thus awareness of thyroid irAEs are necessary for clinicians.

In this symposium, we introduce the mechanism, prompt managements, and future prospects of thyroid irAEs during ICIs therapy.

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## Thyroid hormone-responsive microRNAs in metabolic regulation

**Hao Ying<sup>1</sup>**

1. *Shanghai Institutes for Biological Sciences, CAS, Xuhui, SHANGHAI, China*

Thyroid hormone (TH) plays pivotal roles in metabolic homeostasis. The physiological function of TH is mainly mediated by thyroid hormone receptors (TRs). The role of TH in regulating metabolism has already led to several new therapeutic targets for metabolic disorders. Understanding the mechanisms of the various TH signalling pathways in metabolism will improve our likelihood of identifying novel therapeutic targets. In the past ten years, we profiled the miRNA expression in various metabolic tissues of mice under different thyroid states and identified multiple TH-regulated miRNAs, including miR-133a, miR-182, and miR-378 in skeletal muscle, and miR-378 in liver, respectively. Our recent data suggest that: 1) miR-133a mediates the effect of TH on muscle fiber type conversion and might contribute to the inhibitory effect of TH on myoblast proliferation; 2) miR-182 controls myofiber type determination and fuel selection in skeletal muscle, thereby modulating whole-body glucose homeostasis; 3) miR-378 plays a role in the metabolic regulation of cell death by targeting both autophagy and apoptosis in skeletal muscle; 4) hepatic miR-378 regulates glucose and lipid homeostasis by targeting a critical node in insulin signaling and mediates the TH action in cholesterol and energy metabolism. Based on our studies, we propose that TH is able to achieve its regulatory effect on metabolism through miRNA-mediated mechanisms.

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## Beyond GLP-1. The Future of Diabetes Therapy

**Daniel Drucker<sup>1</sup>**

1. *Mt. Sinai Hospital University of Toronto, Toronto, ONTARIO, Canada*

GLP-1R agonists reduce body weight, decrease glycemia, and reduce the development of cardiovascular disease, establishing this mechanism as foundational for future development of new more effective therapies for cardiometabolic disorders. How to make the next generation therapies even more effective? Here we discuss the emerging field of peptide multi-agonists, highlight promising scientific mechanisms and therapeutic approaches for the treatment of diabetes and associated comorbidities

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## Diabetic drugs that protect the kidney

**Usha Panchapakesan<sup>1</sup>**

1. *Kolling Institute of Medical Research, University of Sydney, Royal North Shore Hospital, St Leonards, NSW, Australia*

Diabetic drugs that inherently offer cardiac and renal protection is now a reality. Recent landmark trials have demonstrated these effects especially with sodium glucose cotransporter 2 inhibitors and glucagon like peptide -1 analogues. This raises further questions regarding which diabetic combination therapy is the best in the clinical setting. The challenges in clinical trials evaluating hard renal endpoints will be discussed followed by the recent evidence around the validity of urinary albumin levels as a surrogate endpoint.

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## Using GLP-1 receptor agonists to improve health outcomes for people with type 2 diabetes

**Richard Maclsaac<sup>1</sup>**

1. *Endocrinology and Diabetes, St Vincent's Hospital & University of Melbourne, Fitzroy, Victoria, Australia*

Glucagon-like peptide 1 (GLP-1) is a gut-derived incretin-hormone that is released in response to eating. Its actions include appetite control, glucose-dependent secretion of insulin and inhibition of glucagon secretion. GLP-1 receptor agonists (GLP-1RAs) have been developed to enhance the incretin effect and have been shown to reduce fasting and post-prandial glucose levels, HbA1c values, blood pressure and weight. The most common side effect of GLP-1RA use is nausea which usually decreases over time. The beneficial effects of some (liraglutide, semaglutide, albiglutide, and dulaglutide) but not all (lixisenatide and extended-release exenatide) GLP-1RAs also extend to the cardiovascular (CV) system. Possibly, the differences in CV outcomes observed in clinical trials with various GLP-1RAs relates to their molecular structure and half-life, but differences in trial design and conduct may also be possible explanations. The exact mechanisms whereby some GLP-1RAs reduce CV events remains unknown but may be due to improved metabolic control, an anti-atherogenic effect and modulation of inflammatory pathways. Furthermore, GLP-1RAs reduce albuminuria and may slow down the progression of renal function loss, but their long-term effects on renal outcomes remains unknown. This presentation will highlight our current understanding of the potential metabolic, CV and renal benefits of the GLP-1RAs.

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## Glycogen in the diabetic kidney: hero or villain?

**Mitchell Sullivan<sup>1</sup>, Ziyi Wang<sup>1</sup>, Qinghua Liu<sup>1</sup>, Liang Wang<sup>1</sup>, Xinle Tan<sup>1</sup>, Bin Deng<sup>1</sup>, Engpeng Li<sup>1</sup>, Robert G Gilbert<sup>1</sup>**

1. *Mater Research Institute - The University of Queensland, Brisbane, QLD, Australia*

Diabetes is one of the leading causes of kidney disease, affecting approximately 30% of individuals with diabetes. Diabetic kidney disease (DKD) ultimately quadruples the risk of cardiovascular disease and mortality. Diabetes is characterised by a fundamental breakdown in glucose homeostasis. It has been observed that diabetes leads to an over-accumulation of the energy storage molecule glycogen (a highly branched polymer of glucose) in kidney tissue. This study was designed to characterise the molecular structure of this glycogen in order to gain insight into its molecular properties, allowing us to better predict possible effects it has on kidney health and blood glucose control.

Sprague-Dawley rats were given a single dose of Streptozotocin (STZ) (30 mg/kg) or a vehicle control at 4 weeks of age (N = 8) and were killed after the age of 16 weeks. The amount and structural parameters of kidney glycogen from the diabetic and control rats were analysed.

There were large accumulations of glycogen in the kidneys of rats with STZ induced diabetes, with very low amounts detected in the non-diabetic control animals. The structural parameters of the glycogen closely resemble muscle glycogen, consisting of the smaller b particles, contrasting with liver and heart glycogen which contains the much larger a particles (which are many b particles attached together). The chain length distribution of these glycogen particles was also consistent with muscle glycogen, providing evidence that while the glycogen accumulates to abnormal levels, it does not resemble the longer-chained glycogen present in numerous glycogen storage diseases such as Lafora disease.

Understanding whether the glycogen that abnormally accumulates is protective (hero), pathological (villain) or inconsequential (bystander) will help determine whether there are therapeutic targets that mitigate diabetic kidney disease.

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## Environmental Epigenetics and Reproductive Compromise

**Linda Giudice<sup>1</sup>**

1. *Center for Reproductive Sciences, University of California, San Francisco, CA, USA*

There has been an alarming global increase in developmental abnormalities, intellectual disabilities, reproductive compromise, respiratory, endocrine and metabolic disorders, and cancer over the past 50-75 years. As this meteoric rise has occurred in a relatively short time-frame, it is unlikely that genetics is their sole cause and likely that environmental factors are significant contributors. Globally, chemicals in agriculture, pesticides, industrial waste, personal care products, household cleaning agents, and nearly ubiquitous plastics, along with particulate matter in indoor and outdoor air pollution are of great concern to human health. Human epidemiologic studies and wildlife, animal studies and laboratory data support plausible causation of these agents in human disease risk. Exposures to environmental toxics at sensitive and critical windows of development have adverse effects on reproductive tract development and function through epigenetic and other mechanisms, resulting in or exacerbating reproductive disorders including, e.g., male and female infertility, endometriosis, polycystic ovarian syndrome, uterine fibroids, and pregnancy outcomes. Additionally, disadvantaged populations have higher likelihood of living in contaminated communities and higher risk occupations, augmenting their risks of poor reproductive outcomes. This lecture will review relevant data supporting environmental toxics and epigenetic and signaling dysregulation putting world reproduction at risk. It will also highlight how reproductive and other healthcare professionals are positioned to advocate for global solutions to prevent these growing harms to the health of this and future generations.

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## The efficacy of dietary interventions in amending polycystic ovary syndrome (PCOS) traits in a PCOS mouse model

**Valentina Rodriguez Paris<sup>1</sup>, Samantha M Solon-Biet<sup>2</sup>, Alistair M Senior<sup>2</sup>, Melissa C Edwards<sup>3,1</sup>, Madeleine J Cox<sup>1</sup>, William L Ledger<sup>1</sup>, Robert B Gilchrist<sup>1</sup>, Stephen J Simpson<sup>2</sup>, David J Handelsman<sup>3</sup>, Kirsty A Walters<sup>3,1</sup>**

1. *School of Women's & Children's Health, University of New South Wales, Sydney, NSW 2052, Australia*

2. *Charles Perkins Centre, University of Sydney, Sydney, NSW 2006, Australia*

3. *ANZAC Research Institute, University of Sydney, Sydney, NSW 2139, Australia*

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder featuring reproductive, endocrine and metabolic abnormalities. Its aetiology is unknown and current medical management is symptom based. Hyperandrogenism is a key characteristic of PCOS, and diet is innately associated as obesity is present in 40-80% of PCOS patients. Dietary interventions are a potentially powerful drug-free treatment to ameliorate PCOS manifestation, but the optimum diet for PCOS remains undetermined. We provided our dihydrotestosterone (DHT)-induced PCOS and control mice with *ad libitum* access to one of 10 diets varying in protein (P), carbohydrate (C) and fat (F) content, to determine the impact of dietary macronutrient balance on the development of PCOS features. All control mice cycled, while most PCOS mice exhibited complete estrous acyclicity. However, cyclicity was restored in PCOS mice consuming a C intake between 20-30kJ/day. Anovulation, as indicated by the presence of corpora lutea in the ovaries, was ameliorated at a C intake >20kJ/day. In contrast, dietary macronutrient composition had minimal effects on PCOS metabolic features. PCOS mice were more sensitive to C and F intake as they gained significantly more weight compared to controls at low C and F intakes of <25kJ/day each (P<0.05). This weight increase was correlated with a significant increase in adipocyte size in PCOS mice compared to controls (P<0.05). Furthermore, serum adiponectin levels were significantly decreased compared to controls at all macronutrient intakes (P<0.001) consistent with adipose tissue dysfunction in PCOS females. Moreover, PCOS mice were unable to regulate serum fasting glucose and cholesterol levels across varying macronutrient intakes, implying the hyperandrogenic PCOS environment impedes metabolic homeostasis in response to dietary changes (P<0.05). These findings provide evidence that PCOS traits can be ameliorated through dietary interventions, although hallmark reproductive and metabolic PCOS traits are differentially sensitive to dietary macronutrient balance.

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### Skin glucocorticoid metabolism in burn injury: towards novel treatments that reduce scarring

**Kevin HY Tsai<sup>1</sup>, Jinwen Tu<sup>1</sup>, Roxanne J Parungao<sup>1</sup>, Huaikai Shi<sup>1</sup>, Xiaosuo Wang<sup>2</sup>, Peter Maitz<sup>1</sup>, Yiwei Wang<sup>1</sup>, Mark S Cooper<sup>1</sup>**

1. Anzac Research Institute, Sydney, NSW, Australia

2. BOSCH MASS SPECTROMETRY FACILITY, Bosch Institute, Sydney, NSW, Australia

The most common and severe complication of burn injury is excessive scarring/tissue fibrosis. No current treatments reduce scarring after burns. Prolonged exposure to high levels of glucocorticoids (Cushing's syndrome) detrimentally impacts skin, with reduced collagen production and impaired wound healing. We previously demonstrated that skin can generate active glucocorticoids locally through expression and activity of the 11 $\beta$ -hydroxysteroid dehydrogenase type 1 enzyme (11 $\beta$ HSD1). We hypothesised that local glucocorticoid activation by 11 $\beta$ HSD1 is an important regulator of wound healing, fibrosis and scarring after burn injury. We additionally proposed that pharmacological manipulation of this system would improve outcomes of burn wound healing.

We examined glucocorticoid metabolism (by RT-PCR, immunohistochemistry and specific enzyme activity assays) in burn and non-burn skin from burn injury patients (n=14) and mouse models of burn injury (1cm<sup>2</sup> full thickness burn). We utilised mice with genetic or pharmacological deletion of 11 $\beta$ HSD1 in skin to evaluate effects of 11 $\beta$ HSD1 on burn injury healing and wound fibrosis. We also developed slow release scaffolds containing therapeutic agents that are selectively reactivated in skin cells expressing 11 $\beta$ HSD1.

Expression of 11 $\beta$ HSD1 in human and mouse skin increased substantially after burn injury (7.1 $\pm$ 1.8 fold increase on days 4-9 compared to non-burn skin, p<0.05). Early after injury expression was primarily in immune cells but at later stages in fibroblasts. Mice with 11 $\beta$ HSD1 deletion experienced faster wound healing post burn (45 $\pm$ 3% wound area healed compared to 28 $\pm$ 4% wildtype at day 7, p<0.001) but when healed these wounds had excessive collagen density and skin thickening, and highly abnormal collagen fibre organisation. In wildtype mice application of scaffolds loaded with inactive glucocorticoid (prednisone) significantly impacted wound healing demonstrating feasibility of using enzyme substrates to improve wound outcomes.

The findings demonstrate the importance of skin 11 $\beta$ HSD1 in wound healing and scarring after burn injury and indicate approaches to prevent excessive scarring.

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### Altered placental androgen signalling contributes to growth restricted outcomes

**Ashley Meakin<sup>1</sup>, Zarqa Saif<sup>1</sup>, Ratana Lim<sup>2</sup>, Martha Lappas<sup>2</sup>, Vicki Clifton<sup>1</sup>**

1. Mater Medical Research Institute - University of Queensland, Woolloongabba, QLD, Australia

2. University of Melbourne, Melbourne

#### Background

Current epidemiological data shows male fetuses have greater birthweight outcomes, but are at a greater risk of being born small for gestational age (SGA), when compared to female fetuses. The mechanisms contributing to these male-specific outcomes remain unclear but may be mediated, in part, by altered placental androgen signalling. Our group recently identified sex-specific expression of multiple androgen receptor (AR) variants in the human placenta that may modulate placental androgen signalling. Specifically, expression of a 45kDa variant, AR-45, was associated with growth outcomes in males only. We have questioned whether altered AR-45 expression and localisation contributes to growth perturbations. Therefore, this study has investigated AR-45 function *in vitro*, and characterised its expression and localisation in SGA placentae.

#### Methodology

AR-45 cellular localisation was measured in response to 0.1nM dihydrotestosterone (DHT), as was the expression of androgen-mediated downstream targets. AR-45-overexpressing trophoblast cell proliferation was also measured in response to DHT. AR variant protein levels were measured in appropriate for gestation age (AGA) (n=28) and SGA (n=84) placentae.

#### Results

AR-45-overexpressing trophoblast cells had reduced proliferation, reduced gene expression of targets involved in the IGF-axis, but significantly increased *VEGF* mRNA expression. Cytoplasmic AR-45 expression was significantly increased in male

placentae from severe SGA neonates (<5<sup>th</sup> birthweight centile (BWC)), when compared to moderate SGA (5<sup>th</sup>–10<sup>th</sup> BWC) and AGA (>10<sup>th</sup> BWC). Cytoplasmic-localised placental AR-45 trended towards a negative association with BWC in males only ( $r^2=0.294$ ,  $p=0.059$ ).

Conclusion

Our data shows AR-45 inhibits growth signalling, but enhances the expression of angiogenic factors. It is postulated that cytoplasmic sequestering of AR-45 alters androgen signalling, thereby contributing to growth restriction via perturbed placental vasculature. Evidently, further studies are needed to understand mechanisms contributing to AR-45 localisation and downstream target gene transcriptional regulation within the placenta, and the implications these may have for fetal growth outcomes.

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## Interrogating Bone Morphogenetic Signalling in cancer cachexia

**Adam Hagg<sup>1,2</sup>, Roberta Sartori<sup>3</sup>, Craig A Harrison<sup>1</sup>, Marco Sandri<sup>3</sup>, Paul Gregorevic<sup>2</sup>**

1. *Physiology, Monash University, Melbourne, Victoria, Australia*

2. *Physiology, University of Melbourne, Melbourne, Victoria, Australia*

3. *Biomedical Sciences, University of Padova, Padova, Italy*

Cancer cachexia is characterised by debilitating frailty and fatigue associated with profound loss of lean and fat mass. Complications arising from cachexia increase morbidity, reducing patients' quality of life, and ultimately account for 1 in 3 advanced cancer deaths. Loss of muscle mass associated with cachexia is a key prognostic indicator in the clinic, and pre-clinical studies have shown that preservation of muscle mass extends the lifespan of cachectic mice independent of tumor progression. The mechanisms underlying cachexia remain incompletely defined.

Recent studies have established that Bone Morphogenetic Protein (BMP) signalling is a key regulator of skeletal muscle plasticity. We sought to investigate the contribution of the BMP pathway to the cachectic phenotype in mice.

In the muscles of multiple cachectic mouse models, we observed diminished Smad1/5/8 phosphorylation (a key BMP effector), and increased expression of the BMP antagonist Noggin. Increasing Noggin expression in the muscles of tumor-free mice resulted in muscle atrophy resembling cachexia. Expression of Interleukin-6 (IL-6) was sufficient to induce Noggin gene expression in cancer-free mice and genetic inhibition of IL-6 signalling reduced Noggin gene expression in tumor-bearing mice.

Seeking to evaluate the therapeutic potential of modulating the BMP pathway, we observed that genetic and pharmacological interventions to increase BMP signalling reduced muscle wasting associated with tumor burden in mice and extended lifespan by over 50%.

As the BMP pathway is a regulator of neuromuscular junction formation in insects, we investigated whether impaired BMP-Smad1/5/8 signalling in cachectic muscles is associated with remodeling of the neuromuscular junction. Our studies revealed that significant neuromuscular junction defects arise with cachexia progression.

Our studies demonstrate a novel role of perturbed BMP signalling in the pathogenesis underlying cancer cachexia, and support further investigation of interventions targeting the BMP pathway as potential treatments.

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## The impact of menstrual cycling on OncotypeDx recurrence scores in premenopausal breast cancer patients

**Sarah M Bernhardt<sup>2,1</sup>, Pallave Dasari<sup>2,1</sup>, Danielle J Glynn<sup>2,1</sup>, Amanda R Townsend<sup>3</sup>, Timothy J Price<sup>3</sup>, Wendy V Ingman<sup>2,1</sup>**

1. *Discipline of Surgery, School of Medicine, The University of Adelaide, Woodville, SA, Australia*

2. *The Robinson Research Institute, The University of Adelaide, Adelaide, SA, Australia*

3. *Department of Medical Oncology, The Queen Elizabeth Hospital, Woodville, SA, Australia*

OncotypeDx is a genomic test used to help guide adjuvant chemotherapy treatment decisions for hormone receptor (HR)-positive breast cancers. There is a scarcity of literature on whether OncotypeDx is suitable for use in premenopausal women, where ovarian hormones estrogen and progesterone fluctuate during the menstrual cycle. This project aimed to determine how variable OncotypeDx recurrence scores are between paired breast cancer samples from premenopausal women, compared to postmenopausal women, and whether recurrence scores are affected by ovarian cycle stage.

To investigate the variability in OncotypeDx recurrence scores within the same tumour collected on different days of the menstrual cycle, paired HR-positive breast cancer samples were collected from premenopausal women (<50 years old; n=19) and compared to non-menstrual cycling postmenopausal women (≥50 years old; n=13). Samples were collected an average of 18 days apart and in the absence of any intervention. OncotypeDx recurrence scores were calculated and compared between paired samples. There was increased variability in recurrence scores between paired samples from premenopausal women ( $3.1 \pm 2.5$ ; mean  $\pm$  stdev) compared to postmenopausal women ( $2.4 \pm 1.7$ ;  $p=0.03$ ).

To determine whether the ovarian cycle contributes to the greater variability in OncotypeDx recurrence scores observed in premenopausal women, HR-positive mammary tumours from naturally cycling MMTV-PyMT mice were dissected at either the estrus or diestrus phase of the ovarian cycle (n=25, 28 respectively). Tumours collected from mice at diestrus showed significant differences in 6/16 OncotypeDx signature genes ( $p \leq 0.05$ ), and a significant increase in their OncotypeDx recurrence score ( $21.1 \pm 2.4$ ; mean  $\pm$  SEM), compared to tumours dissected at estrus ( $15.5 \pm 1.9$ ;  $p=0.03$ ).

This study demonstrates that OncotypeDx recurrence scores are more variable in young women and that ovarian cycle stage significantly alters OncotypeDx recurrence scores in mouse models. We propose that hormonal fluctuations during the menstrual cycle impact OncotypeDx recurrence scores, and may affect the clinical utility of this test in premenopausal women.

## Cellular complexity of the embryonic gonads revealed by single cell transcriptomics

**Martin A Estermann<sup>1</sup>, Sarah Williams<sup>2</sup>, Andrew Major<sup>1</sup>, Craig Smith<sup>1</sup>**

1. Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia

2. Monash Bioinformatics Platform, Monash University, Melbourne, Victoria, Australia

The embryonic gonad is the only organ to have a developmental choice: testis or ovary. We use the chicken as a model organism to understand these developmental decisions. In developing chicken embryos, the gonads form on the medioventral surface of the mesonephric kidneys at embryonic day (E) 3, equivalent to Hamburger and Hamilton stage (HH) 19. Before gonadal differentiation, the gonads are presumed to be bipotential and to have the same structure, cell types and gene expression profiles between sexes. At E6 (HH29), gonads begin morphological differentiation into testes in male (ZZ) embryos or unilateral ovary in female (ZW) embryos. Recently, single cell RNA-seq has been used to identify the emergence of gonadal cell types in the mouse embryo. This data focused on SF1<sup>+</sup> somatic cells and only part of development. It remains unclear whether different cell sub-populations exist, and whether there are other cell types in addition to SF1<sup>+</sup> cells. We performed single-cell RNA sequencing of entire left male and female embryonic chicken gonads at different time points: before gonadal differentiation (E4.5) and during differentiation (E6.5 and E8.5) and after somatic differentiation (E10.5). Prior, gonadal differentiation occurs the same cell populations exist in male and female gonads. The majority of the genes on each cluster were expressed equally in males and females, consistent with the previous mouse data. However, we found that female gonads have more diverse cell populations than males during development. At least nine transcriptionally distinct populations were identified in the female gonads and seven in males throughout the different developmental stages post sexual differentiation. Furthermore, three cell populations were only present in male gonads and four were only present in females. These results reveal greater cellular complexity during vertebrate gonadal sex differentiation than previously thought.

## A regulatory role for CHD4 in maintenance of the spermatogonial stem cell pool

**Brett Nixon<sup>1</sup>, Jon M Oatley<sup>2</sup>, Tessa Lord<sup>1</sup>**

1. University of Newcastle, Callaghan, NSW, Australia

2. Washington State University, Pullman, WA, USA

Precise regulation over spermatogonial stem cell (SSC) function is integral for continuation of spermatogenesis. SSCs must balance self-renewal with the production of progenitor spermatogonia that are poised to enter into a pathway of differentiation, lest the self-renewing reservoir become exhausted, instigating azoospermic infertility. To expand our limited understanding of factors that drive self-renewal and maintenance of the SSC pool, we previously designed and implemented a high-throughput screening pipeline using a large-scale siRNA library in a mouse model. Preliminary data from this siRNA screen identified that knockdown of *Chd4* (Chromodomain Helicase DNA Binding Protein 4); a member of the nucleosome remodelling and deacetylase complex, instigated a 50% reduction in putative SSCs in primary cultures of undifferentiated spermatogonia after 6 days. To validate these findings *in vivo*, we microinjected control or *Chd4*-knockdown spermatogonial populations into the testes of germ-cell ablated recipients, and assessed colonisation efficiency of the donor SSCs. In alignment with our preliminary findings, spermatogonial transplantation experiments revealed that knockdown of *Chd4* significantly impaired SSC function, with the number of donor-derived colonies of spermatogenesis being reduced by 46%. Beyond these functional experiments, the expression profile of *Chd4* was characterised using single cell RNA sequencing (scRNA-seq) of 'whole testis' (i.e. germ and somatic cells). These analyses revealed that expression levels are highest in the germ cell lineage; specifically in spermatogonia, and are most prominently elevated in SSCs. Finally, to explore mechanisms underlying the role of CHD4 in SSC maintenance, we used scRNA-seq to identify differentially expressed genes in 'control' versus '*Chd4*-knockdown' populations. Findings revealed that CHD4 likely acts to repress expression of differentiation-driving genes, such as *Sohlh2*, while activating expression of genes important for maintenance of the undifferentiated state, such as *Pten*. These experiments are the first to depict a key role for CHD4 in SSC function and thus male fertility.

## Cripto: a potential new player regulating spermatogonial stem cell fate

**Guillaume Burnet<sup>1</sup>, Cassy Spiller<sup>1</sup>, Chun-Wei (Allen) Feng<sup>1</sup>, Peter Koopman<sup>2</sup>, Josephine Bowles<sup>1</sup>**

1. School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia

2. Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

We previously demonstrated that Nodal/Activin signalling is active in male germ cells during fetal development. Its role is to drive germ cell towards the male fate, by driving expression of male fate genes such as *Nanos2* and *Dnmt3l*. Others have proposed that Nodal signalling also acts to protect male germ cells from entering meiosis. Nodal is a TGF $\beta$  molecule and signals using the Type I and II TGF $\beta$  receptors; generally the presence of the co-receptor Cripto is also required. *Cripto* expression is driven by FGF9 during a short period of time (11.5dpc to 13.5dpc) in male germ cells and we have linked this to maintenance of the pluripotent state.

Given the importance of FGF signalling and *Nanos2* expression in spermatogonial stem cell (SSC) maintenance in the postnatal testis, we explored whether *Cripto* was also involved in SSC biology. We find that *Cripto* is highly expressed in mouse gonocytes in the juvenile testis while its expression is restricted to a few spermatogonia in the adult. *Cripto* over-expression in

germ cells after birth leads to infertility and a gradual loss of germ cells from P7 onwards. Young adult mutant testis have only a few undifferentiated spermatogonia that are lost over time. The first wave of differentiation is impaired, with germ cells maintaining expression of *PLzf* while lacking *Stra8* expression, showing that mutant cells do not enter meiosis. Preliminary analysis of *Cripto* conditional-KO in germ cells suggests that loss of *Cripto* does not affect spermatogenesis per se but, rather, affects the number of SSCs. Overall, we propose a role for *Cripto* in driving self-renewal (as opposed as differentiation) in undifferentiated spermatogonia, possibly by modulating sensitivity to retinoic acid prior to the start of the first wave of spermatogenesis – when SSCs are specified.

## TGF $\beta$ signaling crosstalk during fetal germ cell development: a role for the nuclear transport protein, IPO5

**Karthika Radhakrishnan<sup>1</sup>, Julia C Young<sup>2,3</sup>, Diana Micati<sup>1</sup>, Josie Iaria<sup>4,5</sup>, Hong-Jian Zhu<sup>4,5</sup>, Kate Loveland<sup>1,2</sup>**

1. Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, VIC, Australia

2. Dept of Molecular and Translational Sciences, Monash University, Clayton, VIC Australia, Melbourne, VIC, Australia

3. Dept of Anatomy and Developmental Biology, Monash University, Clayton, VIC Australia, Melbourne, VIC, Australia

4. Melbourne Medical School, University of Melbourne, Melbourne, VIC, Australia

5. Department of Surgery, Royal Melbourne Hospital, Parkville, Melbourne, VIC, Australia

Testicular germ cell tumors (TGCT) are the most common malignancy affecting males aged 15 to 35. Through unknown mechanisms, they arise from human fetal germ cells that fail to differentiate. They persist in adult testes, forming either a gonocyte-like seminoma, or a differentiated non-seminoma. Several TGF- $\beta$ /BMP signaling pathway ligands (Activins, NODAL, TGF $\beta$ , BMPs) influence mammalian fetal testis development and regulate these germ cells in normal and neoplastic states. Pathway receptors and activated signaling molecules (phosphorylated SMADs) are present in TGCTs, indicating the pathway is active. Aberrant TGF- $\beta$  pathway signaling during embryonic development is associated with other testicular pathologies. This study used the human gonocyte-like TCam-2 seminoma-derived cell line to investigate Activin-BMP signaling crosstalk in fetal germ cell differentiation and TGCTs. A dual luciferase assay was established by transducing TCam-2 cells with lentivirus to measure BMP4-induced activation of a BMP response element (BRE) and an Activin A-responsive promoter (CAGA). Activin A consistently and dose-dependently antagonized BMP4-induced BRE activation, while BMP4 did not alter Activin A-induced CAGA activation. The effects of Activin A and BMP4 on downstream target gene expression were more complex and target-gene specific. TGF $\beta$  signaling requires nuclear transport of phosphorylated SMADs mediated by importin proteins. One of these, IPO5, was recently implicated in BMP signaling; it selectively mediates nuclear localization of the BMP-specific Smads1/5/9 to favor BMP4 target gene activation over Activin/NODAL/TGF $\beta$  targets. The abundance of IPO5 in fetal gonocytes and in TCam-2 cells led us to speculate that it selectively promotes BMP signaling. IPO5 knockdown using siRNA impeded BMP4-induced BRE activation and elevated Activin A-induced CAGA activation in TCam-2 cells. Thus, IPO5 levels may be pivotal for determining gonocyte and TGCT responsiveness to the microenvironment.

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## The severity of microtubule severing: katanins in oocytes and early embryos

**Wai Shan Yuen<sup>1</sup>, Qing-hua Zhang<sup>1</sup>, Jessica Dunleavy<sup>2</sup>, Moira O'Bryan<sup>2</sup>, John Carroll<sup>1</sup>**

1. Biomedicine Discovery Institute, Clayton, VIC, Australia

2. Monash University, Clayton, Victoria, Australia

**Introduction:** Microtubule dynamics are essential for the production of a normal meiotic spindle and fertile oocyte. Katanin is an evolutionarily conserved microtubule-severing complex consisting of a p60 severing enzyme and a p80 regulatory subunit. The aim of this study is to investigate the role of Katanin-mediated microtubule severing in oocyte and embryo development.

**Methods:** We generated oocyte-specific knockouts, using conditional knockout mice (ZP3-Cre), of katanin subunit p80. Breeding trials were conducted to test the effect of p80 on fertility. Meiotic spindles and chromosome organisation were examined in MI and MII stage oocytes using immunofluorescence and hoechst staining. In vitro fertilisation (IVF) and parthenogenetic activation (PA) were performed on control and KO oocytes to determine the role of katanin in oocyte and embryo development.

**Results:** Breeding experiments show infertility in the p80KO. The first meiotic spindle was normal in oocytes of p80KO mice but defects including, monopolar/multipolar spindles, were observed in the MII eggs (60% vs 0%,  $p < 0.0001$ ). After IVF with wildtype sperm, there was no effect on the rate of 2-cell formation and blastocyst development, although there was a significant decrease in the diameter of the blastocyst ( $p < 0.01$ ) which was reflected in a significant reduction in cell number ( $49 \pm 7$  vs  $17 \pm 2$ ,  $p < 0.0001$ ) as well as other morphological abnormalities. To create homozygous p80 KO embryos, oocytes were parthenogenetically activated. This caused a dramatic failure of preimplantation development with only 16% of the p80KO oocytes reaching blastocyst compared to 68% of controls ( $P < 0.0001$ ). Time-lapse imaging of early embryo development revealed that mitotic failure, asymmetric cell division were prevalent in p80KO parthenogenetic embryos.

**Conclusion:** This study shows that katanin is essential for fertility and although these microtubule-severing proteins appear to play a minor role in meiosis, they are critical for the fidelity of early embryonic cell division.

## The development of diagnostic biomarkers in classifying ovary ageing

Emily R Frost<sup>1,2</sup>, Michaela Palmieri<sup>1,2</sup>, Eileen A McLaughlin<sup>2,3,4</sup>, Jessie M Sutherland<sup>1,2</sup>

1. Hunter Medical Research Institute, Pregnancy and Reproduction Program, Newcastle, NSW, Australia

2. Priority Research Centre for Reproductive Science, The University of Newcastle, Callaghan, NSW, Australia

3. School of Science, Faculty of Science & Technology, University of Canberra, Bruce, ACT, Australia

4. School of Biological Sciences, Faculty of Science, University of Auckland, Auckland, New Zealand

Female factor infertility remains the underlying cause in over 40% of infertile cases, with maternal age the most pivotal determinant in the success of assisted reproductive treatments. This is predominately due to the age-related decline in both the quality and quantity of ovarian oocytes. The development of new biomarker-driven diagnostics for ovary age presents an exciting new avenue to facilitate the improved success of female infertility treatments.

The JAK/STAT signalling pathway is known to serve a functional role in early ovary dynamics<sup>1,2,3</sup>. Accordingly, we hypothesised that this pathway may also be important to follicle development in the ageing ovary. To this end, a complete developmental characterisation of the entire JAK/STAT and SOCS pathway (19 members) was undertaken. Each pathway member was subjected to gene (qPCR), and protein expression (immunoblot) and localisation (confocal microscopy) investigations, across pre-pubertal development (PND1, 4 and 8), sexual maturation (6WK) and reproductive decline (10-12MTH), in the C57BL/6 mouse ovary. Notably, distinct changes were observed in the protein expression levels of JAK1 (-1.81FC,  $p < 0.05$ ), STAT3 (+1.63FC,  $p < 0.0001$ ), STAT4 (-1.14,  $p < 0.05$ ), and SOCS4 (+1.22,  $p < 0.05$ ) alongside an increase in the mRNA abundance of *Stat3* (+1.83FC,  $p < 0.05$ ), in aged ovaries (10-12MTH) when compared to young adult (6WK) ( $N=8$ ). JAK1, STAT3 and SOCS4 demonstrated distinctive primordial follicle localisation within the granulosa cells and oocyte during sexual maturation and reproductive ageing. In contrast, STAT4 was localised specifically to the oocyte and found to significantly decrease with age.

These data support the coordinated involvement of JAK1, STAT3 and SOCS4 in primordial follicle regulation and introduce a potentially new role for STAT4 in oocyte maintenance – the underlying mechanisms defining age-related female infertility. Moreover, the functional investigation of these important JAK/STAT pathway members, may warrant the exploitation of this pathway in the development of new prognostic biomarkers of follicle quantity and oocyte health.

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## Ageing modulates expression of oocyte DNA repair genes during the germinal vesicle-metaphase II transition in mice after ovulation induction

Fabrizio Horta<sup>1</sup>, Aravind Ravichandran<sup>1</sup>, Sally Catt<sup>1</sup>, Beverley Vollenhoven<sup>1</sup>, Peter Temple-Smith<sup>1</sup>

1. EPRD, Obstetrics and Gynecology, Monash University, Melbourne, VIC, Australia

Oocyte quality and reproductive outcome are affected by advanced maternal age, ovarian stimulation and method of oocyte maturation in human assisted reproduction. Understanding molecular signatures of the oocyte DNA repair capacity can assist in identifying the mechanisms leading to poor oocyte quality in ageing. The aim of this study was to compare the effects of ageing, method of oocyte maturation (*in vivo/in vitro*) and ovarian stimulation conditions on the relative expression of DNA repair genes in germinal vesicle (GV) and metaphase II (MII) oocytes. The relative expression of ninety DNA repair genes was compared in GV and *in vitro* matured (IVM) MII oocytes from unstimulated and hormone-stimulated (PMSG or anti-inhibin serum [AIS] with an hCG trigger) young (5-8 weeks) and old (42-45 weeks) C57BL6 mice using the Taqman assay. A general pattern of significant down-regulation in DNA repair genes was observed in MIIs compared to GVs from the young mice, irrespective of the method used to produce mature oocytes. The number of significantly down-regulated genes in IVM-MIIs from younger females, were greater than *in vivo*-MII (Young IVM-MII: 38 genes; Young *in vivo*-MII: 25 genes, 95%-IC). In aged oocytes, the expression pattern was reversed, with more DNA repair genes significantly up-regulated in IVM-MII and *in vivo*-MII oocytes than GVs (Old IVM-MII: 22 genes; Old *in vivo*-MII: 12 genes, 95%-IC). Also, in older females more DNA repair genes were down-regulated in IVM-MII oocytes than in *in vivo*-MIIs (Old IVM-MII: 13 genes; Old *in vivo*-MII: 4 genes, 95%-IC), suggesting that IVM has a different effect on MII repair genes than *in vivo* maturation in stimulated oocytes, with potential repercussions on oocyte quality. The key DNA damage response gene, *H2AFX*, was always down-regulated in MII oocytes compared to GV oocytes irrespective of maturation type (95%-IC). Ageing modulates DNA repair gene expression during the GV-MII transition.

## Oocyte SIRT1 deacetylase activity is dispensable for female reproductive capacity during ageing and obesity

Juvita Ilijas<sup>1</sup>, Hayden Homer<sup>1</sup>

1. The Christopher Chen Oocyte Biology Research Laboratory, The University of Queensland-Centre for Clinical Research, Brisbane, Queensland, Australia

Publish consent withheld

## Age-related inflammation in the ovary is associated with oocyte depletion and loss of fertility in females

Carolina Lliberos Requesens<sup>1</sup>, Jason Liew<sup>1</sup>, Ashley Mansell<sup>2</sup>, Karla Hutt<sup>1</sup>

1. Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

2. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

Reproductive ageing in females is defined by a progressive decline in oocyte number and quality. This is a natural process that leads to the loss of fertility and ovarian function, cycle irregularity and eventually menopause. The factors that underlie the natural depletion of oocytes throughout reproductive life are poorly characterised. In this study, we investigated the hypothesis that inflammation contributes to the loss of oocytes as females age. We first determined oocyte numbers and characterised the systemic and local ovarian inflammatory phenotype in C57/Bl6 mice at 2, 6, 12 and 18-months of age. This period of time spans the onset of sexual maturity to the end of female fertility in mice. We observed that the decrease in oocyte numbers over the reproductive lifespan was associated with an increase in the serum concentration and intra-ovarian mRNA and protein levels of pro-inflammatory cytokines IL-1 $\alpha/\beta$ , TNF- $\alpha$ , IL-6, and inflammasome proteins ASC and NLRP3. To gain further insight into the possible role of the NLRP3 inflammasome in oocyte depletion, we compared oocyte numbers in wild type (WT), *nlrp3*<sup>-/-</sup> and *asc*<sup>-/-</sup> mice (n=3-6/genotype). We found that while oocyte numbers were similar in WT and *nlrp3*<sup>-/-</sup> mice at 12 months of age, the primordial follicle reserves remained extremely high in *asc*<sup>-/-</sup> mice (WT= 253 $\pm$ 76 vs *asc*<sup>-/-</sup>= 824 $\pm$ 96, p=0.0051). Notably, levels of some pro-inflammatory cytokines in the serum of *asc*<sup>-/-</sup> mice were found to be significantly lower compared to WT (for IL-6: WT= 4.5 $\pm$ 0.9 vs *asc*<sup>-/-</sup>= 2.0 $\pm$ 0.5, p=0.0341). These data strongly implicate inflammation as a causative factor in the age-associated depletion of oocytes and raises the possibility that ovarian ageing could be delayed, and fertility prolonged, by suppressing inflammatory processes in the ovary.

## The contributions of epididymosomes to sperm function

Hanah M Hart<sup>1,2</sup>, David A Skerrett-Byrne<sup>1,2</sup>, Matthew D Dun<sup>2,3</sup>, Brett Nixon<sup>1,2</sup>, Geoffrey N De Iulius<sup>1,2</sup>

1. Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW, Australia

2. Hunter Medical Research Institute, New Lambton Heights, NSW, Australia

3. School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle, Callaghan, NSW, Australia

The ability of a spermatozoon to recognise and bind to the outer shell of an oocyte, the zona pellucida, is a key determinant of fertility potential. Fully differentiated spermatozoa only acquire the capacity for fertilisation after they have completed epididymal transit. This critical maturation process is achieved remarkably, in the complete absence of *de novo* protein translation. Instead, epididymal maturation is driven by the microenvironment sperm encounter within the lumen of the epididymal tubule. A key element of this dynamic environment are epididymosomes, small membrane-encapsulated vesicles that are secreted from the epididymal epithelial cells lining the lumen of the duct. These vesicles have been implicated in the direct transfer of diverse biomolecular cargo to the maturing gametes, including protein receptors putatively involved in downstream oocyte binding. In this study, we have performed comparative proteomic profiling of mouse epididymosomes isolated from different segments of the epididymis using mass tag based quantification via high resolution LC-MS/MS. A total of 1640 epididymosome proteins were identified and quantified, with 146 proteins being differentially accumulated between caput and corpus epididymosomes, and a further 344 differentially accumulated between corpus and cauda epididymosomes (i.e., fold change of  $\leq -1.5$  or  $\geq 1.5$ ;  $P < 0.05$ ). A subset of the epididymosome proteins that have not been previously curated in current exosome databases, exhibit roles associated with the acquisition of sperm function including binding to the zona pellucida. Together with our demonstration that epididymosomes are able to convey protein cargo to the head of maturing spermatozoa, these data underpin the role of extracellular vesicles in coordinating post-testicular sperm maturation and ultimately the fertility potential of the spermatozoon. Understanding the roles of epididymosomes will advance our knowledge of male infertility and sperm dysfunction, while also opening potential avenues towards enhancing sperm quality for assisted reproductive technologies.



## Non-hormonal IUDs: we haven't even scratched the surface

**Kirsten N Shankie-Williams<sup>1</sup>, Margot L Day<sup>2</sup>, Laura A Lindsay<sup>1</sup>, Christopher R Murphy<sup>1</sup>, Samson N Dowland<sup>1</sup>**

1. *Anatomy and Histology, University of Sydney, Sydney, New South Wales, Australia*

2. *Physiology, University of Sydney, Sydney, New South Wales, Australia*

The mechanism underlying the high contraceptive efficacy of the copper intrauterine device (IUD) is yet to be determined. Copper IUDs have numerous side effects leading to high and early removal rates. Preliminary work on the copper and an alternative (IUD) in our novel rat model indicates that both IUDs are an effective contraceptive, however, the uterus responds uniquely to the different metals contained in the IUD.

Histological studies identified local endometrial inflammation and metaplasia of the uterine epithelial cells (UECs) in response to copper. Energy dispersive x-ray spectroscopy detected accumulated copper ions in the underlying endometrium. The alternative IUD did not cause an inflammatory response or metaplasia of the UECs. An embryo survival assay was performed using embryos from IUD treated vs. non-treated control horns. This demonstrated that all ovulated oocytes were fertilised in both metal IUD horns, discounting the spermicidal effects of copper as the main mechanism of contraception. In the copper treated horn 15% of embryos collected at day 3 developed into blastocysts compared to 60% in the non-treated control horn. The alternative IUD yielded 6% of embryos developing into blastocyst compared to 67% in the control horn.

These findings suggest that copper ions contribute to the arrested development of embryos and in association with local inflammation and metaplasia of UECs, contributing to the high contraceptive efficacy of the copper IUD. The alternative IUD resulted in the lowest number of blastocysts and a lack of endometrial inflammation, indicating a decrease in side effects resulting in a novel non-hormonal long-term contraceptive for women.

## Defining the role of EED in the establishment of oocyte transcriptome and how it contributes to maternally inherited disease.

**Tesha Tsai<sup>2,1</sup>, Heidi Bildsoe<sup>2,1</sup>, Ellen Jarred<sup>2,1</sup>, Stephen Pederson<sup>3</sup>, Mai Truong<sup>4</sup>, David Gardner<sup>4</sup>, David Adelson<sup>3</sup>, Patrick Western<sup>2,1</sup>**

1. *Department of Molecular and Translational Science, Monash University, Melbourne, Victoria, Australia*

2. *Hudson Institute of Medical Research, Melbourne, Victoria, Australia*

3. *Bioinformatics and Computational Genetics, School of Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia*

4. *School of BioSciences, University of Melbourne, Melbourne, Victoria, Australia*

Germ cell development involves extensive epigenetic reprogramming and ultimately results in the establishment of highly specialised epigenetic information in oocytes and sperm, disruption of which can lead to disease in offspring. EED is an essential component of Polycomb Repressive Complex 2 (PRC2) that is involved in the establishment of epigenetic modification H3K27me3 in animals to maintain gene silencing during development. In humans, *de novo* EED mutations in the germline cause Weaver syndrome, which is a disease characterised by overgrowth phenotype associated with skeletal abnormality and intellectual disability. To investigate how EED epigenetically regulates transcription in oocytes and offspring development, we developed a mouse model to delete *Eed* specifically in growing oocytes. Our previous work showed that this mouse model results in overgrowth of offspring that is similar to Weaver syndrome. Here, we isolated fully grown germinal vesicle (GV)- stage oocytes with surrounded nucleolus chromatin configuration and performed RNA-seq. Transcriptome analysis showed that 245 out of 248 (99%) differentially expressed genes are de-repressed by *Eed* oocyte-specific deletion (>2-fold increase; FDR=0.05), demonstrating the importance of EED in gene repression in the oocyte. Gene enrichment analysis using Ingenuity Pathway Analysis and G:Profiler showed that these dysregulated genes are involved in key developmental processes including brain and skeletal development, consistent with characteristics of Weaver syndrome. To further investigate how loss of EED in the oocyte leads to disease, we are analysing offspring gene expression and development in blastocysts, brain and bone with a focus on target genes identified from RNA-seq in oocytes. One candidate is *Zdbf2*, a gene that is epigenetically modified during pre-implantation embryo development and regulates offspring growth. Our work will provide greater understanding of maternal epigenetic programming, and how environmental insults mediated by drugs and diet could impact offspring health.

## Quantitative proteomic profiling of sperm maturation along the epididymal tubule.

**David A Skerrett-Byrne<sup>1,2</sup>, Amanda L Anderson<sup>1</sup>, Matthew D Dun<sup>2,3</sup>, Brett Nixon<sup>1,2</sup>**

1. *Priority Research Centre for Reproductive Science, The University of Newcastle, Newcastle, New South Wales, Australia*

2. *Hunter Medical Research Institute, Newcastle, New South Wales, Australia*

3. *Priority Research Centre for Cancer Research, The University of Newcastle, Newcastle, New South Wales, Australia*

Arguably the most important tissue involved in post-testicular mammalian sperm maturation is the epididymis, a highly specialised ductal system that drives dramatic functional changes in spermatozoa. Indeed, it is during epididymal maturation that spermatozoa acquire the competence to engage in fertilisation and surprisingly, these changes occur in the complete

absence of endogenous gene transcription or *de novo* protein translation. Despite years of study, we have yet to fully resolve the key pathways that facilitate and promote epididymal sperm maturation. To enhance our understanding of the factors that promote sperm maturation, we have sought to characterise the proteome of the caput, non-capacitated (NC) and capacitated (CAP) cauda epididymal spermatozoa. To achieve this we have employed a comparative and quantitative label-based proteomic approach coupled to high-resolution liquid chromatograph tandem mass spectrometry (LC-MS/MS). Preliminary analysis has achieved an improved depth of the sperm proteome, characterising 1,981 unique proteins ( $\geq 2$  unique peptides). Strikingly, a total of 848 of these proteins were differentially expressed between the caput and the NC cauda spermatozoa and a further 144 had altered abundance between in the CAP vs NC spermatozoa (fold changes of  $\leq -1.5$  or  $\geq 1.5$ ;  $p \leq 0.05$ ). Alterations in the former comparison included ADAM3 (2.86 fold change) and threonine aspartase 1 (2.68 fold change) and in the later comparison, calcium and integrin-binding protein 1 (3.25 fold change) and glutathione S-transferase Mu 5 (2.65 fold change). Utilising Ingenuity Pathway Analysis, we assigned canonical pathways and upstream regulators to both comparisons, identifying rapamycin-insensitive companion of mTOR and transcription initiation factor TFIID subunit 7-like as activated upstream regulators with potential roles in sperm maturation. These preliminary analyses are providing us with the capacity to progressively track the proteomic changes associated sperm maturation and will help improve our understanding of human male fertility and ultimately infertility.

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## A microenvironment of high lactic acid and reduced pH created by the blastocyst induces changes in endometrial receptivity

**Kathryn H Gurner<sup>1</sup>, Jemma Evans<sup>2</sup>, Alexandra J Harvey<sup>1</sup>, David K Gardner<sup>1</sup>**

1. University of Melbourne, Parkville, VIC, Australia

2. Hudson Institute of Medical Research, Clayton, VIC, Australia

Successful embryo implantation requires a synchronized dialogue between the receptive endometrium and blastocyst via locally produced soluble mediators. At the time of implantation, the blastocyst produces a significant amount of lactate, creating a microenvironment around the embryo characterized by high lactate and low pH. Whilst historically considered a 'byproduct' of metabolism, the identification of a lactate specific receptor, GPR81, and recent work in cancer cells has established lactate as an important signalling molecule, with roles in angiogenesis, ECM breakdown and immunosuppression. This study aimed to determine the role of blastocyst derived lactate, with and without pH adjustment, on cellular functions essential for establishment of endometrial receptivity and successful implantation.

Hormonally primed ECC-1 cells were exposed to varying concentrations (0mM, 2.5mM, 5mM, 7.5mM and 10mM) of Lactic acid (LA) or LA with neutralised pH (nLA) to mimic the microenvironment created by the blastocyst at implantation. Cells were analysed for cellular tight junction integrity (TER assessment), and changes in cellular proliferation and migration (xCelligence real-time cell function analysis). Stimulated and unstimulated mouse uterine tissue was analysed for GPR81 gene expression by real-time PCR.

Cellular tight junction integrity and cellular proliferation were significantly downregulated in ECC-1 cells exposed to 2.5mM, 5mM and 7.5mM LA ( $p < 0.01$ ) while cellular migration significantly increased in the presence of 2.5mM ( $p < 0.05$ ) and 5mM ( $p < 0.01$ ) LA. A decrease in cellular tight junction integrity (5mM and 7.5mM nLA) and proliferation (5mM nLA) ( $p = 0.07$ ) was still evident in ECC-1 cells exposed to nLA. Expression of GPR81 was detected in mouse uterine tissue, inferring a non-metabolic signalling role for LA *in vivo*.

These data therefore demonstrate a synergistic effect of LA, in combination with LA induced pH change, in enhancement of endometrial receptivity. This suggests that, via creation of a specialised microenvironment, the blastocyst acts to facilitate its own implantation.

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## Nampt-mediated spindle sizing secures a post-anaphase surge in spindle speed required for extreme asymmetry in oocytes

**Zhe Wei<sup>1</sup>, Jessica Greaney<sup>1</sup>, Hayden Homer<sup>1</sup>**

1. The Christopher Chen Oocyte Biology Research Laboratory, Centre for Clinical Research, The University of Queensland, Brisbane, QLD, Australia

Publish consent withheld

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## Health outcomes in offspring following an acute, low dose of prenatal alcohol: Potential reproductive and metabolic impacts in early and adult life

**Lisa K Akison<sup>1</sup>, Tam MT Nguyen<sup>2</sup>, Elizabeth McReight<sup>2</sup>, Sarah E Steane<sup>2</sup>, Karen M Moritz<sup>1</sup>**

1. Child Health Research Centre, Centre for Children's Health Research, The University of Queensland, South Brisbane, QLD, Australia

2. School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia

Alcohol consumption is highly prevalent in women of reproductive age. Our recent systematic reviews highlight the range of health impacts in offspring with prenatal alcohol exposure (PAE). However, few studies have looked at acute, low-dose alcohol exposure. Our aim was to examine offspring glucose metabolism, frequently affected in higher dose models, as well as ovarian reserve and female fertility, not currently reported in the literature, in PAE offspring.

Sprague-Dawley dams were treated with a 1g/kg-BW EtOH-gavage (EtOH) or an equivalent volume of saline (Control) at gestational days 13.5 and 14.5 (peak BAC ~0.06%). Reproductive outcomes: Primordial and early growing follicles were quantified in neonatal ovaries using unbiased stereology; puberty onset was determined by age at vaginal opening; estrous cycles were monitored in adults via vaginal electrical impedance; and fertility assessed by mating success and number of implantations. Glucose metabolism: Fasting blood glucose and plasma insulin were measured in adolescence (day 30) and a glucose tolerance test (GTT) or insulin tolerance test (ITT) was performed at 6-months of age. Only 1 male and/or 1-2 females from each litter were used per experiment (n=8-9).

There was no evidence of growth restriction in EtOH versus control offspring. There was also no impact on follicle numbers, puberty onset or female fertility in adulthood. Glucose metabolism was not altered in EtOH-exposed adolescent offspring compared to controls. However, there was evidence of insulin resistance in adult males, with elevated fasting plasma insulin levels and 1<sup>st</sup>-phase insulin secretion during the GTT, altered indices for insulin resistance/sensitivity, and attenuated blood glucose lowering during the ITT.

Therefore, while some aspects of offspring health were not affected by this relatively modest PAE, there was evidence of a pre-diabetic, insulin-resistant phenotype in male offspring of EtOH-exposed dams. This study highlights the importance of abstaining from alcohol consumption during pregnancy.

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### FIB-SEM tomography as an innovative tool for spermatogenesis studies

**Denis Korneev<sup>1</sup>, Alex de Marco<sup>1</sup>, Moira K O'Bryan<sup>1</sup>**

*1. Monash University, Monash University (Clayton), VICTORIA, Australia*

FIB-SEM tomography is a new developing method, which has opened a new era in electron microscopy – it makes possible to visualize internal three-dimensional organization of biological structures with nanometer scale resolution [1]. This method is very promising for spermatogenesis studies. Indeed, intercellular interactions and complex microtubules machinery can be revealed with high three-dimensional resolution [2].

Proper sample preparation has a crucial importance for preservation of embedded tissue ultrastructures. In this study high-pressure freezing was used as the most delicate method of tissue preservation. The protocols of freezing and freeze-substitution have been optimized for seminiferous tubules preparation. It was shown that tannic acid-mediated osmium impregnation [3] dramatically improves contrast of tissue structure. After freeze-substitution tissue were embedded in Epon resin and examined with FEI Helios DualBeam plasma FIB with Auto Slice & View software, voxel size up to 5 x 5 x 5 nm.

2D images were segmented with iLastic software, 3D structures were rendered with FEI Amira platform. Sertoli cells spatial organisation, germ cell development and three-dimensional microtubules distribution in the cells have been shown in mouse.

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### Global coagulation assays in transgender women

**Ada S Cheung<sup>1,2</sup>, Hui Yin Lim<sup>3,5,4</sup>, Niloufar Torkamani<sup>1,2</sup>, Max Rainier<sup>1</sup>, Mathis Grossmann<sup>1,2</sup>, Harshal Nandurkar<sup>5</sup>, Jeffrey D Zajac<sup>1,2</sup>, Prahlad Ho<sup>3,5</sup>**

*1. Department of Medicine (Austin Health), The University of Melbourne, Heidelberg, VIC, Australia*

*2. Endocrinology, Austin Health, Heidelberg, VIC, Australia*

*3. Department of Haematology/Northern Pathology Victoria, The Northern Hospital, Epping, Victoria, Australia*

*4. Florey Institute of Neurosciences and Mental Health, The University of Melbourne, Parkville, Victoria, Australia*

*5. Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia*

**Background:** As opposed to standard coagulation tests, global [coagulation assays](#) such as [thromboelastography](#) (TEG) and [thrombin](#) generation may be better surrogate measures of venous and arterial thrombosis risk, adverse effects of estradiol therapy. There is minimal data investigating thrombotic risk of estradiol therapies in transgender women. We hypothesised that transgender women on oral estradiol would have TEG parameters of higher clot strength and lower clot lysis, and higher thrombin generation than those on transdermal estradiol.

**Aims:** To evaluate global coagulation assays (thromboelastography; thrombin generation using calibrated automated thrombogram (CAT); fibrin generation with overall haemostatic potential (OHP) assay) in transgender women on estradiol therapy.

**Methods:** This cross-sectional analysis involved transgender women (male-to-female) and cisgender healthy controls. Fasting blood samples were collected and analysed within 4 hours of venepuncture for (i) thromboelastography using citrated whole blood (TEG® 5000), (ii) CAT and (iii) OHP using platelet-poor plasma.

**Results:** 25 transgender women (mean age 39) were compared to 97 controls (68% female; mean age 43). Fourteen (56%) used oral and 11 used transdermal estradiol. Mean duration of estradiol therapy was similar between oral and transdermal routes (26 vs 34 months;  $p=0.41$ ). Route of estrogen delivery did not influence any global coagulation assay parameter. Overall, transgender women on estradiol demonstrated increased clot strength (maximum amplitude 65.2 vs 57.9 mm;  $p<0.001$ ) on whole blood TEG (Table). CAT showed comparable endogenous thrombin potential (ETP) (1399.7 vs 1350.0 nM.min;  $p=0.41$ ) in the transgender individuals. Interestingly, fibrin generation was reduced in the transgender women (overall coagulation potential 51.9 vs 59.6;  $p=0.003$ ) with similar overall fibrinolytic potential.

**Conclusion:** Transgender women on oral and transdermal estradiol had higher whole blood clot strength compared to cisgender controls. Route of delivery did not influence whole blood clot strength. Further study is required with larger sample sizes to assess differing doses of transdermal therapy.

**Table. Global coagulation assay parameters in transgender women compared with female and male controls.**

	<b>All controls (n=97)</b> (p-value all controls vs transgender)	<b>Female controls (n=65)</b> (p-value female controls vs transgender)	<b>Male controls (n=32)</b> (p-value male controls vs transgender)	<b>Transgender women (n=25)</b>
<b>TEG</b>				
R time (min)	7.3 0.008	7.4 0.015	7.2 0.018	5.9
Alpha-angle (°)	56.7 <b>&lt;0.001</b>	56.6 <b>0.003</b>	56.8 <b>&lt;0.001</b>	48.3
Maximum amplitude (mm)	57.9 <b>&lt;0.001</b>	58.2 <b>&lt;0.001</b>	56.8 <b>&lt;0.001</b>	65.1
Lysis 30 (%)	2.0 0.102	2.2 0.075	1.4 0.307	0.9
<b>CAT</b>				
Endogenous thrombin potential (nM.min)	1350.0 0.405	1390.2 0.886	1264.8 0.051	1399.7
Peak thrombin (nM)	222.9 0.863	236.7 0.308	194.2 0.100	220.4
Velocity index (nM/min)	72.8 0.306	80.5 0.069	56.6 0.333	64.6
<b>OHP</b>				
Overall coagulation potential	59.6 <b>0.003</b>	61.4 <b>&lt;0.001</b>	56.5 0.218	51.9
Overall haemostatic potential	29.2 <b>0.001</b>	29.2 <b>&lt;0.001</b>	29.7 <b>0.007</b>	24.1
Overall fibrinolytic potential	49.9 0.098	51.5 0.387	46.8 <b>0.007</b>	53.0

## Risk of polycythaemia with different formulations of testosterone therapy in transgender men

**Brendan J Nolan**<sup>1,2</sup>, **Shalem Y Leemaqz**<sup>3</sup>, **Olivia Ooi**<sup>1</sup>, **Pauline Cundill**<sup>4</sup>, **Nicholas Silberstein**<sup>4</sup>, **Peter Locke**<sup>4</sup>, **Mathis Grossmann**<sup>5,2</sup>, **Jeffrey D Zajac**<sup>1,2</sup>, **Ada S Cheung**<sup>1,2</sup>

1. *Trans Medical Research Group, Department of Medicine (Austin Health), University of Melbourne, HEIDELBERG, VIC, Australia*

2. *Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia*

3. *Robinson Research Institute, Adelaide Medical School, University of Adelaide, ADELAIDE, SA, Australia*

4. *Equinox Gender Diverse Clinic, Thorne Harbour Health, FITZROY, VIC, Australia*

5. *Department of Medicine (Austin Health), HEIDELBERG, VIC, Australia*

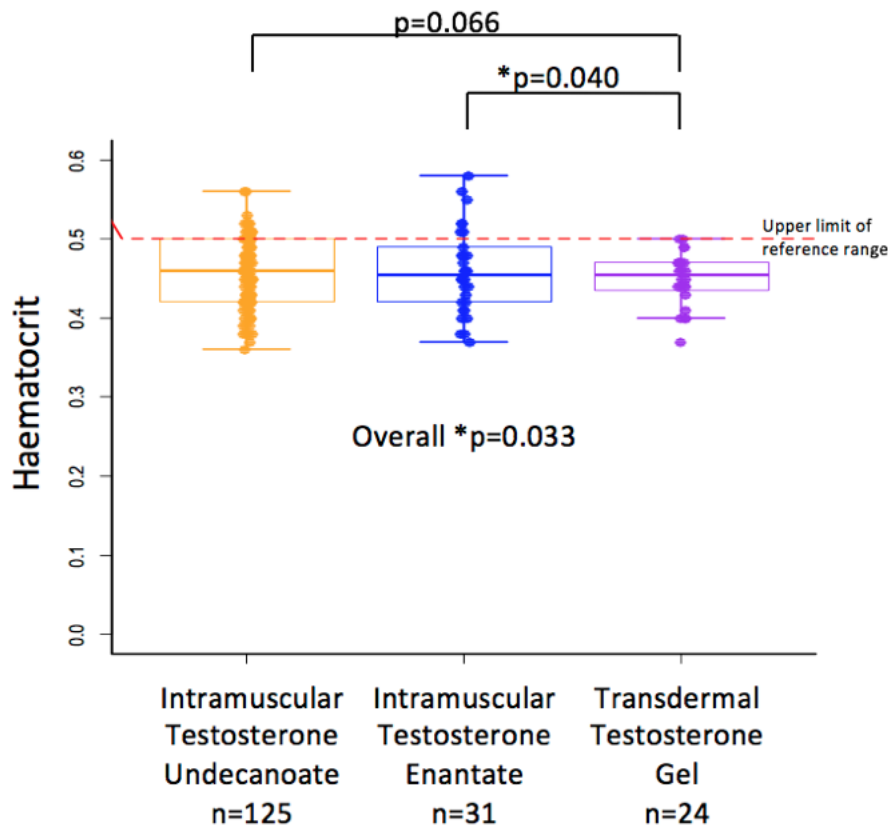
**Background:** Masculinising hormone therapy with testosterone is used to align an individual's physical characteristics with their gender identity. Testosterone therapy can be administered via intramuscular or transdermal routes and polycythaemia is the most common adverse event. We aimed to compare the risk of polycythaemia with different formulations of testosterone therapy in transgender men.

**Methods:** A retrospective cross-sectional analysis was performed of 180 transgender individuals attending gender clinics in Melbourne who were on established testosterone therapy for > 6 months and had haematocrit available. Groups included those receiving (1) intramuscular testosterone undecanoate (n=125), (2) intramuscular testosterone enanthate (n=31), or (3) transdermal testosterone (n=24). Outcomes were haematocrit level and polycythaemia (defined as haematocrit >0.5). Mean (SD) or median (IQR) are reported as appropriate. Kruskal-wallis test was used to compare haematocrit levels between

testosterone therapy groups, and Fisher's exact test was performed for polycythaemia with Bonferroni adjusted pairwise comparisons.

**Results:** 180 individuals (mean age 28.4(8.8) years) had data available for analysis. Median duration of testosterone therapy was 37.7 (24.2) months. 27% were smokers. There was no difference between groups in serum total testosterone levels achieved (mean 12.6 nmol/L (10.8) for testosterone undecanoate, 12.1 nmol/L (10.4) for testosterone enanthate and 10 nmol/L (10.8 nmol/L) for transdermal testosterone, overall  $p=0.347$ ). Box plots of haematocrit levels by group are shown in Figure 1. There was a higher proportion of patients with polycythaemia in those who were on intramuscular testosterone enanthate (23.3%) than on transdermal testosterone (0%),  $p=0.043$ . There was no statistically significant difference in polycythaemia between intramuscular testosterone undecanoate (15%) and transdermal (0%),  $p=0.066$ .

**Conclusions:** Intramuscular testosterone is associated with a higher risk of polycythaemia than transdermal testosterone in transgender men. This highlights the importance of regular monitoring of haematocrit in transgender men treated with testosterone and findings may inform treatment



**Figure 1: Haematocrit by formulation of testosterone**

choices.

### Relationships between body composition and insulin resistance in transgender individuals on established gender-affirming hormone therapy.

Ingrid Bretherton<sup>1,2</sup>, Cassandra Spanos<sup>1</sup>, Shalem Y Leemaqz<sup>1,3</sup>, Mathis Grossmann<sup>1,2</sup>, Jeffrey Zajac<sup>1,2</sup>, Ada S Cheung<sup>1,2</sup>

1. Department of Medicine (Austin Health), The University of Melbourne, Heidelberg, VIC, Australia

2. Endocrinology, Austin Health, Heidelberg, VIC, Australia

3. Adelaide Medical School, The University of Adelaide, Adelaide, South Australia

**Background:** Transgender individuals receiving gender-affirming hormone therapy (GAHT) are at increased risk of adverse cardiovascular outcomes. This may be related to the effects of GAHT on body composition, fat distribution, and insulin resistance.

**Aim:** To evaluate the impact of GAHT on body fat distribution and insulin resistance in transgender individuals.

**Methods:** This cross-sectional study compared 84 transgender individuals (41 'male-to-female' trans women and 43 'female-to-male' trans men) on established GAHT (>12 months) with 85 age-matched cisgender controls (37 men and 48 women, respectively). Body composition was measured using dual-energy x-ray absorptiometry and insulin resistance was estimated from the updated Homeostasis Model of Insulin Resistance (HOMA2-IR). Multiple linear regressions were used to examine

relationships between HOMA2IR and fat mass with gender, adjusting for age and total duration of GAHT. Mean difference (95% confidence intervals) is presented. Pearson correlation was used to analyse correlations of HOMA2-IR with fat mass.

**Results:** Mean age was 30.3 years in trans men and 40.8 years in trans women. Compared to control women, trans men had mean difference of +7.8kg (95%CI 4.0, 11.5) ( $p<0.001$ ) in lean mass and higher android:gynoid fat ratio (0.2 (0.1, 0.3),  $p<0.001$ ), but no difference in overall fat mass or insulin resistance. Compared to control men, trans women had mean difference in lean mass of -6.8kg (95%CI -10.6, -3.1) ( $p<0.001$ ), fat mass of +9.2kg (95%CI 3.9, 14.5) ( $p=0.001$ ), lower android:gynoid ratio -0.1 (-0.2,-0.0),  $p<0.05$ , and higher insulin resistance (+60% (33 – 92),  $p<0.001$ ). Higher HOMA2-IR correlated with higher android ( $r^2= 0.712$ ,  $p<0.001$ ) and gynoid ( $r^2= 0.572$ ,  $p<0.001$ ) fat mass.

**Conclusion:** Feminising hormone therapy is associated with insulin resistance related to higher fat mass compared to cisgender male controls. This may predispose to increased cardiovascular risk in trans women. Despite fat redistribution, no adverse changes in insulin resistance were observed in trans men.

## A novel liver-targeted testosterone-therapy for the prevention of sarcopaenia in androgen deprived men with prostate cancer: a double-blind placebo-controlled study

Handoo Rhee<sup>1</sup>, Anojan Navaratham<sup>1</sup>, Irina Olienkova<sup>1</sup>, Deborah Gilroy<sup>2</sup>, Yolanda Scuderi<sup>3</sup>, Peter Heathcote<sup>1</sup>, Simon Wood<sup>4,1</sup>, Ken Ho<sup>2,4,5</sup>

1. Urology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

2. Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

3. Pharmacy, Princess Alexandra Hospital, Brisbane, Queensland, Australia

4. The University of Queensland, Brisbane, Queensland, Australia

5. The Translation Research Institute, Brisbane, Queensland, Australia

Androgen deprivation therapy (ADT) causes sarcopaenia, increasing frailty and fracture risk in men with prostate cancer (PCa). Liver-targeted testosterone treatment (LTTT) achieved through oral delivery of low dose testosterone stimulates protein anabolism without elevating peripheral androgen levels in hypogonadal men and postmenopausal women (1,2).

### Aim

We investigated whether LTTT prevents sarcopaenia during ADT

### Method

Forty nine men with PCa were recruited into a 6-month double-blind placebo-controlled study of 40mg/day of crystalline testosterone; 19 commencing and 30 on stable ADT for > 18 months. Testosterone and PSA levels, body composition (lean, fat and BMC), daily step count and grip strength were measured. Patients in whom PSA doubled or rose above 4 ng/mL were withdrawn.

### Results

43 patients completed the study. Mean testosterone rose during LTTT but not placebo treatment ( $\Delta +2.34\pm 0.47$  vs  $-0.67\pm 0.41$  nmol/L;  $p<0.01$ ). Mean PSA level did not change significantly during LTTT or placebo treatment ( $+0.59\pm 0.43$  vs  $-0.05\pm 0.37$  ng/ml,  $p=0.27$ ). Blood urea fell during LTTT ( $\Delta -0.41\pm 0.41$ ) but not placebo ( $\Delta +0.05\pm 0.35$  mmol/L.). Lean mass ( $\Delta +0.46\pm 0.36$  vs  $-0.37\pm 0.32$  kg;  $p=0.09$ ) and BMC ( $\Delta +0.01\pm 0.02$  vs  $-0.04\pm 0.02$  kg,  $p<0.03$ ) increased compared to placebo. Neither mean daily step count nor grip strength changed significantly between treatments. Five of 6 patients withdrew from an increase in PSA levels, which returned to baseline. All five were on active treatment.

LTTT increased testosterone slightly but not PSA concentration among those completing the study. LTTT for six months induced beneficial biochemical and body compositional but not physical function effects. 10% withdrew from a reversible increase in PSA.

### Conclusion

LTTT shows promise as simple therapy for preventing sarcopenia and bone loss during ADT in men with prostate Ca. Further studies are required to optimise the dose, safety and efficacy in PCa and to explore potential application in other catabolic states.

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## Plasma insulin-like growth factor-I, IGF binding protein 3 and estradiol are associated with longer leucocyte telomere length, a marker of lower biological age, in older men.

Bu B Yeap<sup>1,2</sup>, Jennie Hui<sup>3</sup>, Matthew W Knuiman<sup>4</sup>, Paul Chubb<sup>5</sup>, Ken KY Ho<sup>6</sup>, Mark L Divitini<sup>4</sup>, Gillian M Arscott<sup>3</sup>, Stephen M Twigg<sup>7</sup>, Susan V McLennan<sup>7</sup>, Leon Flicker<sup>1,8</sup>, John P Beilby<sup>3</sup>

1. The Medical School, University of Western Australia, Perth, WA, Australia

2. Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, WA, Australia

3. PathWest Laboratory Medicine, Sir Charles Gairdner Hospital, Perth, WA, Australia

4. School of Population and Global Health, University of Western Australia, Perth, WA, Australia

5. PathWest Laboratory Medicine, Fiona Stanley Hospital, Perth, WA, Australia
6. Garvan Institute of Medical Research, Sydney, NSW, Australia
7. Department of Endocrinology, University of Sydney, Sydney, NSW, Australia
8. WA Centre for Health and Ageing, University of Western Australia, Perth, WA, Australia

#### Introduction

Telomeres are essential DNA-protein complexes which protect the physical ends of chromosomes. Leucocyte telomere length (LTL) reflects length of telomeres in tissues, and shorter LTL marks advancing biological age. Estradiol is associated with LTL [1,2], but the influence of insulin-like growth factor-I (IGF-I) remains uncertain. We examined associations of plasma IGF-I, its binding proteins 1 and 3 (IGFBP1 and IGFBP3) and estradiol with LTL in 2,999 community-dwelling men aged 70-84 years.

#### Methods

Plasma IGF-I, IGFBP1 and IGFBP3 measured using immunoassay and sex hormones using mass spectrometry. LTL measured by PCR, expressed as the ratio of telomeric to single-copy control gene DNA (T/S ratio). Linear regression models adjusted for age and cardiometabolic risk factors, and median splits were used to define low/high (L/H) groups.

#### Results

Mean age was 76.7±3.2 years. Per decade of age, T/S ratio declined by 0.063 (p=0.0002), IGF-I by 18.4 ug/L (p<0.0001) and IGFBP3 by 467 ug/L (p<0.0001) while IGFBP1 increased by 12.1 ug/L (p<0.0001). IGF-I and IGFBP3 showed age-adjusted correlations with LTL (coefficient 0.059, p=0.001 and 0.045, p=0.013 respectively) IGFBP1 did not. In multivariable-adjusted models IGF-I and IGFBP3 (but not IGFBP1) were associated with LTL (estimated difference in T/S ratio 0.015 per 1SD increase in IGF-I, p=0.007 and 0.011 per 1SD increase in IGFBP3, p=0.049). Men with high IGF-I (>133 ug/L) and high estradiol (>70 pmol/L) (H/H) had longer LTL compared to men with low concentrations (L/L) (multivariable-adjusted T/S ratio H/H 1.20, p=0.007; H/L 1.18, p=0.147, L/H 1.16, p=0.877, L/L 1.16). There was no corresponding finding for IGF-I and testosterone.

#### Conclusions

Higher IGF-I and IGFBP3 are independently associated with longer telomeres, consistent with lower biological age, in older men. Additive influences of higher IGF-I and higher estradiol on telomere length are present. Further work is needed to clarify how hormonal exposures might interactively modulate biological ageing.

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## Nuclear receptor ligand screen to identify new therapies for endocrine-sensitive and endocrine-resistant estrogen receptor positive breast cancers

**Jean M Winter<sup>1</sup>, Theresa Hickey<sup>1</sup>, Wayne Tilley<sup>1</sup>**

1. Dame Roma Mitchell Cancer Research Laboratories, Adelaide Health and Medical Sciences, The University of Adelaide, Adelaide, SA, Australia

Estrogen receptor-alpha (ER) drives the majority of breast cancers (BC), which are treated with endocrine therapy. ER also remains active in endocrine-resistant disease, a major contributor of BC mortality. ER is a DNA transcription factor that can be "reprogrammed" to less oncogenic genetic loci via activation of the progesterone receptor in endocrine-sensitive disease. Whether ER can be reprogrammed in endocrine-resistant BC is unknown. Moreover, DNA hyper-methylation at specific enhancer loci might impede this treatment strategy in the endocrine-resistant context. To determine the optimal conditions for nuclear receptor-mediated growth inhibition of endocrine-sensitive and endocrine-resistant BC, we performed a medium-throughput, image-based screen of 38 novel or FDA-approved ligands using a panel of ER-positive cell lines that were endocrine-sensitive (n=3) or endocrine-resistant (n=3). We further investigated whether epigenetic remodelling is required for growth inhibition. Two ER-negative lines were included as controls. Each ligand was tested at six 10-fold incremental doses, with or without pre-treatment of the DNA demethylation agent Decitabine. Cell number was quantified and cellular morphology analysed using Hoescht staining. Criteria for defining hits included >25% growth reduction, morphological changes, and EC50 <100nM. Pre-treatment with Decitabine did not enhance growth inhibition by any ligand in most BC cell lines. Twelve ligands were selected for subsequent validation. Four ligands, known to act through the androgen, glucocorticoid or ER-beta receptors, inhibited growth across a broader panel of ER-positive, endocrine-sensitive and -resistant cell lines, with the same or better efficacy than progesterone. In summary, epigenetic remodelling using Decitabine is not required for growth inhibition in endocrine-resistant cell line models. Four novel or FDA-approved ligands significantly reduced growth of endocrine-sensitive and -resistant ER positive BC cells, which will be further tested for ER reprogramming capacity by ChIP-seq in vitro and their ability to reduce growth and reprogram ER in patient-derived xenograft models of BC.

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## TMEPAI inhibits SMAD2/3-mediated muscle wasting

**Swati Kharoud<sup>1,2</sup>, Kelly L Walton<sup>1</sup>, Adam Hagg<sup>3</sup>, Georgia Goodchild<sup>1</sup>, Justin L Chen<sup>1</sup>, Hongwei Qian<sup>3</sup>, Paul Gregorevic<sup>3</sup>, Craig A Harrison<sup>1</sup>**

1. Department of Physiology, Monash University, Clayton, VIC, Australia

2. Faculty of Science, Engineering and Technology (FSET), Swinburne University of Technology, Hawthorn, VIC, Australia

Inhibition of activin and myostatin activities using ligand traps such as follistatin, soluble receptors, and propeptides have been shown to ameliorate skeletal muscle wasting in multiple mouse models of cancer cachexia and muscular dystrophies. Though effective, clinical transition of these approaches has been hindered as a result of their systemic activities promoting off-target effects. Toward the goal of seeking tissue-specific activin/myostatin interventions, we explored the ability of membrane bound TMEPAI (transmembrane prostate androgen-induced) to block wasting within skeletal muscle. TMEPAI, a transcriptional target of exogenous activin, is a known inhibitor of TGF- $\beta$ 1 mediated SMAD2/3 signalling. In this study we show that TMEPAI can also block activin A, activin B, myostatin and GDF-11 *in vitro* signalling. Adeno-associated viral (AAV) gene delivery of TMEPAI into healthy C57/BL6 mice increased local muscle mass by as much as 30%. Increased muscle mass was attributed to hypertrophy of fibers in TMEPAI expressing muscles, and coincided with an upregulation in protein synthesis pathway markers. The ability of TMEPAI to block activation of the canonical activin/myostatin SMAD2/3 pathway, was determined by co-delivering activin A and TMEPAI into the muscles of healthy mice. TMEPAI effectively attenuated muscle loss induced by activin A in this setting, and prevented atrophy of muscle fibers. Activin-induced phosphorylation of SMAD3 protein was suppressed in muscles co-expressing TMEPAI, as was transcription of activin target genes. Finally, we show that TMEPAI can protect local muscle wasting in the C26-colon cancer model of cachexia in mice. These results support that viral gene delivery of TMEPAI can effectively increase muscle mass, specifically within skeletal muscle, via inactivation of the activin/myostatin SMAD 2/3 pathway.

## Estrogen receptor alpha controls gene expression via translational offsetting in breast and prostate cancer

Julie Lorent<sup>1</sup>, Richard J Rebello<sup>2</sup>, Vincent van Hoef<sup>1</sup>, Mitchell Lawrence<sup>2</sup>, Krzysztof J Szkop<sup>1</sup>, Eric Kusnadi<sup>3</sup>, Baila Samreen<sup>1</sup>, Preetika Balanathan<sup>2</sup>, Karin Scharmann<sup>4</sup>, Itsuhiro Takizawa<sup>2</sup>, Sebastian A Leidel<sup>4</sup>, Gail Risbridger<sup>2</sup>, Ivan Topisirovic<sup>5</sup>, Ola Larsson<sup>1</sup>, Luc Furic<sup>3</sup>

1. Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden
2. Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia
3. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
4. Cells-in-Motion Cluster of Excellence, Max Planck Institute for Molecular Biomedicine, Munster, Germany
5. Gerald Bronfman Department of Oncology, McGill University, Montreal, Qc, Canada

Estrogen receptor alpha (ER $\alpha$ ) activity is associated with increased proliferation in hormone-dependent cancers. Studies aiming to understand the impact of ER $\alpha$  on cancer-associated phenotypes have largely been limited to its transcriptional activity. Here we demonstrate that ER $\alpha$  coordinates its transcriptional output with selective modulation of mRNA translation. Importantly, translational perturbations caused by depletion of ER $\alpha$  largely manifest as “translational offsetting” of the transcriptome, whereby amounts of translated mRNA and protein levels are maintained constant despite changes in mRNA abundance. Transcripts whose levels, but not polysome-association, are reduced following ER $\alpha$  depletion lack features which limit translational efficiency including structured 5'UTRs and miRNA target sites. In contrast, mRNAs induced upon ER $\alpha$  depletion whose polysome-association remains unaltered are enriched in codons requiring U34-modified tRNAs for efficient decoding. Consistently, ER $\alpha$  regulates levels of U34-modification enzymes, whereas altered expression of U34-modification enzymes disrupts ER $\alpha$  dependent translational offsetting. Our preliminary data also show that ER $\alpha$ -dependent changes in U34-modifications may be associated with drug resistance and our findings therefore may have important implications in understanding alterations in gene expression programs following treatment with ER $\alpha$  antagonists. Altogether, we unravel a hitherto unprecedented mechanism of ER $\alpha$ -dependent orchestration of transcriptional and translational programs, and highlight that translational offsetting may be a pervasive mechanism of proteome maintenance in cancer. Furthermore, our results suggest that tRNA U34 modifications may be a common therapeutic vulnerability shared between hormone-dependent cancers.

## New combination treatments for castrate-resistant prostate cancer targeting DNA damage and repair

Mitchell Lawrence<sup>1,2</sup>, Laura Porter<sup>1</sup>, Nicholas Choo<sup>1</sup>, Hong Wang<sup>1</sup>, David Pook<sup>1</sup>, Shahneen Sandhu<sup>2</sup>, Elaine Sanij<sup>2</sup>, Richard Pearson<sup>2</sup>, Ross Hannan<sup>2,3</sup>, Luc Furic<sup>1,2</sup>, Renea Taylor<sup>1,2</sup>, Gail Risbridger<sup>1,2</sup>

1. Monash University, Clayton, Vic, Australia
2. Peter MacCallum Cancer Centre, Melbourne, Vic, Australia
3. Australian National University, Canberra, ACT, Australia

Advanced prostate cancer is often treated sequentially with monotherapies until tumours inevitably develop resistance. This has created new clinical challenges, including the emergence of aggressive androgen receptor (AR)-null subtypes of prostate cancer. Nevertheless, recent clinical trials demonstrate that combining different treatments for prostate cancer can improve patient outcomes. Consistent with this, we are using the Melbourne Urological Research Alliance (MURAL) collection of patient-derived models to identify novel combination therapies that target diverse phenotypes of prostate cancer.

One of the most promising combinations so far is the small molecule CX-5461, an RNA Polymerase I (Pol I) transcription inhibitor that induces nucleolar localised DNA damage, combined with talazoparib, a PARP inhibitor (PARPi) that prevents DNA damage repair. Together, these compounds synergistically decreased *in vitro* growth of patient-derived organoids and androgen-responsive and -independent cell lines. This was associated with increased DNA damage compared to either agent alone, with upregulation of  $\gamma$ H2AX and Rad51 foci formation and increased phosphorylation of checkpoint kinases 1 and 2



(CHK1 and CHK2). Furthermore, oral administration of talazoparib and CX-5461 significantly attenuated the *in vivo* growth of four different patient-derived xenografts (PDXs). Notably, the decrease in tumour growth was maintained for up to two weeks after treatment, resulting in a significant increase in survival. Importantly, these responses occurred in PDXs from diverse, castration-resistant tumours, including AR-null and neuroendocrine disease from patients who failed currently available treatments in the clinic. All tumours were proficient in homologous recombination, demonstrating that the efficacy of the combination treatment does not require genomic defects in DNA damage repair. Together these studies provide strong preclinical evidence that CX-5461 and PARPi combination therapy may represent a new treatment strategy for aggressive subtypes of advanced prostate cancer. This work provides promise for further clinical development of novel therapies targeting DNA damage in prostate cancer.

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### Obesity is associated with *BRAF*-mutated thyroid cancer

**Sabbir T Rahman**<sup>1,2</sup>, **Nirmala Pandeya**<sup>1,2</sup>, **Donald S A McLeod**<sup>1,3</sup>, **Rachel E Neale**<sup>1,2</sup>, **Susan J Jordan**<sup>1,2</sup>

1. QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

2. School of Public Health, The University of Queensland, Brisbane, Queensland, Australia

3. Department of Endocrinology and Diabetes, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia

**Background:** Thyroid cancer incidence has increased in many parts of the world since 1980s, as has obesity prevalence. Evidence suggests that people with greater body size have greater thyroid cancer risk, but it is unclear whether this association is causal or is driven by over-diagnosis of indolent cancers because overweight/obese people use health services more frequently than those of normal weight and have greater opportunity for incidental diagnosis. Assessing whether obesity is associated with higher-risk thyroid cancers might help clarify this issue.

**Methods:** We recruited 1013 thyroid cancer cases diagnosed between 2013 and 2016 and 1057 population controls, frequency matched by sex and age group. We used logistic regression to assess the association between body mass index (BMI) and overall thyroid cancer risk as well as by tumour *BRAF* mutational status as a marker of potentially higher-risk cancer.

**Results:** In both women and men, higher BMI was associated with greater odds of thyroid cancer overall. Having a BMI  $\geq 30$  kg/m<sup>2</sup> was associated with increased odds of *BRAF*-mutated thyroid cancer (odds ratio =2.22; 95% confidence interval: 1.66-2.97 for obese vs. normal BMI); odds ratios were lower and inconsistent for *BRAF*-negative cancers. The odds of *BRAF*-mutated cancer increased by 18% and 24% for each 5 kg/m<sup>2</sup> increase in BMI in women and men respectively.

**Conclusions:** Greater risk of *BRAF*-mutated thyroid cancers among those with high BMI suggests that the association may not merely reflect greater healthcare service use and indicates an independent relationship between excess body fatness and clinically important thyroid cancer.

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### An up date on HRT

**Roisin Worsley**<sup>1</sup>

1. Alfred Hospital, Prahran, VIC, Australia

After all the many analyses and re-analyses menopausal hormone therapy is once again considered a safe and reasonable treatment option for women with severe menopausal symptoms.

But what should you actually prescribe for the 50 year old woman with severe hot flushes? Are all menopausal hormone therapies created equal or is there a best option? Is there anything new?

This up date will focus on the current state of play of menopausal hormone therapy - the comparative risks and benefits of the various forms of hormone therapy available in Australia today.

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### Managing menopause without hormones

**Martha Hickey**<sup>1</sup>

1. Department of Obstetrics and Gynaecology, University of Melbourne, The Royal Women's Hospital, Parkville, VIC, Australia

Menopausal symptoms include vasomotor symptoms (often described as "hot flushes" or "night sweats") and vaginal dryness. Estrogen-containing hormonal therapies are currently the most effective treatments for these symptoms but are contraindicated for some women and avoided by others. This presentation will review the evidence supporting the efficacy of non-pharmacological and non-hormonal treatments for vasomotor and vaginal symptoms and provide practical advice on evaluating and managing symptoms without hormones.

Learning outcomes for this presentation include understanding the evidence base to support use of non-hormonal and non-pharmacological treatments for menopausal symptoms and understanding the clinical situations where non-hormonal treatments should be offered.

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## Menopausal hormone therapy under difficult circumstances

**Bronwyn Stuckey<sup>1</sup>**

1. *School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia*

Oestrogen-containing menopausal hormone therapy (MHT) is the gold standard for the treatment of vasomotor symptoms of the menopause. Although one may have a favourite formulation of oestrogen or of oestrogen and progestogen, there are times when that formulation does not suit a particular woman. This may be either because of stage of menopause, or medical history, or co-morbidities, or side-effects, or simply patient preference. One size does not always fit all.

This presentation will present real-life case histories where a modification of MHT delivery, dose, or formulation was required, and will discuss the evidence base for efficacy and safety in each case.

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## HRT for fracture prevention: an effective option worth revisiting?

**Emma Duncan<sup>1</sup>**

1. *Queensland University of Technology and Royal Brisbane Women's Hospital, Brisbane, QLD, Australia*

It is hard to overestimate the importance of gonadal hormones – particularly oestrogen – to the skeleton. From pubertal bone accrual to growth plate closure to normal bone turnover to mechano-sensing, oestrogen is of key skeletal importance in both men and women. At menopause, oestrogen deficiency causes rapid bone loss – in the following decade women lose 9-10% of their total bone mass – and it continues to contribute to ongoing bone loss thereafter. It is not unsurprising, therefore, that randomised controlled trials demonstrated that oestrogen-alone and combined oestrogen-progestogen increase BMD and prevent vertebral and non-vertebral fractures in postmenopausal women. Perhaps what is surprising is how controversial menopausal hormone therapy (MHT) is in general, and for osteoporosis specifically. In large part, this is due to the premature closure and, arguably, sensational publications of the Women's Health Initiative [WHI] trials. As a consequence, many endocrinologists are no longer familiar with prescribing MHT and indeed are uncomfortable continuing any form of gonadal hormone replacement in women aged over 50 years.

In recent years a closer analysis of the WHI and the risks vs. benefits of MHT have led to a re-evaluation and a question: in the widespread abandonment of MHT, have we lost a potentially valuable therapeutic option for prevention and/or treatment of osteoporosis? This talk will discuss the evidence for MHT specifically with respect to bone health, and provide a contextualised and nuanced approach to its use in 2019.

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## From lab to paddock: fertility enhancements for the equine breeding industry

**Zamira Gibb<sup>1</sup>, Aleona Swegen<sup>1</sup>, Roisin A Griffin<sup>1</sup>, Stephanie Waugh<sup>1</sup>, Robert J Aitken<sup>1</sup>**

1. *University of Newcastle, CALLAGHAN, NSW, Australia*

The Australian equine industry is dominated by the production of the Thoroughbred (in which assisted reproductive technologies; ART, are prohibited) and the Standardbred (almost exclusively produced via ART) racehorse. These contrary breeding strategies present diverse obstacles for fertility enhancement, particularly for stallions. In the Thoroughbred, in vivo fertility improvements have been implemented through the development of an on-farm diagnostic test to identify periods of stallion subfertility, and via nutritional interventions using carnitine and omega-3 fatty acids to reduce sperm oxidative stress and improve membrane fluidity respectively. Current industry-focussed research is underway to develop an early pregnancy test for the mare and to identify stallions which are at risk of subfertility induced by heat stress. This information allows breeders to strategically manage these animals in such a way that will reduce the need for excessive veterinary interventions and reduce the crucial foaling-to-pregnancy interval so that horses are born as close to the 1<sup>st</sup> of August (and are therefore more mature at age-related sales and races) as possible. In contrast, to improve breeding efficiency for the AI-centric Standardbred, we have developed a synthetic sperm storage medium which preserves fertility for up to two weeks without the need to chill or cryopreserve, thereby overcoming the deleterious effects of cold shock and cryoinjury which are associated with increased sperm DNA damage, reduced longevity following thawing or warming and ultimately, reduced fertility. The fertility of spermatozoa stored in this medium is akin or higher than that of fresh spermatozoa, and being entirely devoid of biologically-derived components, its use will expedite sperm imports, facilitating the dissemination of valuable genetics across the globe. These improvements will translate to an increased rate of genetic gain, improved welfare and enhanced economic stability for this sustainable, culturally significant industry.

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## Nuclear Preparation for Embryonic Genome Activation

**Chris O'Neill<sup>1</sup>**

1. *Human Reproduction Unit, Kolling Institute, Sydney Medical School (Northern), University of Sydney, Camperdown, NSW, Australia*

An essential prerequisite for normal embryo development is the activation of transcription from the new embryonic genome created at fertilization. This process, known as embryonic genome activation, occurs at a specified time after fertilization and is independent of the cell-cycle. This first round of embryonic transcription converts the transcriptome inherited from the gametes into one that encodes the totipotent state of the early embryo. Errors in the fidelity of this process compromises embryo development and post-natal health.

Several processes of remodeling of the embryo are required to achieve embryonic genome activation. These include: (1) remodeling nuclear chromatin from a transcriptionally repressive to a permissive state; (2) epigenetic reprogramming at the genic level to foster transcription of the totipotent transcriptome; (3) the assembly and nuclear localization of transcriptional machinery; and (4) the activation of the zygotic clock that coordinates these processes.

While many aspects of these processes remain unresolved, this presentation will review recent progress in our understanding of these essential requirements for embryonic genome activation. Gaps in our current understanding of this key developmental transition will also be highlighted.

## Building the mid-piece of the sperm tail: unexpected insights from a *Vdac2* knockout mouse

**Jessica Dunleavy<sup>1</sup>, Mari Lehti<sup>1</sup>, Denis Korneev<sup>1</sup>, Jo Merriner<sup>1</sup>, Anne O'Connor<sup>1</sup>, Grant Dewson<sup>2</sup>, Moira O'Bryan<sup>1</sup>**

1. School of Biological Sciences, Monash University, Clayton, VIC, Australia

2. Walter and Eliza Hall Institute of Medical Research, Clayton, VIC, Australia

The mid-piece of the sperm tail is a major site of energy production for sperm motility and defects in its formation are strongly associated with human male infertility. Despite, this the processes by which mitochondria are recruited from the cytoplasm, and then assembled into the characteristic, helical mitochondrial sheath of the mid-piece, remain virtually unexplored. Herein, through the characterisation of a *Vdac2* knockout mouse model, we reveal novel insights into this mysterious process.

VDAC2 is a voltage-gated porin in the outer mitochondrial membrane. It has well-established roles in apoptosis and cellular metabolism, and decreased VDAC2 levels have been previously correlated with human male infertility. Consistent with this, here, we show that VDAC2 is essential for male fertility and that spermatogenesis is uniquely sensitive to even partial VDAC2 reduction. Indeed, mice heterozygous for our *Vdac2* deletion allele (*Vdac2*<sup>+/−</sup>) are male sterile. Unexpectedly however, our analyses suggest this phenotype is not due the disruption of VDAC2 apoptotic function. Instead, *Vdac2*<sup>+/−</sup> male sterility is due to an inability to produce functionally motile sperm. Both the percentage of motile sperm and the percentage of progressively motile sperm were minimal in *Vdac2*<sup>+/−</sup> males (reduced by 62% and 93%, respectively). Intriguingly, these motility defects are structural in origin. Most notably trafficking and incorporation of mitochondria into the sperm flagella mid-piece was severely compromised. Consistent with these data, we also show that VDAC2 is highly testis enriched, and while it is present in all ages of the postnatal mouse testis, it is particularly upregulated after the onset of spermiogenesis. Collectively, our data unequivocally establishes VDAC2 as an essential component of the spermiogenesis machinery and supports a model wherein VDAC2 facilitates mitochondrial loading into the mid-piece. Further, given that the VDAC2 has previously been established to bind the microtubule motor protein, KLC3, these data raise the distinct possibility that VDAC2 functions as an 'adaptor' between motor proteins and mitochondria during mid-piece formation.

## The odyssey (and oddity) of gonadal sex determination

**Peter Koopman<sup>1</sup>**

1. University of Queensland, Brisbane, QLD, Australia

More than 27 years have passed since the discovery of *Sry*, the Y-chromosomal gene responsible for directing male development in eutherian mammals. This landmark discovery in 20<sup>th</sup> century molecular genetics opened the door to detailed study of how genetic pathways channel the development of the initially ambiguous embryonic gonads into testes and so generate males, while suppressing ovarian development.

In this talk, I will draw together current knowledge relating to how *Sry* activates its target gene *Sox9* to set in train the events leading to Sertoli cell differentiation, in turn triggering a cellular cascade of events leading to testis formation. The unusual genetics and biology of *Sry*, and of gonadal development, have led to a number of curious idiosyncrasies, which I will also describe. Our current progress relating to the regulation and structure/function of *Sry* will be presented. Finally, I will describe efforts to gain a better understanding of the causes of disorders or differences of sex development (DSDs), a diverse group of conditions commonly associated with complex healthcare issues, impaired reproductive capacity, and gonadal cancer, and applying this knowledge to the diagnosis and clinical management of DSD, so as to improve outcomes for affected children and adults.

## Multifocality is not a significant risk factor for progression during active surveillance of papillary thyroid microcarcinoma

**Iwao Sugitani<sup>1,2</sup>, Aya Ebina<sup>1</sup>, Kazuhisa Toda<sup>2</sup>, Ryuta Nagaoka<sup>1</sup>, Marie Saitou<sup>1</sup>, Masaomi Sen<sup>1</sup>, Keiko Yamada<sup>3</sup>**

1. Department of Endocrine Surgery, Nippon Medical School, Graduate School of Medicine, Bunkyo-ku, TOKYO, Japan

2. Division of Head and Neck, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Koto-ku, TOKYO, Japan

3. Department of Ultrasonography, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Koto-ku, TOKYO, Japan

**Background:** Prospective trials of active surveillance (AS) have shown progression rates of only about 10% in patients with low-risk papillary microcarcinoma (PMC, cT1aN0M0) of the thyroid. Previous studies demonstrated that younger age and weaker calcification were significant risk factors for progression during AS. However, the significance of multifocality as a prognostic factor is controversial. The aim of this study was to investigate the prognostic value of multifocality on progression of PMC while conducting AS.

**Methods:** Data of 571 patients (mean age: 53.1 years, 495 females) with PMC who underwent AS were reviewed. Progression of the tumor was defined as tumor size enlargement (3 mm or more in the maximum diameter on ultrasonography from the beginning of AS) and/or development of ultrasonographically evident lymph node metastasis (LNM).

**Results:** After a mean of 7.6 years of observation, 49 patients (8.6%) showed tumor enlargement and 8 patients (1.4%) developed LNM. Ten-year progression rate was 12.7%. There were 115 patients (20.1%) with multifocal PMCs (2-5 lesions for each, 262 tumors in total) and 456 patients (79.9%) with unifocal disease. Age, sex and calcification pattern were not

significantly different between the groups; however, patients with multifocal PMCs were more frequently accompanied with Hashimoto's thyroiditis (46.2% vs 34.4%,  $p = 0.026$ ). Between patients with multifocal and unifocal disease, there were no significant difference in 10-year rate of tumor enlargement (7.0% vs 11.2%,  $p = 0.13$ ), LNM development (2.4% vs 1.1%,  $p = 0.27$ ) and progression (14.8% vs 12.2%,  $p = 0.51$ ). Eventually, 10 patients (8.7%) with multifocal PMCs underwent surgery and 9 needed to have total thyroidectomy, although 7 were still T1N0M0 low-risk cancer.

**Conclusions:** Even patients with multiple PMCs (cT1aN0M0) are thought to be good candidates for AS and most of them could avoid total thyroidectomy thanks to the policy.

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### Is Childhood, Adolescent and Young Adult Thyroid Cancer in Fukushima after Fukushima Daiichi Nuclear Power Plant Accident different from Chernobyl's Thyroid Cancer after the accident ?

**Shinichi Suzuki**, Manabu Iwadate<sup>1</sup>, Sho Hasegawa<sup>1</sup>, Yoshiko Matsumoto<sup>1</sup>, Satoshi Suzuki<sup>1</sup>, Hiroshi Mizunuma<sup>1</sup>

*1. Thyroid and endocrinology, Fukushima Medical University School of Medicine, Fukushima, Japan*

After the Fukushima Daiichi Nuclear Power Plant accident that followed the Great East Japan Earthquake and tsunami on March 11, 2011, a large-scale thyroid ultrasound examination (TUE) survey began for people aged 18 years or younger at the time of the disaster. We would like to report the thyroid cancers screened from this survey. We analyzed clinicopathological findings of our operated 145 thyroid cancer.

Among these 145 subjects, 143 papillary thyroid carcinomas, one poorly differentiated thyroid carcinomas, and one other thyroid carcinoma were postoperatively confirmed. Mean age at diagnosis was 18 years, and mean tumor size was 15 mm. Even though the tumor smaller than 10mm, almost cases were not encapsulated PTC. There were no encapsulated cases smaller than 10mm without vascular/capsular invasion and lymph node metastasis. Extra thyroidal invasion and node positive were shown in 45% and 79%, postoperatively. Total thyroidectomy was performed in only 8% unlike Chernobyl. Our operated cases were not included super-low risk cases recommended active surveillance, and also included high risk cases only a little. Thyroid cancer detected in Fukushima does not display specific qualitative changes in tumor histopathology or morphological features of tumor aggressiveness over time unlike Chernobyl. Similarity of pathological characteristics between tumors removed within 4 years after the accident and 4-5 years after it strongly suggest their common etiology, which is unlikely related to radiation and also is in contrast to Chernobyl. Most cases were diagnosed with classical PTC, and there were few cases with solid variant PTC, unlike Chernobyl. Genetic alteration was also different between the children of Chernobyl and Fukushima. Fukushima PTC becomes less invasive at older ages which is similarly to 'radiogenic' and 'sporadic' PTC from Chernobyl.

Conclusively, we are aware of the high prevalence of thyroid cancer detected by sophisticated and large-scale ultrasound screenings following the accident.

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### Prognostic role of the lymphocyte-to-monocyte ratio for clinical outcomes of patients with progressive radioiodine-refractory differentiated thyroid carcinoma treated by sorafenib

**Jonghwa Ahn**<sup>1</sup>, Eyun Song<sup>1</sup>, Won Gu Kim<sup>1</sup>, Tae Yong Kim<sup>1</sup>, Won Bae Kim<sup>1</sup>, Young Kee Shong<sup>1</sup>, Min Ji Jeon<sup>1</sup>

*1. Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Songpa-gu, Seoul, South Korea*

**Background:** The lymphocyte-to-monocyte ratio (LMR) reflects tumor infiltrating immune cell status and host immunity. The LMR has been reported as a prognostic marker in various cancers including anaplastic thyroid carcinoma. The aim of the present study was to evaluate the role of LMR as a prognostic marker in patients with progressive radioiodine-refractory (RAIR) differentiated thyroid carcinoma (DTC).

**Methods:** We retrospectively included forty patients with progressive RAIR DTC who were treated by sorafenib and had available pre-treatment complete blood cell count (CBC) data. We assessed the response rate, progression-free survival (PFS), and overall survival (OS) according to the LMR.

**Results:** The patients were divided into two groups based on their pre-treatment LMR: a low LMR group (<4) ( $n = 22$ , 55%) and a high LMR group ( $\geq 4$ ) ( $n = 18$ , 45%). There was no significant difference in baseline characteristics between two groups. Low LMR was associated with poor response rate and shorter disease control duration to sorafenib. The PFS curves were significantly different based on the LMR values, and the median PFS of the low and high LMR groups ( $P = 0.019$ ). The OS curves were also significantly different based on the LMR values, and the median OS of the low and high LMR groups were 24.3 and 35.7 months, respectively ( $P = 0.015$ ). In multivariate analysis, low LMR was an independent risk factor for all-cause mortality in patients with progressive RAIR DTC (HR, 2.64; 95% confidence interval (CI): 1.04-6.72,  $P = 0.041$ ).

**Conclusions:** Low LMR is associated with poor response rate, PFS and OS in patients with progressive RAIR DTC who were treated by sorafenib. LMR could be a simple prognostic biomarker in patients with progressive RAIR DTC.

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### Stimulated thyroglobulin is a predictor of structural recurrence for microscopic positive margins in papillary thyroid cancer

**Jayani Jayasekara**<sup>1</sup>, Aimee DiMarco<sup>1</sup>, Jia Feng Lin<sup>1</sup>, Adam Aniss<sup>1</sup>, Anthony Gill<sup>1</sup>, Diana Learoyd<sup>1</sup>, Roderick Clifton-Bligh<sup>1</sup>, Anthony Glover<sup>1</sup>, Stan Sidhu<sup>1</sup>, Mark Sywak<sup>1</sup>

1. University of Sydney, Sydney, Australia

**Background:** The implication of microscopic positive margin status in resected papillary thyroid cancer (PTC) is poorly-understood, whereas serum thyroglobulin levels (Tg) are used routinely after total thyroidectomy in PTC as a potential surrogate for early disease recurrence. The aim of this study was to determine whether stimulated Tg is a predictor of structural recurrence in patients with microscopic positive margins in PTC.

**Methods:** This retrospective cohort study reviewed 468 patients who underwent total thyroidectomy +/- central lymph node dissection for PTC >10mm in the period 1985-2016. Exclusion criteria were; metastatic disease, gross residual disease at resection and absence of a post-operative Tg measurement between 3-52 weeks post-operatively. The median follow-up time was 2 years (range; 1-19 years). The primary outcome measure was structural recurrence, defined as disease detected on imaging modalities (including ultrasound, computed tomography or positron emission tomography) and confirmed on biopsy.

**Results:** The median patient age was 49 years (range 20-87). There were 355 females (75.9%). The overall structural recurrence rate was 13.0%. Three hundred and forty-seven patients had uninvolved margins at time of resection with a structural recurrence rate of 11.5% and one hundred and twenty-one patients had microscopic positive margins with structural recurrence rate of 17.4% ( $p=0.101$ ). In patients with microscopic positive margins, there was a significant difference ( $p=0.0007$ ) in the rate of structural recurrence between patients with early post-operative Tg<2ng/mL (5.2%) compared to patients with Tg≥2ng/mL (28.6%). Univariate analysis identified lymph node involvement, vascular invasion and early Tg as significant predictors of recurrence. After multivariate analysis, early serum Tg remained the only significant predictor of recurrence.

**Conclusion:** In patients undergoing total thyroidectomy for papillary thyroid cancer with known microscopic margin involvement, early post-operative stimulated Tg can be used to accurately quantify the risk of disease recurrence.

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## BRAF(V600E) mutation is highly prevalent in the young population in Fukushima

**Manabu Iwate**<sup>1</sup>, **Norisato Mitsutake**<sup>2</sup>, **Michiko Matsue**<sup>2</sup>, **Satoshi Suzuki**<sup>1</sup>, **Hiroshi Mizunuma**<sup>1</sup>, **Yoshiko Matsumoto**<sup>1</sup>, **Sho Hasegawa**<sup>1</sup>, **Keiichi Nakano**<sup>1</sup>, **Izumi Nakamura**<sup>1</sup>, **Toshihiko Fukushima**<sup>1</sup>, **Shunichi Yamashita**<sup>3</sup>, **Shinichi Suzuki**<sup>1</sup>

1. Department of Thyroid and Endocrinology, Fukushima Medical University, Fukushima, Japan

2. Department of Radiation Medical Science, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

3. Fukushima Medical University, Fukushima, Japan

**(Aim)** According to the surveillance, new cases of thyroid cancers in the young population have increased. The thyroid ultrasound screening for children aged 0-18 was performed in Fukushima after the accident at the Fukushima Daiichi Nuclear Power Plant. As a result, many thyroid cancer cases in the young population have been found. To explore the carcinogenic mechanisms of the cancers, we analyzed their clinicopathological and genetic features. **(Methods and Results)** We analyzed 138 patients (52 males and 86 females) operated between 2013 and 2016 at Fukushima Medical University. The median age at operation was 18 years old. The mean size of the tumors was 15.3mm. The lymph node metastasis was observed in 109 (79.0%) cases. We analyzed BRAF(V600E) mutation by direct DNA sequencing. The BRAF(V600E) mutation was observed in 96 (70.0%) cases of the thyroid cancers. On the contrary, the RET/PTC3 rearrangement was observed in 1 (0.7%) cases. **(Conclusion)** The RET/PTC3 rearrangement has been found in pediatric post-Chernobyl thyroid cancer. However, the RET/PTC3 rearrangement with PTC was detected only 0.7% in our cases. The prevalence of the BRAF(V600E) mutation was comparable to Japanese adult sporadic cases, implying that the carcinogenesis mechanism may be similar between young population and adult papillary thyroid cancers.

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## Prognosis of thyroid cancer in patients with Graves disease

**Hyungju Kwon**<sup>1</sup>, **Hae Na Lee**<sup>1</sup>, **Byung-In Moon**<sup>1</sup>, **Woosung Lim**<sup>1</sup>

1. Ewha Womans University, Yangcheon-gu, SEOUL, South Korea

### Background

There is an ongoing debate about the aggressiveness of thyroid cancer associated with Graves' disease. The aim of this study was to investigate the recurrence of papillary thyroid carcinoma (PTC) in patients with Graves' disease, compared with matched control patients.

### Methods

From January 2006 to June 2014, 3,628 patients underwent total thyroidectomy for PTC. Of those, 114 patients had Graves' disease with non-occult PTC (GD group). Recurrence rates were analyzed between GD group and 1:5 matched euthyroid patients (control group), after propensity score matching according to age, gender, and pathological features including tumor size, extrathyroidal extension, resection margin involvement and lymph node metastasis.

### Results

The matched cohorts did not differ in age, gender, body mass index, pathologic features. Recurrence was found in 1 patient (0.9%) in the GD group after median follow-up of 94.1 months, whereas 6 patients (1.6%) experienced recurrences in the control group. No difference was found on 5-year recurrence-free survival between GD group and control group (100% vs. 98.4%,  $P = 0.572$ ). The Cox proportional hazard analysis indicated that only age was a prognostic factor for predicting disease-free survival (hazard ratio 0.898, 95% CI 0.832-0.969,  $P = 0.006$ ).

### Conclusions

Our data suggest that Graves' disease does not affect the prognosis of papillary thyroid carcinoma. Papillary thyroid cancers in patients with Graves' disease showed an excellent prognosis and comparable disease-free survival than those with euthyroid states

## The Effect of Thyroid Stimulating Hormone Suppression Therapy on Bone Mineral Density and Trabecular Bone Score in Postmenopausal Women with Differentiated Thyroid Carcinoma

**Chaewon Chung<sup>1</sup>, Hoonsung Choi<sup>2</sup>, Sun Wook Cho<sup>1</sup>, Jae Hoon Moon<sup>3</sup>, Young Joo Park<sup>1</sup>, Ka Hee Yi<sup>4</sup>, Do Joon Park<sup>1</sup>**

1. Seoul National University Hospital, Seoul, , South Korea

2. Kangwon National University Hospital, Chuncheon, South Korea

3. Seoul National University Bundang Hospital, Bundang, South Korea

4. SEOUL METROPOLITAN GOVERNMENT SEOUL NATIONAL UNIVERSITY BORAMAE MEDICAL CENTER, Seoul, South Korea

Thyroid stimulating hormone (TSH) suppression therapy in differentiated thyroid carcinoma (DTC) has been reported to increase fracture risk and reduce bone mineral density (BMD). However, there are few studies analyzing serial BMD data of a patient over 3 years. Also, the relationship of TSH suppression and trabecular bone score (TBS) is still unclear. The aim of this study is to investigate the effect of TSH suppression therapy on BMD and TBS through serial follow-up data over 3 years.

We conducted a retrospective cohort study including 110 postmenopausal women with DTC who had received TSH suppression therapy and 30 control subjects matched for age, sex, and type of thyroidectomy. BMD in the spine and hip area and TBS were measured by dual energy X-ray absorptiometry (DXA). Data of BMD and TBS was checked at within a year from surgery, 1 year, 2 years, and 4 years after the surgery. All patients had the data at within a year from surgery and at least one follow-up DXA was conducted.

The % difference over time was calculated for BMD of L spine and hip, and TBS. TSH suppression group showed significant % reduction in BMD of L spine and hip earlier than control group. For TBS, control group had no significant change over time, but TSH suppression group showed significant change from 2 years after the surgery. With mixed linear regression analysis, there was significant difference in TBS over time between control and suppression group.

TSH suppression in postmenopausal DTC patient showed earlier reduction in BMD of L spine and hip and TBS.

## Prognostic value of acoustic structure quantification in patients with Hashimoto's thyroiditis

**Ji Ye Lee<sup>1</sup>, Hyun Sook Hong<sup>2</sup>**

1. Eulji Medical Center, Seoul, SEOUL, South Korea

2. Radiology, Soonchunhyang University Bucheon Hospital, Bucheon, Gyeonggi-do, 대한민국

**Objectives** Assessment of thyroid parenchymal echogenicity on ultrasonography is a predictor of future thyroid dysfunction. Our objective was to determine the prognostic value of acoustic structure quantification (ASQ) to predict the outcome of patients with Hashimoto's thyroiditis (HT).

**Materials and Methods** We prospectively evaluated 90 patients with HT using ASQ from May to December 2013. Surveillance for the development of overt hypothyroidism was conducted over a median period of 40 months [3–55]). ASQ were dichotomized based on optimal cutoff values obtained from ROC curve analysis. The probability of developing overt hypothyroidism was compared between the dichotomized subgroups using Kaplan–Meier analysis and log-rank tests. Multivariate Cox regression analysis was performed to determine significant prognostic factors.

**Results** The cumulative rate of overt hypothyroidism was 67.7%. The median interval to overt hypothyroidism was 27.9 months (95% confidence interval, 12.0–38.0 months). There was no significant difference in the risk of overt hypothyroidism using qualitative echogenicity between groups ( $P = 0.669$ ) according to Kaplan–Meier analysis. However, the ASQ average ( $P < 0.001$ ), standard deviation ( $P = 0.015$ ), and focal disturbance ratio ( $P < 0.001$ ) were significantly associated with an increased risk of overt hypothyroidism. Multivariate Cox regression analysis revealed that a higher ASQ average (hazard ratio, 1.03;  $P = 0.03$ ) and higher thyroid-stimulating hormone level (hazard ratio, 1.02;  $P = 0.02$ ) were independent predictors of overt hypothyroidism.

**Conclusions** ASQ has potential as a prognostic biomarker for predicting the risk of overt hypothyroidism in patients with HT.

## Association of other autoimmune diseases in patients with Graves' Disease (with/without Graves' Ophthalmopathy): review of the literature

**Silvia Martina Ferrari<sup>1</sup>, Poupak Fallahi<sup>2</sup>, Ilaria Ruffilli<sup>1</sup>, Giusy Elia<sup>1</sup>, Francesca Ragusa<sup>1</sup>, Salvatore Benvenga<sup>3,4,5</sup>, Alessandro Antonelli<sup>1</sup>**

1. Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

2. Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

3. Department of Clinical and Experimental Medicine, Section of Endocrinology, University of Messina, Messina, Italy

4. Master Program on Childhood, Adolescent and Women's Endocrine Health, University of Messina, Messina, Italy

5. Interdepartmental Program on Molecular & Clinical Endocrinology, and Women's Endocrine Health, University Hospital, A.O.U. Policlinico Gaetano Martino, Messina, Italy

Discordant results have been reported in the literature about the association of thyroid disorders and other systemic, or organ specific, autoimmune diseases. We have carried out a prospective study investigating about the correlation between Graves' disease (GD) and other autoimmune diseases from data obtained by the literature.

The study involved 3209 GD patients [984 of whom with Graves' ophthalmopathy (GO)], in comparison to: 1) 1069 healthy controls; 2) 1069 with multinodular goiter; 3) 1069 patients with autoimmune thyroiditis (AT). All the subjects were matched by age and gender, had a similar iodine intake and came from the same area.

GD patients showed a significant increase of the prevalence of certain autoimmune disorders, compared to controls. The mostly detected associations were: vitiligo<sub>SSEP</sub> (2.6%), chronic autoimmune gastritis (2.4%), rheumatoid arthritis<sub>SSEP</sub> (1.9%), polymyalgia rheumatica (1.3%), multiple sclerosis (0.3%), celiac disease (1.1%), type 1 diabetes (0.9%), systemic lupus erythematosus and sarcoidosis (<0.1%), Sjögren disease (0.8%). Moreover, three associated autoimmune disorders were found in 1.5% patients with GD. GO patients showed higher (18.9%) prevalence of autoimmune disorders, with respect to GD patients not having GO (15.6%). The pattern of the associated autoimmune disorders in GD was quite similar to that observed in AT patients.

These researches shed light on the importance of screening GD patients, who are sick, or who develop new unspecific symptoms (also if in treatment for the hyperthyroidism), for the presence of other autoimmune disorders.

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### **Worsening profiles of cardiometabolic risk factors after thionamide treatment in Graves' disease: A 12-month prospective cohort study**

**Chih-Chen Wang<sup>1</sup>, Ching-Han Lin<sup>1</sup>, Hao-Chang Hung<sup>1</sup>, Hung-Tsung Wu<sup>2</sup>, Horng-Yih Ou<sup>1</sup>**

1. Division of Endocrinology and Metabolism, Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan

2. Graduate Institute of Metabolism and Obesity Sciences, College of Nutrition, Taipei Medical University, Taipei, Taiwan

**Objective:** One most recent study by Onyebuchi *et al.* showed that Graves' disease has an increased risk of cardiovascular events, and early control of hyperthyroidism improves long-term cardiovascular morbidity and all-cause mortality. It has been known that, treatment with antithyroid drugs is usually accompanied with a certain gain in body weight (BW). However, the serial changes of BW and other cardiometabolic risk factors have never been clearly investigated. The aim of this study was to examine the serial changes of these cardiometabolic risk factors in the clinical course of thionamide treatment for Graves' disease.

**Design:** This was a prospective observational study. Patients with newly diagnosed Graves' disease treated with thionamide were followed up to 12 months. At each visit, anthropometric data, clinical features, biochemistry and thyroid function tests were measured.

**Result:** A total of 97 subjects (M/F: 29/68) were recruited. After treatment, BW increased by  $2.4 \pm 3.4\%$ ,  $6.9 \pm 4.6\%$ ,  $10.0 \pm 6.0\%$ ,  $11.2 \pm 6.5\%$  and  $12.4 \pm 6.9\%$  in males, and  $1.7 \pm 3.4\%$ ,  $5.0 \pm 4.9\%$ ,  $5.9 \pm 5.6\%$ ,  $7.0 \pm 6.4\%$  and  $7.3 \pm 6.8\%$  in females, at 1, 3, 6, 9 and 12-month, respectively. Waist circumference and body mass index also increased significantly after treatment in both genders. Furthermore, HDL-Cholesterol decreased significantly after treatment. As for metabolic syndrome components, we found significantly increased percentages of subjects with central obesity, hypertension, and hypertriglyceridemia after treatment. On the contrary, the percentage of hyperglycemia decreased significantly after treatment. Taken together, subjects with metabolic syndrome increased accordingly from 6.3 % to 21.1 % ( $p = 0.017$ ).

**Conclusion:** We demonstrated a rapid BW gain as well as deteriorations of cardiometabolic risk factors shortly after thionamide treatment in both genders, which might contribute to increased cardiovascular mortality in Graves' disease. Further studies are needed to validate this speculation.

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### **The association between low vitamin D status and autoimmune thyroid disease in Korean premenopausal women: The 6th Korean National Health and Nutrition Examination Survey, 2013-2014**

**Choonyoung Kim<sup>1</sup>**

1. Seoul National University Bundang Hospital, Seongnam-si, GYEONGGI-DO, South Korea

Publish consent withheld

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### **Investigating Clinical Characteristics of Cancer Immunotherapy Related Thyroiditis**

**Hana HN Kim<sup>1</sup>, Tae-Yong TY Kim<sup>1</sup>, Do Joon DJ Park<sup>1</sup>, Ka Hee KH Yi<sup>1</sup>, Young Joo YJ Park<sup>1</sup>, Sun Wook SW Cho<sup>1</sup>**

1. Seoul National Univ Hospital, Seoul, YES, South Korea

Immune checkpoint inhibitors such as programmed cell death 1 (PD1) and cytotoxic T lymphocyte antigen 4 (CTLA4) monoclonal antibodies have become a notable treatment for various advanced cancers. One of the most common immune-related adverse events (IRAEs) is thyroid dysfunction. This study aimed to investigate the incidence, disease course, and oncologic outcomes of thyroid IRAEs. We performed retrospective review of 222 patients who used immune checkpoint inhibitors for cancer therapy with thyroid function test before and after treatment. Mean (SD) diagnostic age of study patients was 60.6 (11.5) years and men were 67.6%. Lung (34.7%) and stomach (14.4%) were two most common sites of primary cancer. All types of Immune checkpoint inhibitors were included. The prevalence of thyroid IRAEs was 40.5% during median of



2.0 (range, 0.0-22.0) months of follow-up, and median time from baseline to detection of first thyroid dysfunction was 2.0 (range, 0.0-24.0) months. Among 90 patients of thyroid IRAEs, 37 had transient thyroiditis following spontaneous recovery and 36 and 17 of them had subclinical and overt hypothyroidism, respectively. 16 patients who had overt hypothyroidism received levothyroxine therapy, while none of the subclinical hypothyroidism patients needed it. Baseline TSH levels increased from the thyroid IRAE(-) group to the transient thyroiditis, subclinical hypothyroidism and overt hypothyroidism group, in sequence ( $p$  for trend =0.014). Within the thyroid IRAE(+) group, the 1<sup>st</sup> abnormal TSH over 10 uIU/ml was more frequent in overt hypothyroidism than SCH group (42% vs 0%,  $p < 0.001$ ). Finally, patients with overt hypothyroidism showed better overall survival than patients without thyroid IRAEs (14.7 vs 10.0 months,  $p = 0.074$ ). In conclusion, higher baseline TSH and the first abnormal TSH over 10uIU/ml can predict the occurrence of overt hypothyroidism as thyroid IRAEs which was associated favorable oncologic outcomes.

## Efficacy and Safety of Lithium adjuvant to Radioiodine treatment in Graves' disease

**Nipawan Waisayanand<sup>1</sup>, Usanee Damrongpipatkul<sup>1</sup>, Waralee Teeyasoontranon<sup>1</sup>, Ampica Mangklabruks<sup>1</sup>**

1. Faculty of Medicine, Chiang Mai University, Mueang, CHIANG MAI, Thailand

**Objective:** Radioactive iodine therapy (RAI) is an effective treatment for Grave's disease. Lithium increases the iodine retention in the thyroid gland so that the adjuvant lithium to RAI treatment may increase the efficacy. This study compared the efficacy and safety of RAI given with or without lithium in the treatment of Graves' disease.

**Methods:** This prospective randomized controlled study was performed in the patients with Grave's disease confirming by immunological studies, radioactive nuclear and ultrasound imaging at a tertiary center hospital from June 2015 to June 2016. The patients were randomly assigned to RAI plus lithium (900 mg/day; 5 days before, 7 days after RAI) or RAI alone as a control group. The remission rates, time to remissions, adverse effects were evaluated at the 2nd, 4th, 6th-month follow-up visits.

**Results:** The 60 patients with Grave's disease were included. Mean age was 42.5±12.3 year-old, 73.3% were female, 53.3% were recurrent hyperthyroidism and mean duration of disease was 48.1 months. Thyrotropin-receptor antibodies (TRAb) were positive in 93.3%. Mean thyroids' size was 37.4 grams calculated by ultrasound and mean iodine uptake was 70.0±12.7%. There were 30 patients with RAI plus lithium group and 27 patients of the control group completing the study. The remission rates occurred 23/30 (76.7%) in the RAI plus lithium group vs. 17/27 (63.0%) in the control group ( $P = 0.26$ ). Time to remission was 116.5±23.5 days in the RAI plus lithium group vs. 135.1±35.2 days in the control group ( $P = 0.06$ ). Mean serum lithium level was 0.7±0.3 mEq/L. No patient had a major side effect, however, the RAI plus lithium group reported higher polydipsia (51.6%) and polyuria (13.8%) than RAI group ( $p < 0.01$ ).

**Conclusions:** Lithium as an adjuvant therapy could not show superior benefits and might have higher adverse effects than standard RAI treatment in Graves' disease.

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## Dysbiosis of the gut microbiome is associated with the favorable response to treatment in Graves' disease patients

**Seul Ki Kwon<sup>1</sup>, Nam Il Kim<sup>2</sup>, Hyeon Seok Oh<sup>2</sup>, Sun Wook Cho<sup>1</sup>, Do Joon Park<sup>1</sup>, Jong Sik Chun<sup>2</sup>, Young Joo Park<sup>1</sup>**

1. Seoul National University Hospital, Jongno-gu, Seoul, Korea

2. Chunlab, Inc., Seocho-gu, Seoul, Korea

**INTRODUCTION:** The balance of gut microbiome is associated with the immunologic and metabolic homeostasis in various pathophysiology such as obesity and autoimmune diseases. However, the characteristics and the clinical relevance of gut microbiome in autoimmune thyroid disease are undetermined. This study aimed to determine the characteristics of the gut microbiome in Graves' disease (GD) patients.

**METHODS:** Untreated GD patients who visited a single tertiary center from April-2018 to March- 2019 were recruited. The fecal samples were collected and freshly analyzed by 16S rRNA gene sequencing. Patients who had euthyroidism with less than 10mg/day of antithyroid drugs at 6 month were defined as good response. Additionally, patients were divided into three groups according to the weight changes at 6 month: <1kg, 1-3kg, and > 3kg.

**RESULTS:** A total of 31 GD patients were recruited. The mean age was 40.8 ± 12.3 years and 87% was female. Compared to the healthy controls, GD patients showed significantly lower microbial richness and higher firmicutes to bacteroidetes ratio. Patients with good response to treatment showed higher dysbiosis index and relative abundance of pathobiont than others. Interestingly, patients with lower microbial richness and higher dysbiosis index showed bigger weight gain ( $P$  for trend <0.05). The relative abundance was higher in *Clostridium\_g24* and *Lactobacillus rogosae* and lower in *Dialister* and *Oscillibacter* in patients with weight gain >3kg than others.

**CONCLUSION:** Higher index of dysbiosis in untreated GD patients was associated with good treatment response and higher weight changes. Pre-treatment status of gut microbiome may be used for predicting clinical outcomes in GD patients.

## The Success Rate of RAI for Graves' disease in Korea

**Hyunju Park<sup>1</sup>, Jun Park<sup>1</sup>, So Young Park<sup>1</sup>, Tae Hyuk Kim<sup>1</sup>, Jae Hoon Chung<sup>1</sup>, Sun Wook Kim<sup>1</sup>**

*1. Samsung Medical Center, Gangnam-gu, SEOUL, South Korea*

### Introduction

The initial treatment options for Graves' disease (GD) are anti-thyroid drugs (ATDs), radioactive iodine ablation (RAI) and surgery. The effects of these three modalities are similar. Thus, the choice of treatment was chosen by patient's and physician's preference. In Korea, most patients used ATDs as initial treatment, RAI tends to be used who failed with ATDs. For this reason, we estimated the first RAI success rate in Korea might be different from other countries.

### Methods

The patients underwent the first RAI between January 2007 and January 2017. A total of 247 patients were enrolled, primary outcome was a cumulative success rate in the first year, and factors affecting the success rate. Secondary outcome was delayed response, which is a patient who reached successful RAI during the follow-up period after the first year, and factors affecting the delayed response.

### Results

The cumulative success rate in the first year was 62.8%. On multivariate analysis, recur (Reference (Ref) is recur; HR 0.074,  $P < 0.001$ ), duration of using ATDs (Ref  $\leq 1$ yr;  $< 1$ yr to  $\leq 5$ yr, HR 0.454,  $P = 0.001$ ;  $5$ yr  $<$ , HR 0.062,  $P < 0.001$ ), size of goiter (Ref  $\leq 45$ g;  $45$ g  $<$ , HR 0.512,  $P = 0.027$ ), and fT4 after RAI (Ref  $\leq$  upper normal range (UNL);  $1.5 \times$  UNL  $<$ , HR 0.405,  $P = 0.006$ ) was associated with the RAI success. Twenty-seven patients were showed delayed response, and pre-RAI TRAb ( $P = 0.003$ ) and post-RAI fT4 ( $P = 0.005$ ) were affecting to delayed response.

### Conclusions

The success rate of the first RAI in Korea was lower than other countries, and most of the affecting factors were related to disease severity. In Korea, a patient who received RAI might be more "refractory" case than other countries because of medical accessibility and physician's preference. The delayed response was observed in patients with low pre-RAI TRAb and low post-RAI fT4. In these patients, clinical follow-up with careful monitoring could be an option for the treatment.

## Balancing needs: Placental mechanisms regulating maternal-fetal resource allocation

**Amanda Sferruzzi-Perri<sup>1</sup>**

*1. Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK*

During pregnancy, the fetus requires nutrients supplied by the mother to develop and grow. However, the mother also requires nutrients to maintain her health and help her to support the pregnancy and the subsequent lactation. Failure to appropriately regulate nutrient allocation between the mother and fetus can lead to pregnancy complications, such as abnormal fetal growth and gestational diabetes, with immediate and life-long consequences for maternal and offspring health. The placenta is the main determinant of materno-fetal resource allocation during pregnancy (Sferruzzi-Perri and Camm, 2016). It supplies all the nutrients and oxygen required for fetal growth and secretes hormones that facilitate maternal allocation of nutrients to the fetus. However, we lack information on how the placenta integrates the various signals in the mother and the fetus to modulate resource allocation during pregnancy and its importance for long-term health.

In this presentation I will describe our findings in mice that explore how the environment of the mother modulates placental phenotype and thus fetal resource supply and growth, via changes in the structure, function and metabolism of the placenta. I will also tell you about our genetic studies in mice that examine the significance of signals in the mother versus in the fetus in adapting placental resource allocation during pregnancy. By understanding the factors regulating placental phenotype and materno-fetal resource allocation, we hope to improve our understanding of the development of pregnancy complications and the subsequent increased risk of poor health in the child and mother after a complicated pregnancy. We also hope to identify targets in the placenta for therapeutic intervention in complicated pregnancies.

## Placental ex vivo model infection for pathogenesis studies of Congenital Cytomegalovirus

**William Rawlinson<sup>1, 2, 3, 4</sup>**

*1. Serology and Virology Division, NSW Health Pathology Microbiology, Prince of Wales Hospital, Sydney, NSW, Australia*

*2. School of Women's and Children's Health, University of New South Wales, Sydney, NSW, Australia*

*3. School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia*

*4. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia*

Congenital CMV infection (cCMV) affects ~350 Australian infants annually. It is extremely challenging clinically, particularly with lack of vaccine or effective, licensed pharmaceutical treatments, combined with practical and ethical problems studying fetal infection. Infection may cause fetal malformation and in severe cases fetal and neonatal death. Fetal injury may be caused directly by fetal infection with cell damage or indirectly by placental infection, inflammation in response to infection and fetal undernutrition.

We study how CMV dysregulates trophoblast function, at protein and molecular levels. Our studies are of viral-cellular protein interactions implicated in cCMV pathogenesis including i) cyclin-mediated complex interactions with viral proteins including vCDK pUL97 complexes, ii) DYRK (Dual specificity tyrosine kinases, protein family important in neuronal development) and CDK (cell cycle regulators) interactions with CMV proteins, iii) CMV interactions with dysregulation of the cell protein Wnt (a signalling pathway important in placental development).

We study these events using *ex vivo* first trimester TEV-1 trophoblast cells and *ex vivo* placental explants infected with laboratory-adapted (AD169) or genetically-intact (Merlin) CMV strains, or naturally infected clinical placentae. Infection causes accumulation and relocalisation of DYRK1A proteins to cell cytoplasm and cytoplasmic virion assembly complexes, with sequestration of DYRK1B to nuclear replication compartments. Western blots show this accumulation results from upregulated DYRK1A and DYRK1B protein expression, resulting from transcriptional upregulation. Treatment of TEV-1 and placental explants with novel DYRK inhibitors significantly inhibits HCMV replication, indicating these cellular kinases are essential during HCMV placental replication.

We also show CMV alters expression of receptor tyrosine kinase ROR2 to modulate Wnt5a-stimulated trophoblast migration in the non-canonical pathway. CMV infection increases expression of Wnt-binding receptor ROR2. Ectopic ROR2 expression reduces T-cell-specific (TCF)/lymphoid enhancer-binding factor (LEF)-mediated transcription and inhibits Wnt5a-induced trophoblast migration. Downregulation of ROR2 using siRNA duplexes rescues CMV-induced reduction in trophoblast migration. These data suggest CMV alters cell protein functions, with implications for placental development and function.

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## Combining *in vitro*, *in vivo* and *in silico* approaches to improve the detection of fetal growth restriction

**Jo James<sup>1</sup>**

1. *University of Auckland, Auckland, New Zealand*

Fetal growth restriction (FGR) affects 5-10% of all pregnancies and is one of the leading causes of stillbirth. Concerningly, we cannot predict which pregnancies will go on to develop FGR in early pregnancy, at the time the pathophysiology of the disorder is established, and in fact we fail to detect more than half of FGR babies prior to delivery. This means that we miss the opportunity to apply best clinical practice to manage these pregnancies, and cannot intervene with potential novel therapeutic approaches in early pregnancy when they may be most effective. The Pregnancy Modelling group at the University of Auckland is integrating novel computational and experimental approaches to create a 'virtual pregnancy' in which we can simulate blood flow and oxygen exchange, incorporating dynamic changes in vessel structure and reactivity throughout the materno-fetal circulation throughout gestation. The overall aim of this work is to determine which arteries matter most for adequate delivery of nutrients and oxygen to the baby, and to use this knowledge to improve our ability to predict FGR by focussing on what really matters. In this talk, Dr James will discuss how this work has led them to challenge current dogma that inadequate spiral artery remodelling is the dominant factor influencing the abnormal uterine artery Doppler waveforms and poor placental perfusion in FGR. Rather, their work has highlighted that inadequate outward remodelling of the larger uterine vessels, in particular the radial arteries, may play key roles in the pathogenesis of FGR. Current work to understand how radial artery remodelling is regulated, and the impact of impaired remodelling on placental exchange in FGR, is being used to inform clinical imaging strategies to incorporate radial artery assessment into novel algorithms to predict FGR in early pregnancy.

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## Developing novel devices to detect fetal distress

**Fiona Brownfoot<sup>1</sup>**

1. *Fetal Biomedical Engineering Group, Translational Obstetric Group Mercy Hospital for Women, University of Melbourne, Heidelberg, VIC, Australia*

### Introduction

Pregnancy and birth are among the most dangerous days in your life. Stillbirth tragically ends 3 million pregnancies globally every year whilst fetal asphyxia inflicted by labour is a leading cause of neonatal seizures, cerebral palsy and death. Unfortunately, current measures of fetal wellbeing during pregnancy and direct measures of fetal distress during labour are intermittent and often miss the critical point when a life-saving birth could be performed. Excitingly we are developing two devices to continuously measure markers of fetal distress in pregnancy and directly measure markers of fetal distress during labour.

### Methods

With a team of electronic, material and chemical engineers and physicists we are using novel flexible electronics and a suite of original algorithms to develop a device to detect markers of fetal well-being non-invasively in pregnancy. Furthermore, utilising cutting edge fibre optic and sensor technology we are developing a device to accurately measure direct markers of fetal distress during labour.

### Results

We have developed a suite of algorithms to extract the fetal electrocardiogram (ECG) and have found they more reliably extract the fetal heart rate at 84% of the time compared to traditional algorithms which only extract the fetal heart rate 40% of the time.

We have optimized the physics and chemistry of our sensor and demonstrated it accurately detects a marker of fetal distress in buffers. We have promising data showing it accurately detects markers of fetal distress in biological samples. Currently we are developing an application device and will assess its accuracy in a fetal sheep hypoxia model.

### Conclusion

We are developing technology to continuously assess fetal well-being in pregnancy and labour. These devices have the potential to detect fetal distress and subsequently reduce stillbirth and hypoxic complications of labour.

## Management of Osteoporosis in Underserved Populations

**Frances Milat<sup>1</sup>**

*1. Monash Health, Clayton, VIC, Australia*

To date, clinical research has focused on postmenopausal osteoporosis, while the aetiology and management of osteoporosis in young adults and chronic disease remain poorly understood. Individuals with chronic neurological conditions, premature ovarian insufficiency, transfusion-dependent haemoglobinopathies, diabetes mellitus, renal disease, malignancy and young hip fractures are particularly underserved by current literature. This lecture will explore recent developments in the management of the bone disease in several of these groups.

Premature ovarian insufficiency (POI), whereby menopause occurs before the age of 40, is a life-changing diagnosis with profound physical and psychological consequences. It affects approximately 1-2% of women. Osteoporosis is a well-established complication of POI, with a prevalence of 8-14%; bone loss of up to 26% at the lumbar spine compared with age-matched control populations has been reported. Small studies show that HRT can maintain and restore bone mineral density (BMD) in POI, but the optimal dose and type of oestrogen therapy is unknown. Our current work examines fracture prediction in POI as well as optimising management from a bone, muscle and cardio-metabolic perspective.

Improvements in transfusion medicine have significantly improved life expectancy for patients with thalassemia major. However, osteoporosis and fracture are one of the main causes of morbidity. Multiple factors are implicated in bone disease including marrow expansion, iron toxicity, endocrinopathies and more recently renal tubular dysfunction. Our discovery of accelerated bone loss, renal calculi and deferasirox-associated hypercalciuria in haemoglobinopathies provides a new pathogenic mechanism underlying bone loss in this cohort. Current work explores methods to minimise and manage hypercalciuria as well as fracture prediction in this cohort.

## Mice with myocyte-deletion of vitamin D receptor (VDR) have sarcopenia and impaired muscle function

**Jennifer Chen<sup>1</sup>, Christian Girgis<sup>2,3,1</sup>, Kuan M Cha<sup>1</sup>, Benjamin So<sup>1,4</sup>, Michael Tsang<sup>1</sup>, Peter J Houweling<sup>5,6</sup>, Aaron Schindeler<sup>2,7</sup>, Rebecca Stokes<sup>1</sup>, Michael M Swarbrick<sup>2,1</sup>, Frances J Evesson<sup>2,8</sup>, Sandra T Cooper<sup>2,8</sup>, Jenny E Gunton<sup>2,3,1</sup>**

*1. The Westmead Institute for Medical Research, University of Sydney, Westmead, NSW, Australia*

*2. Faculty of Health and Medicine, University of Sydney, Sydney, NSW, Australia*

*3. Department of Diabetes and Endocrinology, Westmead Hospital, Westmead, NSW, Australia*

*4. Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia*

*5. Murdoch Children's Research Institute, Melbourne, VIC, Australia*

*6. Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia*

*7. Orthopaedic Research & Biotechnology Unit, The Children's Hospital at Westmead, Westmead, NSW, Australia*

*8. Kids Neuroscience Centre, The Children's Hospital at Westmead, University of Sydney, Children's Medical Research Institute, Westmead, NSW, Australia*

Vitamin D deficiency has been linked to decreased muscle strength, and falls. Previous work on the whole-body vitamin D receptor knockout mouse model (VDRKO) found that these mice had reduced grip strength. Whether vitamin D has direct effects in muscle is very controversial.

In normal muscle, the VDR is present in very low levels. In this study, we used mice with myocyte-specific deletion of VDR (mVDR) to clarify whether vitamin D has a direct role in muscle function. Floxed VDR mice were interbred with human skeletal actin (HSA)-Cre mice to generate mVDR mice, and muscle physiology was assessed by grip strength tests and Promethion metabolic cages.

Compared to their floxed control siblings, mVDR mice had reduced grip strength (7-16% decrease,  $P=0.008$ ), voluntary wheel running distance (22% decrease,  $P=0.009$ ) and average wheel running speed. mVDR mice had significantly lower lean mass, measured by DEXA. At sacrifice, their muscles were significantly smaller. There were fewer, but larger myocytes in the quadriceps as well as increased proportions of small angular fibres with central nuclei indicating ongoing regeneration. The expression of endoplasmic reticulum genes which regulate calcium levels such as *Serca2b*, *Serca3*, and the cytosolic  $Ca^{2+}$  buffer *Calbindin-D28k*, both implicated in muscle relaxation, was reduced in mVDR mice. Downregulation of cell cycle progression genes such as *CyclinD1*, *CyclinD2*, *CyclinD3*, *Cdk2* and *Cdk4* may have contributed to the reduced muscle mass.

Together, our results suggest myocyte VDR deletion perturbs normal muscle function in mice. These findings demonstrate that vitamin D signalling has functional effects in muscle, where normal vitamin D signalling is important for maintaining muscle function and mass despite the low levels of VDR protein normally found.

## Effects of diet on human islet function

Heather Burns<sup>1</sup>, Rebecca Stokes<sup>1</sup>, Jennifer Chen<sup>1</sup>, Jenny E Gunton<sup>1, 2, 3, 4</sup>

1. Westmead Institute for Medical Research, Westmead, NSW, Australia

2. Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

3. St Vincent's Clinical School, University of New South Wales, Kensington, NSW, Australia

4. Department of Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

**INTRODUCTION:** While it is well known that obesity increases the risk of type 2 diabetes, the effect of specific diets on human islet function is difficult to determine. In this study, we wish to examine the effects of a high fat diet on human islet function after transplant.

**METHODS:** Human islets were transplanted into immunodeficient mice which had been made diabetic with alloxan prior to transplantation. Ten female Rag-1KO mice (on C57Bl/6 genetic background) were each transplanted with 2000IEQ human islets under the kidney capsule. Eight weeks after the transplant, mice with functioning grafts (n=8) were commenced on high-fat diet (HFD, 45% of calories from fat) or continued on normal chow diet (n=4 each). Glucose and insulin tolerance tests (GTT and ITT) were performed before and after diet and metabolic measurement of energy expenditure was performed using Promethion metabolic cages.

**RESULTS:** 80% of transplant recipients (8 of 10) were normoglycaemic after islet cell transplantation. Mice placed on a HFD gained significantly more weight over the following 8 weeks compared to their chow diet counterparts. HFD mice also had higher daily non-fasting BSL readings than mice on chow diet, with one HFD mouse exhibiting persistent hyperglycaemia (BSL>17mmol/L), however differences in daily random BGL were not significant for the most part. GTT results at 8 weeks post diet commencement showed a significantly greater deterioration in the HFD group compared to the chow group, with a tendency to higher fasting BGL, significantly higher BGL readings at 15 and 30 minutes, and a significant change in overall glucose tolerance (p=0.0022).

**CONCLUSION:** Prolonged exposure to HFD results in significant weight gain and impairment of islet function in human islets transplants into diabetic mice. The onset of dysglycaemia is slower than seen in C57Bl/6 mice.

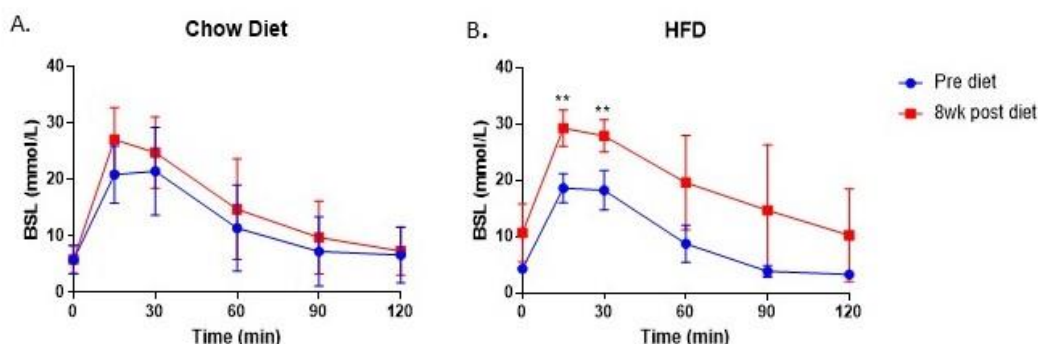


Figure 1. GTT results pre diet vs 8 weeks post diet commencement for mice on chow (A) and HFD (B). Significantly higher BSL readings observed in HFD group at 15 minutes and 30 minutes after glucose administered, \*\* p<0.01

## Changes in resting energy expenditure with different schedules of calorie restriction

Dr Brian T Corley<sup>2, 1</sup>, Christina Khouri<sup>2</sup>, Lorene Theaude<sup>2</sup>, Philippa Hawke<sup>2</sup>, Dr Rosemary M Hall<sup>2, 1</sup>, Prof. Mark Weatherall<sup>2</sup>, Prof. Jeremy D Krebs<sup>2, 1</sup>

1. Endocrinology & Diabetes, Capital and Coast DHB, Wellington, New Zealand

2. University of Otago, Wellington, NA, New Zealand

**Background:** Dietary calorie restriction reverses dysglycaemia in type 2 diabetes in proportion to the degree of calorie restriction. There has been much interest in whether the timing of calorie restriction provides additional benefit to such a diet. One possible mechanism is the attenuation of the drop in basal metabolic rate commonly seen within a few weeks of starting an energy restricted diet, a phenomenon known as adaptive thermogenesis. Adaptive thermogenesis drops the calorie requirements of a fasting individual below that expected for weight, thus reducing weight loss during a low-calorie diet.

**Aims:** To determine if intermittent fasting produces less adaptive thermogenesis compared with continuous daily restriction during a 6-week dietary intervention.

**Methods:** We conducted a randomised controlled trial in obese men of 79% daily restriction versus an intermittent fast consisting of two days of 25% restriction and 5 days of eucaloric intake per week over 6 weeks. Body composition, resting energy expenditure & anthropometry were measured at baseline 3 weeks and 6 weeks. Secondary outcomes were change in HbA1C, blood pressure, fasting lipids, leptin, ghrelin, adiponectin and thyroid function tests. A general linear mixed model (GLMM) was used for the primary outcome. ANCOVA was used for all secondary outcome variables.

**Results:** 32 men completed the diet intervention and lost approximately 1 kg per week. Intermittent energy restriction did not alter weight loss or attenuate adaptive thermogenesis. There was a trend toward fat mass reduction with intermittent fasting.

**Conclusion:** Intermittent fasting does not attenuate adaptive thermogenesis. A GLMM approach is suggested for studies of RCT's examining adaptive thermogenesis.

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## The human gut is a source of extra-pancreatic glucagon

Emily W Sun<sup>1</sup>, Richard L Young<sup>2,3</sup>, David A Wattchow<sup>4</sup>, Damien J Keating<sup>3,1</sup>

1. College of Medicine and Public Health, Flinders University, Adelaide, South Australia, Australia

2. Adelaide Medical School, University of Adelaide, South Australia

3. Nutrition and Metabolism, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia

4. Discipline of Surgery, Flinders University, Adelaide, South Australia, Australia

Publish consent withheld

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## Gut-derived serotonin is synthesised in sensory cells that influence peripheral metabolism

Alyce M Martin<sup>1</sup>, Julian M Yabut<sup>2</sup>, Amanda L Lumsden<sup>1</sup>, Richard L Young<sup>3</sup>, Jocelyn M Choo<sup>4,5</sup>, Amanda J Page<sup>3</sup>, Waliul I Khan<sup>2,6</sup>, Geraint B Rogers<sup>4,5</sup>, Gregory R Steinberg<sup>2,6,7</sup>, Damien J Keating<sup>1,3</sup>

1. Centre for Neuroscience and College of Medicine and Public Health, Flinders University, Adelaide, South Australia, Australia

2. Centre for Metabolism, Obesity & Diabetes Research, McMaster University, Hamilton, Ontario, Canada

3. Nutrition & Metabolism, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

4. Microbiome Research Laboratory, Flinders University, Bedford Park, SA, Australia

5. Microbiome & Host Health, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

6. Department of Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

7. Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada

Gut-derived 5-HT, produced by enterochromaffin (EC) cells within the mucosal lining of the gastrointestinal tract, is a known metabolic regulator and is augmented in human obesity and diabetes. However, very little is known about the cellular changes to EC cells which may contribute to the increase in plasma 5-HT observed under these conditions. The gut microbiome also has a substantial influence on host metabolism, however the mechanisms behind this are also not well understood. EC cells are important nutrient sensors within the gut and are influenced by the gut microbiome. We therefore aimed to (1) determine what changes to nutrient sensing occur in EC cells from obese humans and mice and (2) determine if the gut microbiome influences host metabolism via effects on gut-derived 5-HT.

We find that high fat diet (HFD) consumption alters EC cell nutrient sensing in a region-dependent manner. Specifically, broad sugar sensing by duodenal EC cells is reduced in primary EC cells from HFD mice, while only glucose sensing is affected in colonic EC cells. EC cell proliferation is increased 2-fold in obese humans and mice, and correlates with both fasting plasma glucose and circulating 5-HT. Furthermore, reduction of 5-HT synthesis and antibiotic-induced microbial dysbiosis independently improve glucose handling, but no additive effect of combining the two treatments is observed. This effect is not due to changes in energy expenditure, feeding behaviour or activity levels. This suggests that the gut microbiome acts through gut-derived 5-HT to influence host glucose handling and metabolism.

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## Comparison between liquid chromatography-mass spectrometry and immunoassay in the measurement of serum testosterone in adult men at PathWest, Sir Charles Gairdner Hospital

Sharon Lam<sup>1</sup>, Conchita Boyder<sup>2</sup>, Bronwyn Stuckey<sup>1,2,3</sup>, Ee Mun Lim<sup>1,2</sup>

1. Sir Charles Gairdner Hospital, East Perth, WA, Australia

2. Clinical Biochemistry, Pathwest, Perth, WA, Australia

3. Keogh Institute for Medical Research, Perth, WA, Australia

### Introduction

Androgen deficiency in adult men is characterised by clinical features of hypogonadism and confirmed by low morning serum total testosterone (T) [1]. Hence, accurate determination of T is essential.

Immunoassay technique (IAT) is used in T measurement as it is economical and provides rapid information. IAT can reliably measure T at higher levels, but is less accurate at lower levels, in infants, children and women. The ratio 10:1 of T:dihydrotestosterone (DHT) was established using radioimmunoassay (RIA).

Currently, mass spectrometry (LCMS) is preferred given better accuracy and less variability compared to IAT [ii] [iii] [iv]. However, there is operator and inter-method variability affecting precision and accuracy [v]. It is also labour intensive and therefore costlier with longer processing times.

Therefore, there is debate whether IAT is preferred in measuring T in adult men [vi].

#### Methods

Primary: Retrospective audit comparing T measured by LCMS and IAT in men >17 years (n=49), from February 2016 to August 2018.

Secondary: We compared T:DHT relationship using LCMS (n=81). Samples from men (n=8) on testosterone replacement were excluded.

#### Results

T measured using LCMS and IAT demonstrated good correlation ( $R^2=0.9758$ ). Even when  $T<10$  nmol/L, this relationship was maintained.

Relationship between LCMS T and DHT showed only 27% (n=22) had a ratio of approximately 10:1, with poor correlation ( $R^2=0.6932$ ).

#### Conclusion

Given good correlation between LCMS and IAT (even when  $T<10$  nmol/L), quicker processing and lower costs, IAT could be considered as first line method in measuring T in adult men. Relationship between T and DHT established using RIA was not observed with LCMS. Larger studies to further validate T measurement using IAT in adult males are recommended.

1. [i] (Frederick C.W. Wu 2010)
2. [ii] (Christina Wang 1 February 2004)
3. [iii] (Valérie Moal, Elisabeth Mathieu, Pascal Reynier, Yves Malthièry, Yves Gallois November–December 2007)
4. [iv] (Joëlle Taieb July 2003)
5. [v] (Yuesong Wang 2014)
6. [vi] (Huhtaniemi IT1 and Group. June 2012)

## An intrinsic gut melanocortin signalling complex regulates L-cell secretion in humans

**Emily W Sun<sup>1</sup>, Eva W Iepsen<sup>2,3</sup>, Nektaria Pezos<sup>4,5</sup>, Amanda L Lumsden<sup>1</sup>, Godrun Schober<sup>4,5</sup>, Alyce M Martin<sup>1</sup>, Nicole J Isaacs<sup>4,5</sup>, Chris Rayner<sup>4</sup>, Nam Nguyen<sup>4</sup>, Dayan De Fontgalland<sup>6</sup>, Philippa Rabbitt<sup>7</sup>, Paul Hollington<sup>6</sup>, David A Wattchow<sup>6</sup>, Torben Hansen<sup>8</sup>, Jens-Christian Holm<sup>2</sup>, Alice P Liou<sup>9</sup>, Margaret V Jackson<sup>9</sup>, Jens J Holst<sup>2,3</sup>, Signe S Torekov<sup>2,3</sup>, Richard L Young<sup>4,5</sup>, Damien J Keating<sup>5,1</sup>**

1. College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia
2. Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark
3. The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark
4. School of Medicine, Adelaide University, Adelaide, SA, Australia
5. Nutrition and Metabolism Theme, South Australia Health and Medical Research Institute (SAHMRI), Adelaide, South Australia, Australia
6. Department of Surgery, Flinders University, Bedford Park, SA, Australia
7. Department of Surgery, Flinders Medical Centre, Bedford Park, SA, Australia
8. Department of Endocrinology, Hvidovre University Hospital, Hvidovre, Denmark
9. Pfizer Worldwide Research and development, Cardiovascular and Metabolic diseases Research Unit, Boston, MA, USA

Publish consent withheld

## Iodine Deficiency and Iodine Excess: What are the Implications for Thyroid Health?

**Angela Leung<sup>1</sup>**

1. Division of Endocrinology, Diabetes, and Metabolism Department of Medicine David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA

Adequate iodine nutrition may be achieved from dietary intake of iodine-fortified foods, including iodized salt, or ingestion of iodine-containing multivitamins or supplements. Inadequate iodine has been associated with hypothyroidism, as well as adverse obstetric and developmental and cognitive outcomes among offspring born to women with insufficient iodine during pregnancy. Requirements for iodine intake are increased during pregnancy and lactation in order to provide adequate iodine nutrition for the developing fetus and breastfed infant.

However, excess iodine exposure also carries the risk of iodine-induced thyroid dysfunction, particularly among individuals with pre-existing thyroid disease, the elderly, and in the fetus and neonate, in whom thyroid gland development is still immature. Sources of excess iodine include use of iodine-containing medications, topical iodine antiseptics, and radiographic iodinated contrast media. Biochemical hypothyroidism or hyperthyroidism resulting from an acute iodine load may be transient or permanent, either subclinical or overt, and the source of the excess iodine might not be readily apparent. With the exception of specific medical indications supporting the use of supraphysiologic iodine, chronic excessive iodine ingestion and/or exposure should be avoided.

## Dissecting the contribution of androgen-mediated mechanisms in driving endocrine, reproductive and metabolic traits of polycystic ovary syndrome (PCOS)

**Kirsty Walters<sup>1</sup>**

1. Ovarian Biology Laboratory, School of Women's & Children's Health, University of New South Wales Sydney, Sydney, NSW, Australia

Polycystic ovary syndrome (PCOS) is a common endocrine disorder characterised by reproductive hormone dysregulation involving luteinising hormone (LH) hypersecretion and hyperandrogenism, as well as reduced fertility, due to ovulatory disturbance. In addition, women with PCOS are also predisposed to metabolic disturbances such as obesity, insulin resistance, and dyslipidemia, with an increased risk of cardiovascular disease and type 2 diabetes. Currently, as the origins of PCOS remain unknown, mechanism-based treatments are not feasible and management relies on the treatment of symptoms only. However, if the underlying mechanisms involved in the development of PCOS were uncovered then this would pave the way for the development of new interventions for PCOS. Hyperandrogenism is the most consistent PCOS characteristic, and as androgens mediate their actions via the androgen receptor, we have combined a hyperandrogenised PCOS mouse model with transgenic androgen receptor knockout mouse models to unravel the role of androgens in PCOS. These studies have revealed that androgen actions play an important role in mediating the development of PCOS, and have highlighted the importance of non-ovarian (neuroendocrine and adipose) androgen receptor-mediated androgen actions in the origins of PCOS. In particular, we identified that a specific loss of androgen receptor signalling in the brain protects hyperandrogenised PCOS mice against the development of key reproductive and metabolic PCOS characteristics. These findings support excess androgen receptor-mediated actions in the brain as a key mechanism underpinning the development of PCOS. Hence, our data strongly supports targeting androgen actions in the brain in the development of targeted pharmacological approaches. Collectively these findings provide new insights into how evidence-based interventions may be developed in the future to treat PCOS.

## The metabolic and developmental impact of murine embryo culture in a novel microfluidic device

**Paul McKeegan<sup>1</sup>, C Savva<sup>2</sup>, V Mancini<sup>2</sup>, F Colucci<sup>2</sup>, C Espinilla Aguilar<sup>1</sup>, H M Picton<sup>1</sup>, V Pensabene<sup>2</sup>**

1. Reproduction and Early Development Research Group, Discovery and Translational Science Department, Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, Leeds, UK

2. School of Electronic and Electrical Engineering, University of Leeds, Leeds, UK

Mammalian embryos are exquisitely sensitive to the *in vitro* culture environment, which must support cell division, metabolism, and genetic and epigenetic development. Microfluidic devices offer a mechanism to control this environment, potentially improving *in vitro* embryo development and quality. We report the optimisation and developmental impact of a recently developed polydimethylsiloxane microfluidic device for *in vitro* culture of murine embryos to the blastocyst stage<sup>1</sup>.

To test the impact of microfluidic culture on embryo developmental competence, cryopreserved C57BL/6N mouse zygotes (MRC Harwell, UK) were thawed and cultured in groups of 8-10 in 400nl chamber devices or control drops under oil (1µl media/embryo) at 37°C, 5%CO<sub>2</sub>/5%O<sub>2</sub>/90%N<sub>2</sub>. After 72h, embryos were removed to individual 4µl drops for 24h to profile glucose, pyruvate and lactate turnover<sup>2</sup>. Blastocysts were subsequently transferred to fibronectin-coated dishes for 72h to evaluate attachment and outgrowth<sup>3</sup>. To define the limitations of microfluidic culture, parallel groups of 10-40 2 cell mouse embryos were cultured to the blastocyst stage before metabolic profiling.

Microfluidic culture was non-embryotoxic and similar blastocyst formation, hatching, attachment and outgrowth rates were achieved between devices and controls (n=15/15, p>0.05). However, individual blastocyst pyruvate consumption reduced following microfluidic culture (8.4±0.6, n=139) vs controls (10.9±0.5pmol/embryo/hr, n=144, p<0.0001), while glucose consumption significantly increased in device blastocysts (7.2±0.6pmol/embryo/hr, n=139) vs controls (5.2±0.5pmol/embryo/hr, n=144, p=0.004). Energy substrate turnover did not predict blastocyst outgrowth capacity in either system.

Blastocyst hatching rate in devices significantly decreased with increased group size (40/group: 2.2±2% compared to 10/group: 30±4%, n=4, p=0.02). Embryos cultured in groups of 40 had significantly reduced pyruvate (0.37±0.1pmol/embryo/hr) and glucose consumption (0.05±0.03pmol/embryo/hr, n=3) than groups of 10 (1.4±0.08pmol/embryo/hr, and 0.8±0.08pmol/embryo/hr, n=3, respectively p=0.02).

Murine embryo developmental competence and metabolism were comparable between novel microfluidic device and conventional drop culture systems. Further validation of microfluidic culture efficacy will be provided through ongoing embryo transfer trials.

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## Novel insights into TGF $\beta$ superfamily regulation of FSH synthesis

**Daniel Bernard<sup>1</sup>**

1. Department of Pharmacology and Therapeutics Centre for Research in Reproduction and Development, McGill University Quebec, Quebec, Canada

The activins and inhibins are structurally-related members of transforming growth factor  $\beta$  (TGF $\beta$ ) superfamily that selectively stimulate or suppress follicle-stimulating hormone (FSH) synthesis in pituitary gonadotrope cells. According to current dogma, gonadotrope-derived activin B binds to and signals via complexes of activin type II and type I receptors to stimulate transcription of the FSH $\beta$  (*Fshb*) subunit gene. Gonadal inhibins are thought to antagonize activins, and thereby suppress FSH, by forming high affinity ternary complexes with activin type II receptors and the TGF $\beta$  type III receptor, betaglycan. Results from our lab challenge these concepts. First, activin B is a homodimer of inhibin  $\beta$ B subunits (product of the *Inhbb* gene). However, FSH levels are elevated or normal in global or gonadotrope-specific *Inhbb* knockout mice. Moreover, conditional deletion of the canonical activin type I receptor, ALK4, in gonadotropes does not affect FSH production *in vivo*. These (and other) results suggest that activin B is not an essential regulator of FSH production in mice. Second, FSH synthesis is unaltered in mice lacking betaglycan in their gonadotropes. When pituitaries of these mice are challenged with inhibin A or inhibin B, only inhibin A's suppressive actions on FSH production are impaired. These data indicate that betaglycan functions as an obligate inhibin A, but not inhibin B co-receptor in murine gonadotropes. In our ongoing research, we are trying to identify: 1) the TGF $\beta$  ligand that stimulates FSH production *in vivo*, and 2) the inhibin B co-receptor in gonadotropes.

## RUNX1 as a potential co-regulator of progesterone receptor in mouse peri-ovulatory granulosa cells

**Thao Dinh<sup>1</sup>, Barbara Nicol<sup>2</sup>, Karina Rodriguez<sup>2</sup>, Jimmy Breen<sup>1</sup>, Rebecca Robker<sup>1</sup>, Humphrey Yao<sup>2</sup>, Darryl Russell<sup>1</sup>**

1. Robinson Research Institute, Adelaide, South Australia, Australia

2. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Progesterone receptor (PGR), the transcription factor target for progesterone, is a pleiotropic regulator of reproductive functions and is the essential mediator of ovulation in the ovary. PGR interacts with target chromatin through the PGR response element (PRE). Recently our lab showed that PGR also exhibits unique interactions with non-canonical motifs in granulosa cells, implying the involvement of other co-regulators in PGR activities. Among the discovered motifs was RUNT, commonly recognised by the RUNX family including RUNX1. RUNX1 and PGR are known to interact with co-regulators – however the potential partnership between these transcription factors has never been shown. Here we propose RUNX1 to be a co-regulator of PGR by characterising the RUNX1 cistrome in mouse peri-ovulatory granulosa cells and demonstrating a striking interaction between PGR and RUNX1 that appears unique to granulosa cells. RUNX1 ChIP-seq identified more than 18000 RUNX1 binding sites, three-quarters of which were associated with transcriptionally active chromatin and highly enriched in proximal promoter regions. Motif analysis indicated an enrichment of non-canonical motifs at RUNX1 binding sites, including PRE and others previously identified in PGR ChIP-seq. RUNX1 and PGR chromatin binding patterns showed a remarkably high level of correlation and that the promoter binding preference of each depends on an interaction between the two transcription factors. Proximity ligation assay in granulosa cells was used to demonstrate the presence of RUNX1 in the transcription complex involving PGR. These are the first cistromic characterisation of transcriptional control in granulosa cells and in the ovulation process and the first characterisation of PGR and RUNX1 cooperative chromatin binding. Our results suggest an interplay between PGR and RUNX1, likely through the tethering of PGR to target promoters via RUNX1. This provides a novel understanding in the molecular mechanism behind ovulation and has implications for contraceptives and specific reproductive cancer therapeutics.

## Regulation of nuclear gene expression by mitochondria in oocyte

**Deepak Adhikari<sup>1</sup>, Inwon Lee<sup>1</sup>, Jun Liu<sup>1</sup>, Usama Alzubaidi<sup>1</sup>, Qing-hua Zhang<sup>1</sup>, Wai Shan Yuen<sup>1</sup>, Rebecca Robker<sup>2</sup>, John Carroll<sup>1</sup>**

1. Department of Anatomy and Developmental Biology and Development and Stem Cells Program, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

2. The Robinson Research Institute, School of Medicine, The University of Adelaide, Australia 5005, Adelaide

Mitochondrial biogenesis occurs throughout oocyte growth by Mitochondrial DNA (mtDNA) replication and mitochondrial division, during which the number of mitochondria increases from about 1000 to up to 500,000. mtDNA replication is driven by upregulation of Transcription Factor A, Mitochondrial (TFAM). The aim of this study is to address the role of mtDNA proliferation on mitochondrial function in oocytes and embryos.

To investigate this, we have generated oocyte-specific TFAM KO mice and studied oogenesis and embryo development. We find that TFAM KO oocytes have greatly reduced levels of mtDNA ( $12369 \pm 718$ ,  $n = 11$ ) than control ( $114398 \pm 7602$ ,  $n = 11$ ) oocytes but surprisingly similar levels of mitochondrial mass (control  $41.4 \pm 2$ ,  $n = 7$  Vs mutant  $46.5 \pm 2.5$ ,  $n = 12$ ). Mutant oocyte morphology was apparently normal and no difference in the levels of ATP were found. Upon fertilization with WT sperm, embryo development to the blastocyst stage was not affected. However, breeding experiments reveal that no offspring were derived from the KO oocytes due to post-implantation loss around mid-gestation despite these embryos recovered normal

TFAM protein expression from WT paternal allele. We found that nuclear gene expression in TFAM KO oocytes was disrupted suggesting effects of mitochondrial biogenesis on oocyte nuclear regulation.

In conclusion, these studies show that inhibition of mtDNA replication specifically during oocyte growth results in embryo loss after implantation. Mitochondrial function in oocytes is clearly extending beyond normal role of 'power supply' for the purposes of maintaining oocyte function.

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### Nicotinamide mononucleotide alleviates hepatic steatosis and insulin resistance in the DHT-induced PCOS mice model.

**Ali Aflatounian<sup>1</sup>, Melissa C Edwards<sup>1,2</sup>, Valentina Rodriguez Paris<sup>1</sup>, Michael J Bertoldo<sup>1</sup>, William L Ledger<sup>1</sup>, Lindsay E Wu<sup>1</sup>, Robert B Gilchrist<sup>1</sup>, Kirsty A Walters<sup>1,2</sup>**

1. School of Women's & Children's Health, University of New South Wales, Sydney, NSW, Australia

2. ANZAC Research Institute, University of Sydney, Sydney, NSW, Australia

Polycystic ovary syndrome (PCOS) is a common and complex endocrine disorder. Apart from the characteristic reproductive and endocrine traits, PCOS patients also suffer from metabolic features including obesity, insulin resistance, liver steatosis and type 2 diabetes. Although insulin sensitizer agents such as metformin are commonly administered to ameliorate PCOS metabolic traits, the beneficial effects of metformin are still questionable. Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) plays a key role in energy metabolism. Moreover, animal and human studies have shown that NAD<sup>+</sup> precursors can have beneficial effects on insulin resistance and liver damage. Therefore, we aimed to assess the efficacy of nicotinamide mononucleotide (NMN), a precursor of NAD<sup>+</sup>, in treating features of PCOS in a dihydrotestosterone (DHT)-induced PCOS mice model. Prepubertal mice were implanted s.c with blank (n=14) or DHT (n=14) implants. After 12 weeks, control and PCOS mice (8/group) were treated with NMN in drinking water while the remaining mice received normal water (NW). All mice were collected 8 weeks after administration of NMN/NW. NMN treatment had no effect on reproductive traits of PCOS. However, oil red O absorption, a marker of liver steatosis, was significantly lower in NMN- versus NW-treated PCOS mice (PCOS+NW: 13.4±2.3; PCOS+NMN: 2.6±1.7; P<0.01). Fasting insulin levels and homeostatic model assessment of insulin resistance (HOMA-IR) were also decreased in PCOS+NMN mice compared to PCOS+NW mice (fasting insulin levels: PCOS+NW, 0.85±0.1ng/mL; PCOS+NMN, 0.52±0.1ng/mL; P<0.05. HOMA-IR: PCOS+NW, 10.6±1.9; PCOS+NMN, 6.9±0.5; P<0.05). While there was no significant difference in body weights, inguinal fat pad weights of PCOS+NMN mice were significantly decreased (PCOS+NW: 17.1±1mg/BW; PCOS+NMN: 12.7±1mg/BW; P<0.001). These findings suggest that boosting NAD<sup>+</sup> may represent a novel therapeutic strategy to target metabolic features of PCOS.

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### Proteomic and functional analysis of the human fallopian tube matrisome

**Yazmin Brown<sup>1</sup>, Muhammad B. Jamaluddin<sup>1</sup>, Arnab Ghosh<sup>1</sup>, Pradeep S. Tanwar<sup>1</sup>**

1. School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW, Australia

Publish consent withheld

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### Sperm cryopreservation prior to gonadotoxic treatment: experience of a single academic centre over 4 decades

**Nandini Shankara Narayana<sup>1,2</sup>, Irene Di Pierro<sup>1</sup>, Carolyn Fennell<sup>1</sup>, Lam P Ly<sup>1,2</sup>, Fay Bacha<sup>1</sup>, Ljubica Vrga<sup>1</sup>, Sasha Savkovic<sup>1</sup>, Leo Turner<sup>1</sup>, Veena Jayadev<sup>1</sup>, Ann J Conway<sup>1,2</sup>, David J Handelsman<sup>1,2</sup>**

1. Andrology Department, Concord Hospital, Hospital Road, Concord Hospital, NSW 2139

2. ANZAC Research Institute, University of Sydney

**Background:** Gonadotoxic treatment for cancer or non-cancer diseases damages spermatogenesis and impairs male fertility whereas pre-treatment sperm cryopreservation can preserve future male fertility. Sperm cryopreservation is an established technique to preserve male fertility prior to gonadotoxic treatment.

**Methods:** Clinical, anthropometric, semen analysis and hormonal data were analysed from 1978-2017 involving 2717 men comprising 2085 men with cancer, 234 non-cancer disease and 398 healthy controls to define sperm output by diseases, the feasibility of sperm cryostorage notably for adolescents, regional access to an urban cryostorage facility, the determinants of sperm output and time-dependent disposal of cryostored sperm. Semen samples were assessed by contemporaneous WHO methods.

**Findings:** Of 2085 men with cancer, 904 (43%) had haematological malignancies, 680 (33%) testicular cancers and 136 (6.5%) were adolescents. Most men (89%) and adolescents (80%) could collect sperm. Sperm output for all cancers and non-cancer diseases was lower than controls. Sperm output correlated positively with total testicular volume (r=0.44, p<0.0001) and negatively with serum FSH and LH (r=-0.24, -0.12 respectively, both p<0.0001) but not testosterone. For all stored samples, the median time in cryostorage was 8.5 years, 7% were transferred for use to induce pregnancy (median time 2.5 years) and 62.2% were discarded as no longer needed (return of fertility, 35.9% median 3.5 years; death, 26.3%, median 6.5 years), the high disposal rate reflecting regular annual follow-up to establish ongoing need for continued cryostorage. Cryostorage facilities are not available in remote and rural areas of the State and the proportion of outer regional and remote area residents cryostoring sperm was only about half that compared with urban residents.

**Conclusion:** Sperm cryostorage is feasible for virtually all men, including sufficiently mature adolescents, who can collect semen to insure future paternity as well as making positive psychological preparation for the patient's survival. Disposal is efficient with regular follow-up. Sperm cryopreservation should be an integral part of comprehensive treatment plan in men receiving gonadotoxic treatment but remains underutilised.

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## Treatment of advanced thyroid cancers

### Hang-Seok Chang<sup>1</sup>

1. *Department of Surgery, Yonsei University College of Medicine, Gangnam-gu, SEOUL, South Korea*

Thyroid cancer is an indolent disease, and usually present as a limited disease in neck. Invasion to vital organs is rarely observed, but even in well-differentiated thyroid cancers, it can be a major cause of mortality of thyroid cancer. The common sites of local invasion are strap muscle, recurrent laryngeal nerve, laryngo-tracheal tree, esophagus, and great vessels in lateral compartment and mediastinum. Uncontrolled invasion to vital organs in neck and mediastinum can cause significant morbidity, affect the quality of life, and finally affect the survival. Limited involvement of the aero-digestive tract can be controlled by conservative surgical treatments such as shaving-off procedures, otherwise, radical resection and following reconstructive procedures is the best choice in this setting. In planning the treatment, the risk-benefit ratio should be carefully evaluated to reduce the morbidity, and also achieve maximal therapeutic effects. Postoperative adjuvant therapies have been the matter of controversy, but there is a general consensus, especially for the high-risk patients, exists that radioiodine(RI) therapy and TSH suppression after radical resection are beneficial. The benefit of external beam radiation therapy is unclear, but it should be considered in the patients with microscopic residual disease. In cases of refractory to RI, we should consider the targeted therapies, but there should be more elaborate approach considering the special status of each patient. Precision medicine can play an important role. In conclusion, radical eradication of lesion followed by proper adjuvant therapy is the treatment of choice for locally advanced thyroid cancers.

**Keywords:** locally advanced thyroid cancers, refractory thyroid cancers, molecular targeted therapy, precision medicine

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## Active surveillance for papillary thyroid microcarcinoma

### Tomohiko Nakamura<sup>1</sup>, Yasuhiro Ito<sup>1</sup>, Akira Miyauchi<sup>1</sup>

1. *Kuma Hospital, Chuo-Ku, Kobe, HYOGO, Japan*

In many countries, the incidence of thyroid cancer, especially small papillary carcinoma, increased without increase in thyroid cancer mortality during the recent 3 decades. Thus, how to treat small papillary carcinoma became a big clinical issue. In 1993, active surveillance (AS) for low-risk papillary thyroid microcarcinomas (PTMCs) was initiated by Akira Miyauchi at Kuma Hospital under the hypothesis that most low-risk PTMCs do not grow and it is not too late to treat PTMCs after seeing progression signs such as size enlargement and novel appearance of nodal metastases.

At 10 years of AS only 8.0% of patients showed enlargement of  $\geq 3$  mm, while only 3.8% showed nodal metastasis. None of the patients, including those who underwent rescue surgery after the detection of progression, showed life-threatening recurrence or died of thyroid carcinoma. Adverse events such as vocal cord paralysis and hypoparathyroidism were significantly more frequent in patients who underwent immediate surgery than in those who had AS. In addition, the total medical cost of immediate surgery with postoperative management for 10 years was 4.1 times the total cost of AS for 10 years.

Patients' point of view or emotional issue on AS is also important. Our questionnaire survey on the patients on AS at Kuma Hospital revealed that 37% of patients had some cancer worry, which decrease over time and that 83% of patients replied that choosing AS was the best decision for them personally.

Therefore, currently at Kuma Hospital, we recommend AS as the first-line management for the patients with low-risk PTMCs. AS of low-risk PTMCs was approved in JAES guidelines in 2010, in its revised version in 2018 and ATA guidelines in 2015.

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## Management of Medullary Thyroid Cancer in 2019 and beyond

### Bruce Robinson<sup>1</sup>

1. *The University of Sydney, Sydney, NSW, Australia*

Management of medullary thyroid carcinoma has been revolutionised by the advent of *RET* receptor tyrosine kinase inhibitors. The results of trials using these agents will be discussed.

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## A novel point of vulnerability in male development

### Josephine Bowles<sup>1</sup>, Allen Feng<sup>1</sup>, Andrew Civitarese<sup>1</sup>, Cassy Spiller<sup>1</sup>, Peter Koopman<sup>1</sup>

1. *School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia*

Disorders of human male reproductive health are common and growing in prevalence. These include conditions that present at birth, such as cryptorchidism (undescended testes) and hypospadias (malformed penis), as well as problems that arise in

young adulthood, such as infertility/subfertility, testicular germ cell cancer (TGCC) and primary hypogonadism. The 'testis dysgenesis syndrome' (TDS) hypothesis proposes that these problems have a shared origin during fetal life: if the testis does not develop appropriately during a critical window of time, whilst in the womb, then both somatic and germ cell function can be affected with ramifications into adult life. Abnormalities of human sexual development ('disorders of sex development' or DSD), including gonadal and genital, are noted in about 1 in 200 to 1 in 300 newborns. In XY individuals, these range from mild hypospadias to complete male-to-female sex reversal.

Working in the mouse model, we have found a novel mechanism that is active during fetal life and is essential for correct development of all three major cell types present in the fetal testis. In brief, we find that a P450 enzyme, CYP26B1, must be induced at the onset of testis determination and that, in its absence, endogenous retinoic acid (RA) is not degraded leading to abnormal function of Sertoli cells (which orchestrate testis development), fetal Leydig cells (which produce steroid and peptide hormones) and germ cells (sperm precursors). Hence, we have now revealed a simple yet critical mechanism that impacts on fetal testis development as a whole. We hypothesise that this mechanism may be vulnerable to the effects of environmental chemicals.

## Disorders of Sex Development in Adolescence

### Jenny Batch<sup>1,2</sup>

1. Department of Paediatrics, School of Medicine, University of Queensland, Brisbane, QLD, Australia

2. Queensland Children's Hospital, South Brisbane, QLD, Australia

Disorders of sex development (DSD) encompass a group of congenital conditions associated with atypical development of internal and external genital structures.

Affected individuals often present at birth due to ambiguity of the external genitalia. Others may present with postnatal virilisation, delayed/absent puberty, primary amenorrhoea or infertility.

Adolescents may typically present with a suspected DSD in three ways

1. A girl with primary amenorrhoea (+ breast development)
2. A girl who virilises at puberty
3. A boy with pubertal delay

Diagnosis of a DSD in adolescence requires initial rapport building with both the young person and family and involvement of members of the multidisciplinary team including paediatric endocrinologist, paediatric surgeon/gynaecologist, clinical psychologist/psychiatrist as needed. Discussion with families and young people need to occur on multiple occasions to allow the MDT and the family to develop a shared understanding of investigations, results, diagnosis, treatments and the value of ongoing psychological support.

Physical examination is essential to define external genital anatomy and presence/absence of gonads. Delineation of internal genitalia is best done either radiologically (ultrasound and/or MRI) or under anaesthesia at the time of laparoscopy and gonadal biopsy. Investigations may include karyotype (or SNP array), endocrine testing both baseline and dynamic, antimüllerian hormone, ultrasound/MRI and genetic studies. Exploratory laparoscopy and gonadal biopsy may be needed to confirm internal anatomy and determine gonadal status/presence of gonadal malignancy.

Management issues include pubertal induction and longer-term hormone replacement therapy, gonadectomy and possible gonadal malignancy. Decisions regarding gonadectomy should include members of the MDT including Clinical Ethics if available with recommendations discussed with the young person and family. Discussion regarding future fertility options requires sensitivity. Psychological support and transition to appropriate adult services are essential to ensure good long-term outcomes.

## Clinical and ethical challenges of gonadectomy in children and adolescents with DSD – for whom, when, why (and why not)?

### Michele O'Connell<sup>1</sup>

1. The Royal Children's Hospital, Melbourne, Melbourne, VIC, Australia

The past decade has seen a significant shift in the clinical landscape of management of Differences of Sex Development (DSD). Gonadectomy, particularly in the paediatric age group, is one of the more potentially contentious surgical interventions that may be considered for a given individual. Historically, prophylactic gonadectomy has been recommended for a number of underlying variations due to an increased risk of malignant change in dysgenetic or intra-abdominal gonads; its consideration was also advocated where sex of rearing differs from biological sex in some 46XY DSD. In recent years, new information gleaned from both lived experience as well as improved genetic diagnoses has called these criteria into question and uncertainties as to the need for and optimal timing of gonadectomy remain for many DSD. In tandem with this, intersex

advocacy groups and human rights agencies have been increasingly vocal in their criticism of many surgical interventions in children too young to give their own consent.

Assessing what is 'medically necessary' and ethically appropriate for an individual at a given timepoint can be difficult. At the Royal Children's Hospital Melbourne, complex management decisions for children and adolescents with DSD are made following multiple clinical reviews and multidisciplinary team discussion. This presentation will review our MDT clinical approach and decision-making process in relation to gonadectomy, with reference to clinical cases to highlight the clinical and ethical challenges involved. Trends in gonadectomy for children with DSD at our institution in recent years and the uncertainties and unknowns relating to both conservative management and active intervention for different variations will also be discussed.

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## Human sex reversal is caused by duplication or deletion of core enhancers upstream of SOX9

**Andrew H Sinclair**<sup>1, 2</sup>

1. Murdoch Childrens Research Institute, Parkville, Vic, Australia

2. Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

Disorders of sex development (DSDs) are conditions affecting development of the gonads or genitalia. Until recently only 13% of DSD patients received a diagnosis. Using genomic sequencing approaches we have improved diagnostic rates for DSD to 43%. However, nearly 60% of patients still lack a diagnosis. Consequently, we explored whether disruption of the regulatory regions of gonad genes may account for a significant proportion of these undiagnosed patients.

Variants in two key genes, *SRY* and its target *SOX9*, are an established cause of 46,XY DSD, but the genetic basis of many DSDs remains unknown. *SRY*-mediated *SOX9* upregulation in the early gonad is crucial for testis development, yet the regulatory elements underlying this have not been identified in humans. We analysed copy number variations (CNVs) in the upstream regulatory region of *SOX9* in DNA from patients with DSD, allowing us to define several minimal critical regions for sex-reversal. We redefined the upstream regulatory landscape of human *SOX9*. Using new patient data, we refined the 32.5 kb *XYSR* and 24 kb *RevSex* intervals and analysed these genomic regions using bioinformatic and luciferase tiling approaches, to identify three putative enhancers 5' of *SOX9*. In cell-based reporter assays these enhancers responded to different combinations of testis-specific regulators including *SRY*, *SF1* and *SOX9* itself. When combined, all three enhancers show synergistic activity, significantly increasing their individual enhancer activity. *In vivo*, deletion of these three enhancers in mice resulted in different outcomes ranging from: no apparent effect to reduced *Sox9* transcription and complete sex reversal.

This is the first study to identify *SOX9* enhancers that, when duplicated or deleted, result in 46,XX or 46,XY sex reversal, respectively. These enhancers provide a hitherto missing link by which *SRY* activates *SOX9* initiation, upregulation and maintenance in humans, and establish *SOX9* enhancer mutations as a significant cause of DSD.

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## Tissue specific regulation of gene expression in endometrium and association with reproductive pathologies

**Sally Mortlock**<sup>1</sup>, **Raden I. Kendarsari**<sup>1</sup>, **Jenny N. Fung**<sup>1</sup>, **Fei Yang**<sup>1</sup>, **Restuadi Restuadi**<sup>1</sup>, **Jane E. Girling**<sup>2</sup>, **Sarah J. Holdsworth-Carson**<sup>3</sup>, **Wan Tinn Teh**<sup>3</sup>, **Samuel W. Lukowski**<sup>1</sup>, **Martin Healey**<sup>3</sup>, **Ting Qi**<sup>1</sup>, **Peter A.W. Rogers**<sup>3</sup>, **Jian Yang**<sup>1</sup>, **Brett McKinnon**<sup>1</sup>, **Grant W. Montgomery**<sup>1</sup>

1. University of Queensland, Brisbane, Australia

2. The University of Otago, Dunedin, New Zealand

3. University of Melbourne, Melbourne, Australia

Human endometrium is a highly specialised tissue lining the inside of the uterus and is essential for female reproduction. Understanding genetic regulatory mechanisms shared between, and specific to, tissue types can aid identification of target genes relevant to disease pathways. Endometrium is considered a source of cells initiating lesions in endometriosis which occurs when tissue, similar to the endometrium, forms lesions outside the uterus. The disease affects 10% of reproductive aged women and costs the Australian economy \$7.4 billion annually. Genome-wide association analyses have identified 27 endometriosis risk loci. However, specific gene targets and genetic mechanisms behind the disease remain to be identified.

To better understand tissue specific genomic regulation in endometrium we analysed RNA-sequencing data from 206 endometrial samples identifying novel effects of genetic variation on gene expression in endometrium and compared these with datasets in other tissues. A total of 444 sentinel *cis*-eQTLs ( $P < 2.57 \times 10^{-9}$ ) were detected including 327 novel *cis*-eQTLs that have not been reported in endometrium previously. A large proportion (85%) of endometrial eQTLs were shared between tissues within the GTEx consortium and with the eQTLGen blood dataset. Genetic effects on gene expression in endometrium were highly correlated with reproductive (eg. uterus, ovary, vagina) and digestive tissues (eg. salivary gland, stomach), supporting evidence that genetic regulation of gene expression is shared between biologically similar tissues and cell types. Tissue enrichment analysis using endometriosis GWAS summary statistics showed that reproductive tissues were significantly enriched for expression of genes in endometriosis risk loci. Summary-data-based Mendelian Randomisation analyses identified putative functional genes associated with reproductive traits and diseases including endometriosis.

We identify strong genetic effects on transcription in endometrium. The results can be applied together with publicly available datasets to identify targets for endometrium-related traits and pathologies.

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## Genetics of Mayer-Rokitansky-Kuster-Hauser syndrome: role of Hnf1b in Mullerian duct development

**Emanuele Pelosi<sup>1</sup>, Katie Ayers<sup>2</sup>, Enya Longmuss<sup>1</sup>, Gorjana Robevska<sup>2</sup>, Jocelyn van den Bergen<sup>2</sup>, Ella Thomson<sup>1</sup>, Rebecca Deans<sup>3</sup>, Andrew Sinclair<sup>2</sup>, Peter Koopman<sup>1</sup>**

1. Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia
2. Murdoch Children's Research Institute, Melbourne, Victoria, Australia
3. University of New South Wales, Sydney, New South Wales, Australia

Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome results from the incomplete development of the embryonic Müllerian ducts, which give rise to the female reproductive tract including oviducts, uterus, and upper third of the vagina. MRKH syndrome affects 1 in 4500 women and accounts for 10% of primary amenorrhea cases. Type I MRKH is characterized by the absence of a fully developed uterus and vagina without any associated malformations. Type II MRKH is syndromic, and features additional malformations, mostly in the renal, skeletal, and cardiovascular systems. To date, the aetiology of MRKH syndrome remains unexplained due to the lack of: 1) genome-wide approaches on large patient cohorts, and 2) functional analyses using specific mouse models. Thus, we established a recruiting program for women affected by MRKH and through their samples, we identified novel candidate genes using a combination of microarray and whole exome sequencing. Network analysis of selected variants led us to focus on Hnf1b, a transcription factor expressed in the epithelial compartment of the urogenital system, but with unknown function during Müllerian duct development. We ablated Hnf1b specifically in the epithelial cells of the Müllerian ducts by crossing a mouse line harboring a floxed Hnf1b gene (Hnf1b<sup>fl/fl</sup>) with a Wnt7a<sup>Cre</sup> mouse strain. Hnf1b<sup>fl/fl</sup>;Wnt7a<sup>Cre/+</sup> mice displayed a shorter uterus and uterine hypoplasia similar to MRKH. Additionally, 15% of Hnf1b mutant mice displayed additional malformations, including unilateral kidney agenesis and skeletal abnormalities providing the first model of MRKH type II. Gene expression analysis revealed dysregulation of the Wnt pathway suggesting its importance in the development of the Müllerian ducts. In conclusion, we developed a novel strategy to identify and functionally validate genetic variants associated with MRKH syndrome. Our findings suggest that Hnf1b is critical in Müllerian duct development and a significant causative factor for MRKH syndrome.

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## Oestrogenic actions on the endometrium are mediated through $\beta$ -catenin

**Isabella G Moore<sup>1</sup>, Claire I Richards<sup>1</sup>, Arnab Ghosh<sup>1</sup>, Pradeep S Tanwar<sup>1</sup>**

1. The University of Newcastle, Callaghan, NSW, Australia
- Publish consent withheld

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## Quantification and clonogenicity of stem/progenitor cells in menstrual fluid from healthy women

**Caroline E Gargett<sup>1,2</sup>, Sophie Suke<sup>1</sup>, Caitlin Filby<sup>1,3</sup>, Jemma Evans<sup>4</sup>**

1. Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia
2. The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Victoria, Australia
3. The Ritchie Centre, Hudson Institute of Medical Research, Clayton, VIC, Australia
4. Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, Victoria, Australia

Shedding and regeneration of the endometrium is critical for embryo implantation. Disordered shedding (endometriosis, adenomyosis) and/or regeneration (Asherman's Syndrome) is associated with debilitating menstrual disorders. Endometrial stem/progenitor cells, likely responsible for endometrial regeneration, are SUSD2+ mesenchymal stem cells (eMSCs) and NCAD+ epithelial progenitors (eEPC). NCAD+ eEPC form gland-like structures in organoid cultures, self-renew and are located in the bases of endometrial glands. SUSD2+ cells are perivascular cells in the endometrium. SUSD2+ eMSC and NCAD+ eEPC are shed during menstruation in women with/without endometriosis (unpublished). Our aim was to determine if there are biological variations in endometrial stem/progenitor cell concentrations and clonogenicity in menstrual fluid between menstrual cycles in normal women and between women.

Menstrual fluid (day 2) was collected from healthy women (not on hormones; regular cycles) in a menstrual cup. Endometrial cells were dissociated with enzymes, leukocytes depleted by CD45 magnetic beads and red blood cells by hypotonic lysis. The %SUSD2+ and %NCAD+ cells were determined by flow cytometry and clonogenicity by colony forming assays (cell seeding, 50/cm<sup>2</sup>).

Menstrual fluid was collected from 3 women over 3 cycles. SUSD2+ cells were present in all menstrual fluids (1.0-7.1% of CD45<sup>-</sup> cells; median 5.4%). NCAD+ cells were detected in 5 of 7 samples (0-3.0% of CD45<sup>-</sup> cells; median 0.6%) with no variation between cycles or participants (p=0.4). The clonogenicity of CD45<sup>-</sup> endometrial cells (3.9% - 17.8%) was also similar across cycles and between women.

In this pilot study, there was a lower frequency of NCAD+ cells compared to SUSD2+ cells in menstrual fluid as expected from their location. Further samples are required to fully determine the variability of endometrial stem/progenitor cells shed between women and between cycles of individual women. Quantification of endometrial stem/progenitor cells in menstrual blood may be a simple, non-invasive method for prognosis of fertility and endometrial disorders.

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## Big GIGnorous TERrific Placenta Data

**Claire Roberts<sup>1,2</sup>, Melanie Smith<sup>1,2</sup>, Jimmy Breen<sup>1,3</sup>, Qianhui Wan<sup>1,2</sup>, K Justin Bogias<sup>1,2</sup>, Dale McAninch<sup>1,2</sup>, Tanja Jankovic-Karasoulos<sup>1,2</sup>, Dylan McCullough<sup>1,2</sup>, Shalem Leemaqz<sup>1,2</sup>, Gus Dekker<sup>1,2</sup>, Tina Bianco-Miotto<sup>1,4</sup>**

1. Robinson Institute, University of Adelaide, Adelaide, Australia

2. Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

3. Bioinformatics Hub, University of Adelaide, Adelaide, SA, Australia

4. School of Agriculture Food and Wine, University of Adelaide, Adelaide, SA, Australia

We now live in exciting times for placenta research. Technological innovations in recent years are enabling unprecedented acquisition of data at an increasingly affordable price. We are assessing placental differentiation, growth and function across gestation in health and disease using multi-omics and reflecting those in matched maternal blood samples obtained at the same time as placenta is accessed or in serial samples across gestation. To date we have undertaken miR-seq, RNA-seq, Illumina DNA EPIC Array analyses in placenta, and miR-seq in maternal plasma and Illumina DNA EPIC arrays in maternal circulating leukocytes for 288 pregnancies. The volume of data is at the terabyte scale. For example, three omics in 96 placenta samples from 6-23<sup>+6</sup> weeks of gestation obtained with consent from elective terminations of pregnancy result in about 500GB of data. Add to that two omics in maternal blood and data from another 192 preterm and term pregnancies and we have a dataset that requires High Performance Computing, multiple bioinformaticians and a biostatistician to enable integration and accurate bio-inference of these data with reference to clinical and lifestyle data and pregnancy and birth outcomes.

Three-omic analysis of the first 96 placenta samples shows that differential expression of coding and non-coding RNAs occurs simultaneously with that of micro RNAs and DNA methylation demonstrating epigenetic regulation of expression. These are changing dynamically week by week, trimester by trimester, before and after maternal blood begins to flow into the placenta at 10 weeks' gestation, in a fetal sex specific fashion, and in response to environmental exposures such as maternal obesity and smoking. In time, non-invasive screening of placental, hence fetal, health in real time will become a reality and may be used to identify and monitor pregnancies at risk.

Supported by NIH NICHD R01 HD089685-01 Maternal molecular profiles reflect placental function and development across gestation PI Roberts

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## New insights into ovarian development and function

**Julia Kaburaki<sup>1</sup>, Pascal Bernard<sup>2</sup>, Stefan Bagheri-Fam<sup>1</sup>, Renata Phyland<sup>1</sup>, Jeremy Le<sup>1</sup>, JoAnne S Richards<sup>3</sup>, Andrew J Pask<sup>2</sup>, Dagmar Wilhelm<sup>1</sup>**

1. Department of Anatomy & Neuroscience, University of Melbourne, Melbourne, VIC, Australia

2. School of BioSciences, University of Melbourne, Melbourne, VIC, Australia

3. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

Normal development of the ovaries during embryogenesis is critical for their function and fertility of the adult female. The WNT4/RSPO1 signalling pathway is critical for normal ovarian development and function. Recently, it has been shown that the prorenin receptor (PRR), encoded by the *Atp6ap2* gene, functions as a bridge between the WNT receptor LRP6 and the vacuolar H<sup>+</sup>-ATPase (V-ATPase). This interaction is crucial for canonical WNT signalling after binding of the ligand. These data, together with our observation that *Atp6ap2* is highly expressed in the developing gonads, led to our hypothesis that PRR is important for ovarian development. In addition to the regulation of canonical WNT signalling PRR is best known for its role in the renin-angiotensin system. In addition, PRR is also important for other signalling pathways that play a role in ovarian development and/or function such as non-canonical WNT signalling and the ERK and p38-MAPK signalling pathways, demonstrating that PRR is a multi-functional protein. Hence, it is not surprising that complete loss of PRR in mouse, *Drosophila*, and zebrafish results in early embryonic lethality. To test our hypothesis that PRR is important for ovarian development we generated mice with conditional deletion of *Atp6ap2* in the somatic cells of fetal gonads using the *Nr5a1-Cre* line. Here, we present the ovarian phenotype of these mice and show that PRR is important for granulosa cell differentiation and the cross-talk between granulosa cells and oocytes.

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## Next generation sequencing of a large international cohort of patients with Disorders/Differences of Sex Development reveals diagnostic and novel genetic variants

**Katie Ayers<sup>1,2</sup>, Stefanie Eggers<sup>3</sup>, Simon Sadedin<sup>1</sup>, Gorjana Robevska<sup>1</sup>, Jocelyn van den Bergen<sup>1</sup>, Ingrid Knarston<sup>1</sup>, Ben Rollo<sup>4</sup>, Nadia Davidson<sup>1</sup>, Josephine Bowles<sup>5</sup>, Liang Zhao<sup>5</sup>, Katherine Smith<sup>6</sup>, Melanie Bahlo<sup>5</sup>, Ann Jorgenson<sup>7</sup>, Gary Hime<sup>8</sup>, Peter Koopman<sup>5</sup>, Melissa Little<sup>1,2</sup>, Alicia Oshlack<sup>1</sup>, Naomi Weintrob<sup>9</sup>, Bruria Ben Zeev<sup>10</sup>, Andrew Sinclair<sup>1,2,3</sup>**

1. Murdoch Childrens Research Institute, Parkville, Vic, Australia

2. Department of Pediatrics, University of Melbourne, Melbourne, Vic, Australia

3. Victorian Clinical Genetics Services, Melbourne, Australia

4. The Florey Institute, Melbourne, Australia

5. The University of Queensland, St Lucia, Australia

6. Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

7. Department of Growth and Reproduction, Copenhagen University Hospital, Denmark

8. Department of Anatomy and Neuroscience, The University of Melbourne, Melbourne

9. Sackler school of Medicine, Tel-Aviv University, Israel

10. Safra Pediatric Hospital, Chaim Sheba Medical Center, Ramat-Gan, Israel

Disorders/Differences of sex development (DSD) represent a major paediatric concern and clinical management of these conditions is often difficult. Uncertainty about a child's gender can be traumatic and may carry profound psychological and

reproductive consequences. Providing a genetic diagnosis for patients with a DSD and their families can serve multiple purposes: naming the underlying cause contributes to acceptance, reduces stigma, and provides crucial guidance for clinical management, including information on the malignancy risks associated with some types of DSD. It is also integral to genetic counseling and family planning. To improve diagnosis rates in DSD we developed a massively parallel sequencing targeted DSD gene panel of 64 known diagnostic DSD genes and 1000 candidate genes. Analysis of DNA from the largest reported international cohort of patients with DSD (327 patients) identified a likely genetic diagnosis in 43% of patients, a significant improvement on the 13% previously achieved in a clinical setting. We found variants in a total of 28 diagnostic genes, with 93 previously unreported variants. Functional testing of many of these has led to improved curation and reporting. This DSD gene panel has now been implemented as a diagnostic test at the Victorian Clinical Genetics Laboratories, Melbourne. Our research focus has shifted to identifying and testing novel DSD genes in the large cohort of patients that we not diagnosed by the panel. Innovative human stem cell technologies and animal models including *Drosophila*, are being employed to characterize the role of these novel genes in sex development. This includes the gene *SART3*, in which homozygous variants were identified in patients with sex reversal and intellectual disability. Identification and validation of novel genes such as *SART3* will further increase diagnosis rates and improve the health and wellbeing of patients with DSD and their families.

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## Opportunities and challenges in analysis of emerging datasets for reproductive genomics

### Grant Montgomery<sup>1</sup>

1. *Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia*

The pathogenesis of many reproductive traits and diseases is complex and influenced by genetic and environmental risk factors that can contribute to overlapping diseases. Advances in technology and high-throughput computing provide unparalleled opportunities to study regulation of these complex systems. Questions can be addressed on a genome-wide scale and down to single cells. However, individual genetic risk factors have small effects requiring large studies. In the last 10 years, genetic studies have demonstrated the advantages and increased power for gene discovery from combining data in large international consortia. Studies on regulation of gene expression and epigenetic signals are now following this example. Increasingly, the results of these large projects are made available in the public domain so individual studies can combine results from local data and large publicly available datasets. Our studies in endometriosis and other traits illustrate some of the opportunities and challenges in the rapidly changing field of reproductive genomics.

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## High-resolution 4D live-oocyte imaging identifies a new model of asymmetric division

### Jessica Greaney<sup>1</sup>, Zhe Wei<sup>1</sup>, Hayden Homer<sup>1</sup>

1. *The Christopher Chen Oocyte Biology Research Laboratory, Centre for Clinical Research, The University of Queensland, Herston, QLD, Australia*

Mammalian oocytes undergo highly asymmetric meiotic divisions, enabling the oocyte to retain the overwhelming majority of cytoplasmic contents for supporting embryogenesis. Inactivation of the major cell-cycle kinase, Cyclin-dependent kinase 1 (Cdk1), is critical for meiotic exit events including chromosome segregation and spindle midzone-directed furrowing of the overlying cortex. It is widely believed that by displacing the future midzone to an off-centre position, spindle migration *before* anaphase is the sole determinant of asymmetric oocyte division.

Here we simultaneously monitor chromosomes, spindles and membranes in live mouse oocytes at the highest temporal resolution to-date. For the first time, we detail the events occurring during anaphase of meiosis I. We find that anaphase-I lasts 25.4 min and is comprised of two unique phases; spindle elongation followed by spindle narrowing during both of which, chromosomes travel pole-wards. Unexpectedly, we discovered that the spindle migrates 8-9µm (12% of the oocyte diameter) *after* anaphase-onset, defining a new post-anaphase-onset phase of spindle migration. Strikingly, we found that the spindle migrated directly into the membrane. Spindle ingression into the membrane correlated strongly ( $R^2=0.9884$ ) with growth of a membrane protrusion over the leading spindle pole, eventually placing half of the spindle beyond the oocyte boundary before midzone-induced furrowing at the base of the protrusion. Intriguingly, Cdk1 inactivation not only triggered anaphase, it also induced spindle movement by generating a cytoplasmic force behind the spindle via a 47.3% increase in cytoplasmic F-actin polymerisation. Remarkably, we find that canonical pre-anaphase spindle migration is dispensable for normal asymmetric division provided that post-anaphase-onset spindle migration and protrusion remain intact.

Here we define a new model in which, Cdk1 inactivation induces an F-actin-dependent cytoplasmic force that propels the anaphase spindle into the membrane thereby inducing a protrusion, ultimately allowing half of the chromosomes to be ejected into the smallest possible polar body.

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## Granulosa cell transcriptome of neonatal mice provides new clues for primordial follicle activation

### Emmalee A Ford<sup>1,2,3</sup>, Emma L Beckett<sup>3,4</sup>, Shaun D Roman<sup>2,3,4,5</sup>, Eileen M McLaughlin<sup>2,6,7</sup>, Jessie M Sutherland<sup>1,2,3</sup>

1. *School of Biomedical Science and Pharmacy, University of Newcastle, Callaghan, New South Wales, Australia*

2. *Priority Research Centre for Reproductive Science, Newcastle, NSW, Australia*

3. *Hunter Medical Research Institute, New Lambton, NSW, Australia*

4. *School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia*

5. *Priority Research Centre for Drug Development, University of Newcastle, Callaghan, NSW, Australia*



6. School of Biological Science, University of Auckland, Auckland, New Zealand

7. School of Science and Technology, University of Canberra, Bruce, ACT, Australia

The dormant population of primordial follicles is determined at birth and serves as the reservoir for future fertility. Of equal importance to fertility is the rate that primordial follicles activate and enter folliculogenesis, yet our understanding of the biochemical processes underpinning primordial follicle activation remains limited. The survival of primordial follicles relies on the correct complement and morphology of granulosa cells, which provide signalling factors essential for oocyte and follicular survival. To investigate the contribution of granulosa cells in the primordial-to-primary follicle transition, ovaries from C57BL/6 mice were enzymatically dissociated at two time points spanning the initial wave of primordial follicle activation. Post-natal day 1 (PND1) ovaries yielded primordial granulosa cells, and post-natal day 4 (PND4) ovaries yielded a mixed population of both primordial and primary granulosa cells. RNA was isolated from these two time points and sequenced using the Illumina NextSeq 500 System. Differential expression was defined as a  $\log_2$  fold change of 1 in either direction, and a significance level of  $p \leq 0.05$ . Differential expression analysis revealed a total of 132 differentially expressed genes between PND1 and PND4 granulosa cells. Biological network modelling was performed using Ingenuity Pathway Analysis and confirmed the upregulation of regulatory genes present within pathways, established (*Figla*, *MapK*) and mostly unexplored (*Wnt*, *Eif4e*), during the primordial-to-primary transition. Validation of transcriptomic findings was carried out via quantitative RT-PCR of candidates among the top 8 differentially expressed genes. We provide preliminary evidence of a novel role for *Fzrb*, a precursor to the Wnt signalling pathway, as contributing to **promoting primordial follicle activation**. The transcript showed a 3.5-fold increase in PND4 granulosa cells relative to PND1 ( $p=0.023$ ), with the same trend in qPCR ( $p<0.001$ ). Advances in primordial follicle activation research may contribute to the development of novel fertility preservation strategies for women at risk of premature reproductive decline.

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## The roles of TGF $\beta$ superfamily ligands in gonocyte fate choices

**Sarah C Moody**<sup>1,2</sup>, **Patrick Western**<sup>1</sup>, **Kate L Loveland**<sup>1,2</sup>

1. Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Clayton, VIC, Australia

The signalling milieu in the developing testis is complex, involving many TGF $\beta$  superfamily ligands. Elucidating how fetal male germ cells (gonocytes) differentiate in response to the somatic environment will aid our understanding of how male infertility and testicular germ cell tumours arise. This study examined the potential influence of activin A and other TGF $\beta$  superfamily ligands on germ cell differentiation during the important developmental window after sex determination. Whole embryonic (E)13.5 mouse testes expressing a germ cell-specific Oct4-GFP transgene were cultured for 48 hrs in media containing 10  $\mu$ M SB431542, an activin/Nodal/TGF $\beta$  inhibitor, or in 5 ng/mL activin A, with appropriate vehicle controls. Transcripts were measured by qRT-PCR ( $n=5$ ), and germ and Sertoli cell proliferation measured following EdU incorporation, antibody staining and flow cytometry ( $n=3$ ). To assess direct actions on germ cells, E13.5 gonocytes isolated by FACS were cultured for 24 hrs in these conditions and collected for transcript analysis ( $n=5-6$ ). SB431542-treated testes had obviously altered cord structure, and although *Sox9* transcript levels were higher in this group, no change in Sertoli cell proliferation was measured. A significantly higher number of germ cells escaped mitotic arrest (2.7%) compared to vehicle control (<1%). Germ cell differentiation-associated genes *Nanos2*, *Dnmt3a*, *Dnmt3l* and *Piwil4* were significantly lower, and the early germ cell marker *Kit* was significantly higher. The only change identified in activin A-treated testes was a 10% reduction of *Sox9*. In cultures of isolated gonocytes, SB431542 treatment resulted in significantly higher *Kit* and lower *Nanos2* levels, while activin A exposure resulted in the reciprocal outcomes of significantly lower *Kit* and significantly higher *Nanos2*. These data suggest that activin/Nodal/TGF $\beta$  inhibition delays germ cell differentiation in whole gonads and gonocyte cultures, and that gonocytes can respond directly to changes in activin A and TGF $\beta$  superfamily signalling, with activin A promoting a more differentiated phenotype.

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## Heparan sulphate: Regulator of growth differentiation factor 9 (GDF9) signalling during oocyte maturation

**Darren JX Chow**<sup>1,2,3</sup>, **Kylie R Dunning**<sup>1,2,3</sup>, **Jeremy G Thompson**<sup>1,2,3</sup>, **Darryl L Russell**<sup>1,2</sup>

1. University of Adelaide, Adelaide, SA, Australia

2. Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

3. Australian Research Council Centre for Nanoscale Biophotonics, University of Adelaide, Adelaide, SA, Australia

Oocyte-secreted growth differentiation factor-9 (GDF9) plays critical roles in oocyte development by directing differentiation of the cumulus cell lineage from the granulosa cells to provide essential metabolic support for the oocyte. How oocyte-secreted GDF9 effects are restricted to cumulus cells is unknown. Recent reports show that GDF9 effects on cumulus cells can be disrupted with exogenous heparin. Heparan sulfate proteoglycans (HSPGs) are cell-surface glycosaminoglycans with a similar structure to heparin. We hypothesised that endogenous HSPGs sequester GDF9 at cumulus cell surfaces thereby restricting signalling during oocyte maturation. To explore this, we first determined the temporal expression of heparan-sulfate synthesising enzymes during maturation of cumulus oocyte complexes (COCs). We found that *Ext1* was significantly induced by 3- and 6-fold at 4- and 16-h of *in vivo* maturation (IVV), respectively, when compared to immature COCs ( $P<0.05$ ). However, *Ext1* was dysregulated during *in vitro* maturation (IVM) with significantly less *Ext1* transcript at 4- and 16-h of maturation compared to IVV. Similarly, *Ext2* was significantly reduced in IVM at 8- and 16-h of maturation compared to IVV ( $P<0.05$ ). This was supported by a significant increase in sulphated glycosaminoglycan (including heparan sulfate) during IVV with no increase observed during IVM. To confirm the role of endogenous heparan sulfate, we conducted a CRISPR-Cas9 knock-down of *Ext1* in murine COCs using lentiviral delivery during pre-maturation. Murine COCs were collected 44 h post-eCG and transduced with lentivirus containing *Ext1*-specific guide RNA and Cas9 protein. COCs were maintained in meiotic arrest in maintenance culture media containing  $1 \times 10^{-8}$  M estradiol and 1  $\mu$ M milrinone. Thirty hours post-transduction, GFP

fluorescence was observed to confirm viral transduction. Successful gene knockdown was verified by cumulus expansion and qPCR for cumulus- and granulosa-cell specific mRNA transcripts. Collectively, the data suggest that heparan sulfates play an essential role in GDF9-signalling during oocyte maturation.

## Binding affinities of high and low affinity corticosteroid-binding globulin; effect of fever and acidosis.

**Emily J Meyer<sup>1,2</sup>, David J Torpy<sup>1,2</sup>, Anastasia Chernykh<sup>3</sup>, Morten Thaysen-Andersen<sup>3</sup>, John Lewis<sup>4</sup>, Wayne Rankin<sup>1,5</sup>, Steven Polyak<sup>6</sup>**

1. Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA, Australia

2. Discipline of Medicine, University of Adelaide, Adelaide, South Australia, Australia

3. Department of Molecular Sciences, Macquarie University, Sydney, New South Wales, Australia

4. Steroid & Immunobiochemistry Laboratory, Canterbury Health Laboratories, Christchurch, New Zealand

5. Chemical Pathology Directorate, SA Pathology, Adelaide, South Australia, Australia

6. School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia

Corticosteroid-binding globulin (CBG) is the principal transport protein for cortisol in the circulation<sup>1</sup>. Secreted high-affinity (ha) CBG undergoes an irreversible conformational change to low-affinity (la) CBG following proteolytic digestion of an exposed reactive centre loop (RCL) by neutrophil elastase (NE). Cortisol is liberated from haCBG upon NE proteolysis at inflammatory sites or elevated body temperatures<sup>2</sup>. CBG also binds other hormones including progesterone, 17-hydroxyprogesterone and cortisone<sup>3-5</sup>.

A homogenous ligand binding assay using Surface plasmon resonance (SPR) technology measured binding affinities of haCBG and laCBG to a panel of 19 steroid ligands at temperatures 25°C, 37°C and 39°C, and neutral (7.4) and acidic (7.0) pH to mimic the pathophysiological conditions of septic shock. 9G12, an in-house monoclonal antibody recognising the RCL epitope encompassing the NE cleavage site, was used to access the RCL integrity across temperature ranges.

Our newly developed SPR binding assay demonstrated a 4-8-fold decrease in relative binding affinity between haCBG and laCBG for cortisol, cortisone, corticosterone, 11-deoxycortisol, progesterone, 17-hydroxyprogesterone and prednisolone, the former expectedly displaying the highest binding affinity. Hormone binding affinity also decreased 7-12-fold at higher temperatures and acidic pH for both haCBG and laCBG. This is the first study to determine the binding affinity of haCBG and laCBG to a large panel of steroid ligands and importantly demonstrate intact thermo-coupling even after NE cleavage. The 9G12 antibody recognition was preserved for haCBG across the temperature range confirming that the RCL remains intact and available for NE proteolysis, providing insight into the mechanism at which haCBG loses ligand affinity during pyrexia due to temperature-dependent flexibility of the hormone binding pocket.

These findings demonstrate the modifiable hormone binding characteristics of CBG in (patho-)physiological conditions, supporting its significance in cortisol delivery in obviating systemic inflammation and multi-organ failure in patients with septic shock and its association with mortality<sup>6</sup>.

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## ADT in prostate cancer patients: the benefits of a 12-month home-based progressive resistance training program

**Teresa Lam<sup>1,2,3</sup>, Bobby Cheema<sup>4</sup>, Amy Hayden<sup>5,6</sup>, Stephen Lord<sup>7</sup>, Howard Gurney<sup>6</sup>, Shivanjini Gounden<sup>4</sup>, Navneeta Reddy<sup>2</sup>, Haleh Shahidipour<sup>2,3,7,8</sup>, Scott Read<sup>2,3</sup>, Glenn Stone<sup>9</sup>, Mark Mclean<sup>2,3</sup>, Vita Birzniece<sup>2,3,10,7,8</sup>**

1. Department of Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

2. Department of Diabetes and Endocrinology, Blacktown Hospital, Blacktown, NSW, Australia

3. School of Medicine, Western Sydney University, Penrith, NSW, Australia

4. School of Science and Health, Western Sydney University, Penrith, NSW, Australia

5. Department of Radiation Oncology, Westmead Hospital, Westmead, NSW, Australia

6. Crown Princess Mary Cancer Centre Westmead Hospital, Westmead, NSW, Australia

7. School of Medicine, University of New South Wales, Randwick, NSW, Australia

8. Translational Health Research Institute, Sydney, NSW, Australia

9. School of Computing, Engineering and Mathematics, Western Sydney University, Penrith, NSW, Australia

10. Garvan Institute of Medical Research, Sydney, NSW, Australia

## Introduction

Treatment with Androgen Deprivation Therapy (ADT) has detrimental effects on body composition and quality of life (QOL). Exercise interventions, including progressive resistance training (PRT) may ameliorate these effects. Existing studies focus on reversing established changes using supervised programs which have their limitations. In prostate cancer, IGFBP-3 is a proapoptotic protein and IGF-1 mediates tumour cell growth. We investigated the effect of ADT on growth factors, and whether a home-based PRT program, instituted at the start of ADT, could prevent adverse effects over a 12-month period.

## Patients and Methods

Twenty-five patients scheduled to receive at least 12 months of ADT were assigned to either usual care (UC) (n=12) or PRT (n=13) starting after their first ADT injection. Body composition, body cell mass (BCM; a functional component of lean body mass), insulin sensitivity, QOL, muscle function, metabolic biomarkers and growth factors were measured at 6 weeks, 6- and 12 months.

## Results

In the total cohort at 12 months, PSA decreased from  $9.6 \pm 1.6$  to  $0.4 \pm 0.2$  nmol/L ( $p < 0.001$ ) indicating a biochemical response to ADT. This was associated with an increase in IGFBP-3 ( $r = 0.27$ ;  $p = 0.01$ ). The IGF-1/IGFBP-3 ratio decreased ( $p = 0.02$ ), whereas serum leptin ( $p < 0.01$ ) and adiponectin ( $p = 0.03$ ) increased. PRT patients preserved BCM (% total mass) compared to UC ( $-2.3 \pm 0.5\%$  vs  $-4.6 \pm 0.5\%$ ;  $p = 0.02$ ). Gains in fat mass were lower in the PRT versus UC group ( $2.1 \pm 0.9\%$  vs  $5.5 \pm 0.8\%$ ;  $p < 0.01$ ). QOL improved in PRT patients at 12 months compared to UC, particularly in the mental health ( $3.6 \pm 1.6$  vs  $-3.0 \pm 1.9$ ;  $p = 0.01$ ) and vitality ( $2.2 \pm 1.5$  vs  $-4.2 \pm 1.8$ ;  $p = 0.02$ ) domains.

## Conclusion

A biochemical response to ADT is associated with an increase in serum IGFBP-3, involved in suppression of prostate cancer metastasis. Conversely, its toxic effects on body composition and QOL can be reduced with the use of a home-based PRT program instituted at the start of treatment.

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## Associations between reproductive factors and bone health outcomes in women with diabetes: a 15-year longitudinal study

**Eleanor P Thong**<sup>1,2</sup>, **Frances Milat**<sup>1,3</sup>, **Anju E Joham**<sup>1,2</sup>, **Sanjeeva Ranasinha**<sup>2</sup>, **Peter R Ebeling**<sup>1,4</sup>, **Gita D Mishra**<sup>5</sup>, **Helena Teede**<sup>1,2</sup>

1. Dept of Endocrinology and Diabetes, Monash Health, Clayton, VIC, Australia

2. Monash Centre for Health Research & Implementation, Melbourne, VIC, Australia

3. Hudson Institute of Medical Research, Clayton, VIC, Australia

4. Department of Medicine, School of Clinical Sciences, Monash University, Clayton, VIC, Australia

5. Centre for Longitudinal and Life Course Research, School of Public Health, University of Queensland, St Lucia, QLD, Australia

**Aims:** To evaluate associations between reproductive health with incident fracture and new osteoporosis diagnosis in women with self-reported type 1 diabetes (T1D) and type 2 diabetes (T2D) compared with controls.

**Methods:** Longitudinal observational study using data from the Australian Longitudinal Study in Women's Health (ALSWH). Women aged 45 to 50 years were followed up every three years, for up to 15 years.

**Outcomes:** Self-reported fracture and osteoporosis diagnosis.

**Statistical analyses:** Poisson regression analyses for evaluation of incidence rate ratios for fracture and osteoporosis, and multiple linear regression analyses for evaluation of associations between time-to-fracture/osteoporosis between diabetes types.

**Results:** 107 women with T1D, 1232 with T2D and 10874 controls were included. 1260 incident fractures and 1085 new cases of osteoporosis occurred over 165357 person-years of follow-up. Mean age at baseline was 47 years in all groups. Women in the T1D group had lower mean BMI, ( $25.5 \pm 4.9$  vs.  $27.6 \pm 6.3$  vs.  $25.8 \pm 5.0$  kg/m<sup>2</sup>,  $p < 0.001$ ) and reduced reproductive lifespan (period between menarche and menopause) compared with the T2D and control groups [ $34.7 \pm 3.8$  vs.  $37.4 \pm 4.8$  vs.  $37.4 \pm 4.0$  years,  $p < 0.001$ ]. Age of natural menopause was significantly lower in T1D ( $47.7 \pm 3.4$  vs.  $50.1 \pm 4.5$  vs.  $50.3 \pm 3.7$ ,  $p < 0.001$ ). Neither T1D nor T2D were associated with incident fracture, although osteoporosis incidence was significantly higher in T1D and T2D, compared with controls (T1D: IRR 1.86, 95%CI 1.02–3.39; T2D: IRR 1.33, 95%CI 1.04–1.70). T1D status was independently associated with a two- and five-year earlier onset of fracture and osteoporosis diagnosis, respectively (fracture:  $\beta$  -2.04 years,  $p = 0.004$ ; osteoporosis:  $\beta$  -4.91 years,  $p < 0.001$ ).

**Conclusions:** Women with T1D have a reduced reproductive lifespan and shorter time to fracture and osteoporosis, compared with both T2D and controls. Diabetes is associated with osteoporosis, and this work confirms the need for consideration of bone health assessment in diabetes, especially around the midlife transition.

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## Understanding Bone Fragility in Adults with Cerebral Palsy through Longitudinal Analysis

**Anne Trinh**<sup>1,2,3</sup>, **Phillip Wong**<sup>1,2,3</sup>, **Michael C Fahey**<sup>1,2,3</sup>, **Justin Brown**<sup>2,3</sup>, **Boyd Strauss**<sup>3</sup>, **Peter Ebeling**<sup>2,3</sup>, **Peter Fuller**<sup>1,2,3</sup>, **Fran Milat**<sup>1,2,3</sup>

1. Hudson Institute of Medical Research, Melbourne, Victoria, Australia

2. Monash Health, Clayton, Victoria, Australia

### 3. Monash University, Clayton, Victoria, Australia

Cerebral palsy (CP) is a motor disorder resulting from damage to the foetal or infant brain. With increasing life expectancy, preserving bone and muscle health is key to maintaining independence. In young adults with CP, 40% have documented osteopenia/osteoporosis<sup>1</sup>, with prevalent fractures in up to 38%<sup>2</sup>. It is unclear whether low bone mass in adults with CP is predominantly due to less bone accrual during childhood, earlier onset of bone loss or accelerated bone loss in adulthood.

We conducted a retrospective longitudinal study of 45 subjects with CP aged >10 years to investigate changes in areal bone mineral density (aBMD) during adolescence and young adulthood. The effect of ambulation, nutrition and hypogonadism on longitudinal changes in aBMD was also examined.

Mean age at first DXA was 19.4 years (range: 10 - 36 years), 57.8% were male and 80% were non-ambulatory. Mean Z-scores at baseline were <-2.0 at all sites, however these remained stable over time. The median change in aBMD was +1.2 to 1.9%/year in all subjects but in those <20 years of age, the median change was 4 to 8%/year. Peak bone mass was achieved late in the third decade of life/early in the fourth decade and plateaued thereafter. Reduced functional state as measured by the gross motor functional classification scale (GMFCS) had a negative effect on aBMD over time.

Low baseline Z-scores remain stable over time suggesting bone mass deficits occur early in childhood. Bone accrual during puberty and subsequent maintenance parallels that of typically developing adolescents/adults, albeit at a lower set-point. This has important implications for timing of treatment, with early childhood intervention to optimise nutrition, mobility or use of pharmacological therapy likely key to adult bone health. Puberty and its associated changes in bone mass should be monitored and timely pubertal induction given if necessary.

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## Parental multiple endocrine neoplasia type 1 (MEN 1) is associated with increased offspring mortality postpartum: the impact of maternal MEN 1 and antenatal hypercalcemia

Michael Thompson<sup>2,1</sup>, Prue Hogg<sup>3</sup>, Tony De Paoli<sup>1,4</sup>, John Burgess<sup>1,5</sup>

1. School of Medicine, University of Tasmania, Hobart

2. Austin Health, Heidelberg, VICTORIA, Australia

3. General Medicine, Royal Hobart Hospital, Hobart

4. Department of Paediatrics, Royal Hobart Hospital, Hobart

5. Department of Diabetes and Endocrinology, Royal Hobart Hospital, Hobart

Context: Information regarding the impact of parental multiple endocrine neoplasia type 1 (MEN 1) on neonatal outcomes is limited to case reports.

Objective: To determine the impact of parental MEN 1 on neonatal outcomes.

Methods: Retrospective cohort analysis of the Tasman 1 MEN 1 kindred stratified by whether birth occurred before ('historical cohort') or after ('contemporary cohort') prospective screening commenced. The historical cohort included kindred members born between 1825-1984 ( $n=341$  children with a MEN 1 positive (MEN 1<sup>+</sup>) parent and  $n=314$  children with MEN 1 negative (MEN 1<sup>-</sup>) parents). The contemporary cohort included all neonates ( $n=52$ ) of MEN 1<sup>+</sup> women ( $n=28$ ) born at a tertiary referral hospital between 1985-2018. Data was retrieved from births, deaths and marriages registries, medical records and the Australian Institute of Health and Welfare.

### Results:

Historical cohort: compared to MEN 1<sup>-</sup> parents, children of MEN 1<sup>+</sup> parents were more likely to die before 15 years of age in multivariable analysis (HR 4.6,  $p=0.005$ ). Excess mortality was attributable to children of MEN 1<sup>+</sup> mothers (HR 4.52,  $p=0.021$ ) and fathers (HR 3.76,  $p=0.029$ ) and primarily accrued postpartum (HR 4.48,  $p=0.039$  at 3 months). Deaths due to neoplasia were not significantly increased (1 vs 0 death,  $p=1.0$ ).

Contemporary cohort: neonates of MEN 1<sup>+</sup> mothers were more likely to be low birth weight (28.9% vs 6.7%,  $p=0.01$ ), admitted to a higher care nursery (40.4% vs 17%,  $p=0.02$ ) and had a longer median postnatal length of stay (5 vs 4 days,  $p=0.009$ ) compared to the Australian average. Neonatal hypoglycemia (76%) and infection (15%) occurred frequently whereas hypocalcemia requiring intravenous calcium treatment did not (3.8%). Isolated antenatal hypercalcemia did not significantly alter neonatal outcomes.

### Conclusion:

Children of MEN 1<sup>+</sup> parents are disproportionately vulnerable and most at risk postpartum. The excess risk was not attributable to maternal MEN 1 or antenatal hypercalcemia alone.

## Chronic activation of arcuate GABA neurons leads to reproductive dysfunction: potential implication for PCOS

**Elodie Desroziers<sup>1</sup>, Sabine Hessler<sup>1</sup>, Melanie Prescott<sup>1</sup>, Chris Coyle<sup>1</sup>, Allan E Herbison<sup>1</sup>, Rebecca E Campbell<sup>1</sup>**

*1. Center for Neuroendocrinology and Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand*

Polycystic ovary syndrome (PCOS) is the most common form of anovulatory infertility worldwide, affecting 1 in 10 women. Although commonly considered an ovarian disorder, the brain is a critical contributor to PCOS pathogenesis. Women with PCOS exhibit elevated cerebrospinal fluid GABA levels and preclinical models of PCOS exhibit increased GABAergic input to gonadotropin-releasing hormone neurons (GnRH-N), which orchestrate the hypothalamo-pituitary-gonadal axis. The arcuate nucleus (ARN) is postulated as the anatomical origin of elevated GABAergic innervation; however, the functional role of this circuit is undefined. The present study aimed to test the hypothesis that increased activity in ARN GABA neurons underpins the reproductive dysfunction of PCOS. To investigate the effect of selective activation of ARN GABA-N on GnRH-N activity and fertility we used chemogenetic tools coupled with a Cre-lox approach in mice. The designer receptor hM3Dq was specifically expressed in ARN GABA-N via stereotaxic injection in vesicular GABA transporter (VGAT-Cre) mice. The delivery of the designer drug (CNO) to activate hM3Dq was coupled with serial tail-tip blood sampling to measure luteinizing hormone (LH) secretion as a readout of GnRH secretion. Acute stimulation of ARN GABA fibers adjacent to GnRH neurons resulted in a significant and long-lasting increase in LH secretion. Chronic activation of ARN GABA neurons impaired estrous cyclicity, decreased corpora lutea number, increased circulating testosterone and resulted in a trend toward increased LH pulse frequency similar to the PCOS condition. Altogether, these results support the hypothesis that ARN GABA neurons are a functional component of the GnRH neuronal network and suggest that elevated activity in this circuit can drive reproductive dysfunction similar to PCOS.

## Adverse effects of maternal stress on fetal neurosteroid levels and late gestation brain development

**Jonathan J Hirst<sup>1</sup>, Julia C Shaw<sup>1</sup>, Hannah K Palliser<sup>1</sup>, Gabrielle K Crombie<sup>1</sup>**

*1. University of Newcastle, Newcastle, NSW, Australia*

Exposure to maternal prenatal stress that leads to elevated glucocorticoid levels has been shown to have adverse long-term effects on the offspring. This exposure results in markedly increased incidences of neurobehavioural disorders with the greatest impact on males. The links between stress exposure and behavioural disorders remain unclear but may involve glucocorticoid-induced persisting changes in inhibitory pathways. Neurosteroids are elevated during fetal life as the synthesis of these steroids in the brain is supplemented with production by the placenta. Fetal neurosteroids have major roles in neurodevelopment, particularly in promoting normal development of myelination. The placenta has a key role in protecting against the effects of prenatal stress, with placental expression of 5 $\alpha$ -reductase type 2 (SRD5A2) leading to increased concentrations of the key neurosteroid, allopregnanolone in the fetus. Placental expression of 11 $\beta$ -hydroxysteroid dehydrogenase 2 (HSD11B2) that converts cortisol to less active cortisone also protects the fetus from rises in maternal cortisol. We have examined the effect of moderate psychosocial stress achieved by strobe light exposure for 2 h/day in pregnant guinea pig dams every 5 days at between gestational age 35-60 (term 70 days) on placental protective responses and long-term outcomes. Both male and females displayed an increase in placental allopregnanolone production and circulating concentrations in mid and late-gestation following repeated stress events. The female placenta displayed a greater protective response to the stress with markedly increased expression of SRD5A2 and HSD11B2, which was not seen in the male placenta. The female fetuses also displayed evidence of brain sparing at term, with normal brain weights and an increase in the brain to liver ratio. Females offspring from prenatally stressed pregnancies displayed markedly less adverse patterns of hyperactive behaviour compared to males. The greater placental responses and neurosteroid changes seen in females may explain these sex differences in long-term behavioural outcomes.

## Kisspeptin neurons are central regulators of fertility and metabolism

**Jeremy Smith<sup>1</sup>**

*1. School of Human Sciences, University of Western Australia, Nedlands, WA, Australia*

Kisspeptin neurons are found in the hypothalamus and are critical for fertility through stimulation of gonadotropin-releasing hormone (GnRH) neurons. In addition to key roles in puberty onset, kisspeptin neurons govern underlying mechanism for sex steroid positive- and negative-feedback, and it is now commonly accepted – at least in rodents – that the ARC kisspeptin neurons act as the GnRH pulse generator. Moreover, kisspeptin neurons are now recognized as a central pathway responsible for conveying key homeostatic information to GnRH neurons to modulate fertility. Thus, in states of severely altered energy balance (either negative or positive) fertility is compromised, as is kisspeptin gene (Kiss1) expression in the arcuate nucleus. Furthermore, in addition to being expressed in GnRH neurons, the kisspeptin receptor (Kiss1r) is also expressed in other areas of the brain, as well as in the periphery, suggesting kisspeptin may have additional functions outside of governing reproductive status. Evidence is building for a direct role for kisspeptin in regulating energy balance and metabolism. Interestingly, kisspeptin neurons located in the arcuate nucleus are anatomically linked to anorexigenic POMC neurons and orexigenic NPY neurons.

Thus, kisspeptin may have a role in energy balance and our observations indicated that Kiss1r knockout mice displayed late onset obesity and reduced energy expenditure. Moreover, recent data suggest that this obesity may be primarily due to altered uncoupling protein-1 (UCP1) mRNA expression in brown adipose tissue (BAT). Kisspeptin receptor is expressed in BAT, but its role there does not appear to be consistent with the obesity in Kiss1r knockout mice. Overall, in addition to regulating reproduction, kisspeptin signaling may also be an important regulator of metabolism and adiposity but the precise mechanistic pathways are yet to be determined.

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## Sexual dimorphism in the control of body weight and metabolic function in healthy men and women

**Belinda Henry<sup>1</sup>**

1. *Metabolism, Diabetes and Obesity Program, Biomedicine Discovery Institute, Department of Physiology, Monash University, Melbourne, VIC, Australia*

Women typically possess more adipose tissue than men, but they are relatively protected against developing cardiometabolic diseases, including cardiovascular disease and type 2 diabetes. Across the menopausal transition, women tend to gain weight and become susceptible to metabolic and cardiovascular diseases. Such weight gain and loss of cardiometabolic protection is due, at least in part, to declining levels of estrogen. Body weight is determined by energy intake and energy expenditure, wherein total energy expenditure is determined by basal metabolic rate, physical activity and adaptive thermogenesis. Adaptive thermogenesis refers to the dissipation of energy through cellular heat production and occurs in mitochondrial enriched tissues, such as brown adipose tissue (BAT). Numerous animal studies have demonstrated that estrogen acts within the brain to regulate both reproductive and metabolic functions. Regarding the latter, estrogen acts on neurons in the hypothalamus to reduce food intake and to increase energy expenditure. In particular, estrogen acts to increase thermogenesis. Despite this, little is known of how sex steroids modulate thermogenesis in healthy adults. This symposium presentation will explain sexual dimorphism in relation to thermogenesis and will examine the role of sex steroids in the regulation of the same in healthy young men and women. I will highlight a possible role for brown adipose tissue in conferring protection against cardiometabolic disease in women.

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## Global deletion of glucocorticoid receptor increases placental weight and impairs placental hemodynamic function in late gestation in the mouse

**Emma N Panting<sup>1</sup>, Jessica R Ivy<sup>2</sup>, Adrian Thomson<sup>2</sup>, Carmel M Moran<sup>2, 1</sup>, Karen E Chapman<sup>2, 1</sup>, Caitlin S Wyrwoll<sup>1</sup>**

1. *School of Human Sciences, The University of Western Australia, Perth, WA, Australia*

2. *Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland*

Maturation of fetal tissues requires glucocorticoid binding to the glucocorticoid receptor (GR). GR is also expressed in the placenta though a functional role in placenta is not established. We hypothesised that glucocorticoids act via GR to modify placental function to meet fetal demand, particularly in late gestation. Here, we tested the effect of GR deletion upon placental growth and hemodynamic function. Heterozygous GR mice, with C57BL/6J background, were time-mated to produce litters of WT, Het and GRKO fetuses. The morning of vaginal plug was designated E0.5. *In vivo* pulsed-wave Doppler ultrasound scanning was conducted at E14.5 or E17.5 to measure umbilical artery (UA) blood flow and fetal heart function. Immediately after scanning, tissues were collected. Data were analysed by two-way ANOVA with post-hoc Bonferroni's test. Global GR ablation did not alter fetal wet weight at E14.5 or E17.5, although placental wet weight was increased at E17.5 ( $p=0.003$ ). At E14.5, UA blood flow was largely unchanged. Conversely, at E17.5, GRKO exhibited reduced UA systolic/diastolic ratio ( $p=0.002$ ) and diastolic flow was detectable in only 38% of GRKO compared to 83% of WT. UA resistance index was increased in GRKO ( $p=0.0004$ ). Moreover, whilst reversed end-diastolic flow (REDF) was undetectable in WT by E17.5, it persisted in GRKO. Minimal effects were seen on fetal heart function. GR ablation increased placental weight in late gestation, when endogenous glucocorticoid levels peak, perhaps reflecting the removal of normal growth inhibitory effects of glucocorticoids. Further, the compromised UA blood flow associated with persistence of REDF and high resistance index in GRKO indicates a functionally immature placenta at E17.5. Interestingly, despite impaired placental blood flow, fetal wet weight was maintained in GRKO. This is consistent with our previous report of fetal oedema in GRKO. Investigation is underway to determine how absent GR signalling affects placental morphology and fetal-placental interactions.

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## New generation antiplatelet drugs for prevention of pre-eclampsia: novel roles in regulating maternal vascular constriction

**Natasha de Alwis<sup>1</sup>, Sally Beard<sup>1</sup>, Yeukai Mangwiro<sup>1</sup>, Natalie Binder<sup>1</sup>, Tu'uhevaha Kaitu'u-Lino<sup>1</sup>, Stephen Tong<sup>1</sup>, Natalie Hannan<sup>1</sup>**

1. *University of Melbourne, Carlton, VIC, Australia*

INTRODUCTION: Pre-eclampsia (PE) is a severe complication of pregnancy, characterised by maternal hypertension, associated with constriction of the vasculature - an important target for PE treatment. Currently, the antiplatelet drug aspirin is prescribed to prevent PE in women at high risk but is only effective in approximately 10% of cases. New generation antiplatelet drugs (clopidogrel, prasugrel and ticagrelor) are used in clinical cardiovascular disease. More recently we have demonstrated they reduce pathophysiological aspects of PE in both the mouse and human models, revealing their potential to prevent PE and outperform aspirin.

**OBJECTIVE:** Determine whether new generation antiplatelet treatment could mitigate vascular constriction in systemic maternal vessels (mouse and human). Importantly, we assessed vascular function in direct comparison with aspirin.

**METHODS:** Mesenteric arteries were dissected from pregnant CBA/C57BL6 (F1) mice on D17.5 gestation (n=4-7). Arteries were dissected from human omental fat biopsies obtained from women at Caesarean Section (n=5). Vessels were mounted onto the Wire Myograph (DMT 620M), normalised and >80% intact endothelium was confirmed. Each vessel was treated with 100uM aspirin, 100uM clopidogrel, 100uM prasugrel, 12.5uM ticagrelor or vehicle control for 30 minutes. Increasing doses of constricting agents, U46619 (human;  $10^{-10}M - 10^{-5}M$ ) or phenylephrine (mice;  $10^{-9}M - 10^{-5}M$ ) were added and constriction assessed.

**RESULTS:** Mesenteric arteries pre-treated with clopidogrel, prasugrel and ticagrelor demonstrated significantly reduced vascular constriction compared to control, while aspirin did not significantly alter constriction. Likewise, human omental arteries pre-treated with ticagrelor and prasugrel demonstrated reduced vascular constriction to U46619, whereas aspirin and clopidogrel had no significant effect.

**CONCLUSION:** In contrast to aspirin, new generation antiplatelets demonstrate a novel role in reducing vascular constriction, a pivotal characteristic of PE. These data further demonstrate the distinct multifaceted actions of these agents and their potential to prevent PE, thus translation to clinical trials should be considered.

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## A placenta-specific protease that is critical for trophoblast syncytialization

**Mary Mansilla**<sup>1,2</sup>, **Yao Wang**<sup>1,2</sup>, **Guiying Nie**<sup>1,2,3</sup>

1. *Implantation and Placental Development, Hudson Institute of Medical Research, Melbourne, Victoria, Australia*

2. *Department of Molecular and Translational Science, Monash University, Melbourne, Victoria, Australia*

3. *Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, Australia*

**INTRODUCTION:** The placenta is a pregnancy-specific organ that functions to nourish the developing fetus. The outer layer of the placenta contains the syncytiotrophoblast (STB), which forms through fusion of cytotrophoblasts and the process is called syncytialization. Impaired syncytialization may lead to abnormal release of STB-derived proteins into the maternal circulation, causing pregnancy complications such as preeclampsia (PE). HtrA4 is a placenta-specific protease that is highly expressed in STB and significantly up-regulated in PE. However, the functional importance of HtrA4 during normal syncytialization is unknown. The aim of this study was to determine whether HtrA4 is essential for syncytialization.

**METHOD:** Primary human cytotrophoblasts were isolated from healthy term placentas, and cultured under a condition that promotes spontaneous syncytialization. Trophoblast BeWo cell line was treated with forskolin to induce syncytialization. Real-time RT-PCR, western blot (WB) and immunocytochemistry (ICC) were performed to monitor changes in HtrA4 as well as syncytial markers in both cell models. HtrA4 was also analysed by ELISA. To determine if HtrA4 is critical for syncytialization, a stable HtrA4 knockout (KO) BeWo line was established using the CRISPR/Cas9 technology, and used to determine the consequences of HtrA4 KO on syncytialization.

**RESULTS:** Primary cytotrophoblasts spontaneously syncytialized in culture as expected. BeWo cells also syncytialized following forskolin treatment as reported. HtrA4 expression was significantly up-regulated during syncytialization in both cell models. When treated with forskolin, control BeWo cells displayed an expression profile of syncytial markers similar to that of un-transfected BeWo cells. However, HtrA4 KO BeWo cells failed to syncytialize following forskolin treatment, as real-time RT-PCR, ELISA, WB and ICC all revealed minimal changes in syncytial markers in these cells.

**CONCLUSION:** HtrA4 increased during syncytialization in both primary trophoblasts and BeWo cells. Knockout of HtrA4 prevented BeWo cells from syncytialization. These data strongly suggest that HtrA4 plays an essential role in syncytialization.

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## Lipopolysaccharide induced inflammation in choriodecidual explants is sex dependent

**Sarah J Delforce**<sup>1</sup>, **Eugenie R Lumbers**<sup>1</sup>, **Grace Burns**<sup>1</sup>, **Tamas Zakar**<sup>1</sup>, **Kirsty G Pringle**<sup>1</sup>

1. *University Of Newcastle, New Lambton Heights, NSW, Australia*

Preterm birth (PTB) is the single largest cause of death in infants and young children. The prevalence of preterm birth is however, significantly higher in pregnancies carrying male babies. Inflammation has long been implicated as a key mediator in the onset of parturition. Therefore, we propose that in pregnancies carrying male babies, there is increased expression of pro-inflammatory cytokines in the decidua, and an increased cytokine response to infection, placing male babies at increased risk of preterm birth.

Term non-labouring choriodecidual explants were treated with lipopolysaccharide (LPS, 5µg/ml, O55:B5) for 24h and culture medium was collected for cytokine analysis (n=5/sex). Levels of Interleukin (IL)-1β, IL-6, and IL-10 as well as tumour necrosis factor (TNF) was measured via a BD Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit.

Baseline levels of TNF were significantly higher in 'female' choriodecidual explants (p=0.03) compared with 'male' explants whereas levels of IL-1β, IL-6 and IL-10 were not significantly different between 'male' and 'female' explants. Treatment with LPS significantly increased the secretion of pro-inflammatory cytokines: IL-1β, IL-6 and TNF but only from 'male' choriodecidual explants (p=0.0004, 0.0001 and 0.03 respectively). The anti-inflammatory cytokine IL-10 was not affected by treatment with LPS in 'male' or 'female' explants.

These data show that term non-labouring choriodecidia from 'male' pregnancies are more sensitive to induced inflammation by LPS when compared with 'female' choriodecidia. This suggests that 'male' decidua may have a higher inflammatory response to infection and, therefore, increased susceptibility to preterm labour.



## The effect of commonly used antipsychotics and benzodiazepines on placental viability and function.

**Roxanne Hastie<sup>3,1,2</sup>, Fiona C Brownfoot<sup>3,1,2</sup>, Laura Tuohey<sup>1,2</sup>, Josepine Power<sup>4</sup>, Stephen Tong<sup>1,2</sup>**

1. *Translational Obstetrics Group, Melbourne*

2. *Mercy Perinatal, Melbourne*

3. *University of Melbourne, Heidelberg, VICTORIA, Australia*

4. *Mercy Hospital for Women, Heidelberg, Vic*

Introduction:

Maternal mental health is an area of great unmet need. Currently, there is scarcity of data relating to the safety of commonly used medications to treat mental health disorders during pregnancy. Additionally, the effect of these drugs on human placental function has not been previously explored. Thus, we aim to determine the effect of a panel of antipsychotics and benzodiazepines, which are prescribed during pregnancy, on human placental viability and function.

Methods and Results:

Primary cytotrophoblast cells were isolated from term placental tissue and treated with increasing doses of a panel of antipsychotics or benzodiazepines that are prescribed during pregnancy. Following treatment cell viability was assessed via MTS assay. The antipsychotics olanzapine, clozapine, haloperidol and chlorpromazine all significantly induced primary cytotrophoblast death when treated at top doses (5-100uM). The remaining antipsychotics, including paliperidone and lurasidone, had no effect on placental cell viability. Interestingly, of the benzodiazepines temazepam, oxazepam and diazepam, temazepam increased absorbance of the MTS assay, suggesting either increased variability or altered cellular metabolism. No effect of oxazepam or diazepam was seen.

Conclusion:

Commonly prescribed drugs to treat maternal mental health alter placental cell viability. Given the importance of placental function for a healthy continuing pregnancy, further interrogation of these medications is required, which may help identify which drugs cause harm and those that present as safer alternatives.

## Angiotensin converting enzyme 2 (ACE2) and pregnancy complications: preeclampsia and small for gestational age.

**Sonia Tamanna<sup>1,2,3</sup>, Vicki L. Clifton<sup>4,5</sup>, Kym Rae<sup>1,6,7</sup>, Dirk Van Helden<sup>2</sup>, Eugenie R. Lumbers<sup>1,2,3</sup>, Kirsty G. Pringle<sup>1,2,3</sup>**

1. *Pregnancy and Reproduction Program, Hunter Medical Research Institute, University of Newcastle, Newcastle, New South Wales, Australia*

2. *School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Newcastle, New South Wales, Australia*

3. *Priority Research Centre for Reproductive Sciences, University of Newcastle, Newcastle, New South Wales, Australia*

4. *MaterMedical Research Institute and Translational Research Institute, University of Queensland, Brisbane, QLD, Australia*

5. *Australia Robinson Research Institute, School of Medicine, University of Adelaide, Adelaide, SA, Australia*

6. *Department of Rural Health, University of Newcastle, Tamworth, Newcastle, NSW, Australia*

7. *Priority Research Centre for Generational Health and Ageing, University of Newcastle, Newcastle, NSW, Australia*

Preeclampsia (PE) and small for gestational age (SGA) are common pregnancy complications and remain a major health burden to both mother and fetus. Nevertheless, the pathophysiological causes of PE and SGA are not very well understood or documented. Therefore, early detection and identification of precise blood biomarkers are required to treat these diseases better. During pregnancy, the renin-angiotensin system (RAS) plays significant roles in the regulation of blood pressure. An imbalance in the RAS peptides may contribute to the pathophysiology of PE and SGA. We aimed to examine maternal levels of angiotensin (Ang) peptides and enzymes across gestation and in PE and SGA pregnancies. Plasma samples were collected from non-pregnant women (n=10) as well as from women with uncomplicated pregnancies (n=80), SGA (n=25) or PE (n=14) across gestation (13-36 weeks). Angiotensin converting enzyme (ACE) and ACE2 levels were measured by ELISA, Ang II and Ang-(1-7) were measured by RIA. Plasma ACE and ACE2 levels were significantly higher in healthy pregnant women compared with non-pregnant women (p<0.05) and remained high throughout gestation. Conversely, Ang II was decreased and Ang-(1-7) increased in pregnant women compared with non-pregnant women, thus Ang II/Ang-(1-7) was decreased. Women with SGA had levels of ACE, Ang II and Ang-(1-7) that were similar to healthy pregnant women but ACE2 levels were reduced (p<0.005). In women with PE, ACE levels were decreased compared to healthy pregnant women (p<0.003) but Ang II levels were increased (P<0.003). These studies show for the first time that plasma ACE2 levels increase in pregnancy. Therefore, ACE2 is likely playing a role in producing Ang-(1-7), thus stimulating the vasodilatory RAS pathway in pregnancy. Women with PE have decreased plasma ACE levels but increased Ang II suggesting that other enzymes (e.g. chymase) are producing Ang II in women with PE.

## Non-coding RNAs as therapeutics in cancer – dawn of a new era

**Peter J Leedman**<sup>1</sup>

1. *Laboratory for Cancer Medicine, Harry Perkins Institute of Medical Research, Centre for Medical Research, The University of Western Australia, Nedlands, WA*

Non-coding RNAs, including small interfering RNAs (siRNAs), microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are gaining prominence in clinical medicine as potential diagnostics and therapeutics. In addition, given that some of these ncRNAs can be efficiently transferred between cells via extracellular vesicles (EVs), they can also have “hormone-like” effects acting at a distance from their secretory cells (eg. miR-221/222 in mediating resistance to tamoxifen). We have previously identified and characterised several novel nuclear receptor coregulators (SLIRP, PACT, TRBP) that bind SRA, a lncRNA, and regulate hormone action (oestrogen and androgen) in breast and prostate cancer, respectively. In addition, we have characterised several miRNAs that inhibit androgen action and prostate cancer tumourigenesis. With the introduction of 2<sup>nd</sup> generation synthetic chemistry, the stability of short double stranded RNAs (dsRNAs, siRNAs and miRNAs) has been dramatically enhanced, producing profound effects on target gene expression, and leading to the FDA approval in late 2018 of the first siRNA drug, *Patisiran*. This landmark decision paves the way for subsequent dsRNA drug development. We have applied 2<sup>nd</sup> generation synthetic chemistry to develop a novel miRNA mimic for the treatment of epithelial cancers. In addition, we have engineered a liver-specific version of the miRNA mimic to treat hepatocellular carcinoma (HCC). The application of these emerging technologies to the treatment of endocrine and liver cancer will be discussed.

## Elf5 and endocrine resistance in breast cancer

**Christopher Ormandy**<sup>1</sup>

1. *Garvan Institute, Darlinghurst, NSW, Australia*

In preparation for lactation the ETS transcription factor ELF5 drives cell-fate decisions within the mammary luminal progenitor cell population that force differentiation toward the estrogen receptor negative alveolar cell lineage. In estrogen receptor positive luminal A breast cancer, early disease progression is predicted by high levels of ELF5, and in preclinical models elevated ELF5 is associated with its two key features, the acquisition of resistance to endocrine therapy and increased metastasis. We hypothesized that persistence in luminal A breast cancer of the normal developmental role of ELF5 provides a mechanism by which ELF5 can drive progression to endocrine insensitivity, and here we detail molecular mechanisms by which this may occur. Using chromatin immunoprecipitation sequencing we found that ELF5 binding overlapped with FOXA1 and ER at enhancers and promoters, and when elevated, caused FOXA1 and ER to bind to new regions of the genome involved in resistance to endocrine therapy. RNA sequencing demonstrated that these changes altered estrogen-driven gene expression and the expression of ER transcription-complex members. Using rapid immunoprecipitation mass spectrometry of endogenous proteins and proximity ligation assays we found that ELF5 interacted physically with members of the ER transcription complex, such as DNA-PKcs. These data provide a mechanistic basis by which ELF5 may influence the progression of luminal breast A cancer to endocrine insensitivity.

## The highs and lows of androgen signalling in prostate cancer

**Renea A Taylor**<sup>1</sup>

1. *Monash Biomedicine Discovery Institute, Department of Physiology, Monash University, Melbourne, Victoria, Australia*

A current challenge in cancer therapeutics is incomplete response to treatment and emergence of therapy-resistant disease. Androgen deprivation therapy (ADT), the standard treatment for advanced prostate cancer, and effectively reduces the tumour burden in most patients. Yet, residual tumour cells that withstand ADT eventually develop lethal castration-resistance. We have taken multiple different experimental approaches to understand the response of prostate cancer to androgen signalling and/or blockade at different stages of progression, including hormone sensitive and castrate-resistant prostate cancer (CRPC). To study this, we have combined novel androgen treatment strategies with contemporary prostate cancer patient-derived xenografts (PDXs). Firstly, we examined whether cancer cells that survive castration (i.e. castrate-tolerant cells) can be effectively targeted by combination treatment at the time of ADT to delay or even prevent the development of CRPC. Using single cell transcriptomics, we identified several gene expression changes induced in castrate-tolerant cells following ADT, which offer novel therapeutic targets for upfront co-treatment. Secondly, we have examined two treatment approaches for CRPC, where androgen signalling remains a key driver of disease. This involves i) effective targeting of the androgen receptor (AR) amino-terminal domain (NTD), which is distinct from the ligand-binding domain (LBD), using peptidomimetics that inhibit specific NTD:coregulator interactions essential for AR activity and CRPC growth and ii) Bipolar Androgen Therapy (BAT), which involves rapid cycling between castrate and supraphysiological androgen levels. Collectively, our data show that both NTD AR-targeting and high-dose testosterone using BAT are both effective treatments for prostate cancers, particularly for those with specific alterations in the AR, including AR variants, and DNA repair pathways. Thus, the AR continues to be a major therapeutic target in prostate cancer at all stages of disease progression.

## Untangling the complex relations between hormones, obesity and risk of ovarian and endometrial cancer

**Penelope Webb<sup>1</sup>**

1. QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

We have known for many years that oestrogen causes the most common type of endometrial cancer (type 1, sometimes described as oestrogen-dependent) and, for this reason, women with an intact uterus are not normally prescribed unopposed oestrogen therapy. Similarly, the positive association between obesity and type 1 endometrial cancer and the strong protective effects of pregnancy and the contraceptive pill are well established. In contrast, other types of endometrial cancer have been labelled as non-oestrogen-dependent with the assumption they are less sensitive to hormonal exposures. Cancers of the ovary (and fallopian tube and peritoneum) are, histologically, very similar to endometrial cancers and, although the same strong protective effects are seen for pregnancy and the contraceptive pill, they have not been associated with menopausal oestrogen therapy or obesity to the same extent as endometrial cancer. There is also a marked contrast between the strong protective effects seen for combinations of an oestrogen plus progestin taken in the form of oral contraceptives during the reproductive years, and the effects of oestrogen plus progestin menopausal hormone therapy. Furthermore, the effects of these hormones on the breast are often opposite to what is seen for endometrial and ovarian cancers. This presentation will discuss the relationships between exogenous oestrogens and progestins, factors that affect endogenous hormone levels and risks of ovarian and endometrial cancer and will compare and contrast these associations with those seen for breast cancer. It will also consider the potential effects of changing trends in obesity and contraceptive and menopausal hormone use on future rates of these cancers.

## Application of Deep Learning to the Diagnosis of Cervical Lymph Node Metastasis from Thyroid Cancer with CT: External Validation and Clinical Utility in Resident Training

**Eun Ju Ha<sup>1</sup>**

1. Ajou University Medical Center, Suwon, Gyeonggi-Do, South Korea

**Purpose:** A deep learning-based CAD system was developed for use in the CT diagnosis of cervical LN metastasis in patients with thyroid cancer and freely available on a website (<http://cdss.co.kr/>). This study aimed to validate the CAD system in a large population and to evaluate its role as a training tool to help trainees.

**Materials and method:** A total of 3838 axial CT images (benign: n = 3606 and malignant: n = 232) were collected from 698 patients with thyroid cancer. We validated the model's diagnostic performance using the DenseNet121 algorithm. We compared the diagnostic performance of the model with those of two trainees on test set (n=241) and evaluated the changes in the level of confidence on a scale of 1-5 in the interpretation before and after the CAD information was provided.

**Results:** The sensitivity, specificity, and AUROC of the CAD system were 83.6%, 81.4%, and 0.874, respectively. On test set, the sensitivity of the CAD system was not significantly different from those of the two trainees (P =0.500 and P =0.500); however, the specificity and accuracy were higher than those of the two trainees (all P <0.001). When the CAD system was used to assist the trainees, the sensitivities did not change (97.4% vs. 97.4% and 100.0% vs. 97.4%); but both the specificity and accuracy increased (58.3% vs. 63.7%, 62.7% vs. 67.6%) (64.5% vs. 69.0%, 68.6% vs. 72.3%). Confidence level was higher with the CAD system (from 3.0 ± 0.9 to 3.8 ± 1.1 for trainee 1, from 4.3 ± 0.8 to 4.6 ± 0.7 for trainee 2).

**Conclusion:** A deep learning-based CAD system could accurately classify cervical LN metastasis in patients with thyroid cancer with an AUROC of 0.874. This approach may have clinical utility as a training tool to help trainees to gain confidence in diagnoses.

1. Application of deep learning to the diagnosis of cervical lymph node metastasis from thyroid cancer with CT. Lee JH, Ha EJ, Kim JH. Eur Radiol. 2019

## Rapid detection of papillary thyroid carcinoma by fluorescence imaging using a $\gamma$ -glutamyltranspeptidase-specific probe: A pilot study

**Rumi Hino<sup>1</sup>, Naoko Inoshita<sup>2</sup>, Toyoki Yoshimoto<sup>2</sup>, Makiko Ogawa<sup>2</sup>, Daisy Miura<sup>3</sup>, Ryoko Watanabe<sup>4</sup>, Kenta Watanabe<sup>4</sup>, Mako Kamiya<sup>5</sup>, Yasuteru Urano<sup>5</sup>**

1. Sports and Health Science, Daito Bunka University, Higashimathuyama-shi, Saitama, Japan

2. Department of Pathology, Toranomon Hospital, Minato-ku, Tokyo, Japan

3. Akasaka Miura Clinic, Akasaka, Minato-ku, Japan

4. Department of Otolaryngology, Toranomon Hospital, Minato-ku, Japan

5. Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

**Background:** Nodular lesions of the thyroid gland, including papillary thyroid carcinoma (PTC), adenomatous nodule and follicular adenoma, may be difficult to diagnose by imaging, such as in ultrasonic echo testing, or by needle biopsy. Definitive diagnosis is made by pathological examination but takes several days. A more rapid and simple method to clarify whether

thyroid nodular lesions are benign or malignant is needed. Fluorescence imaging with  $\gamma$ -glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) uses  $\gamma$ -glutamyltranspeptidase (GGT), a cell-surface enzyme, to hydrolyze the  $\gamma$ -glutamyl peptide and transfer the  $\gamma$ -glutamyl group. GGT is overexpressed in several cancers, such as breast, lung, and liver cancers. This imaging method is rapid and useful for detecting such cancers. In this study, we tried to develop a rapid fluorescence detection method for clinical samples of thyroid cancer, especially papillary carcinoma.

**Methods:** Fluorescence imaging with gGlu-HMRG was performed to detect PTC using 23 surgically resected clinical samples. A portable imaging device conveniently captured white-light images and fluorescence images with blue excitation light. Hematoxylin-eosin (HE) staining was used to evaluate which fluorescent regions coincided with cancer, and immunohistochemical examination was used to detect GGT expression.

**Results:** All 16 PTC samples exhibited fluorescence after topical application of gGlu-HMRG, whereas the normal sections of each sample showed no fluorescence. HE staining revealed that each fluorescent region corresponded to a region with carcinoma. The PTC samples also exhibited GGT expression, as confirmed by immunohistochemistry.

**Conclusions:** All PTC samples were detected by fluorescence imaging with gGlu-HMRG. Thus, fluorescence imaging with gGlu-HMRG is a rapid, simple, and very useful detection tool for PTC.

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### Computer-aided diagnosis system for evaluation of thyroid nodules on ultrasonography: prospective non-inferiority study according to the experience level of radiologists

**Sae Rom SR Chung<sup>1</sup>, Jung Hwan JH Baek<sup>1</sup>, Young Jun YJ Choi<sup>1</sup>, Tae-Yon TY Sung<sup>1</sup>, Dong Eun DE Song<sup>1</sup>, Tae Yong TY KIM<sup>1</sup>, Jeong Hyun JH Lee<sup>1</sup>**

1. Asan Medical Center, Seoul, N/A, South Korea

**Background:** A computer-aided diagnosis (CAD) system was recently introduced for the characterization and interpretation of the US features of thyroid nodules.

**Purpose:** To determine whether a CAD system for the evaluation of thyroid nodules is non-inferior to radiologists with different levels of experience.

**Materials and methods:** Institutional review board approval and informed consent were obtained for this prospective non-inferiority study. Patients with thyroid nodules with a decisive diagnosis, whether benign or malignant, were consecutively enrolled from November 2017 to September 2018. Three radiologists with different levels of experience (1 month, 4 year, and 7 years) in thyroid ultrasound reviewed the thyroid ultrasound with and without the use of a CAD system. Statistical analyses included non-inferiority testing of the diagnostic accuracy for malignant thyroid nodules between the CAD system and the three radiologists with a non-inferiority margin of 10%, comparison of the diagnostic performance, and the added value of the CAD system on the radiologists.

**Results:** A total of 197 patients (165 patients with a benign nodule [84.8%] and 25 patients with a malignant nodule [15.2%]) were included in the study cohort. The diagnostic accuracy of the CAD system was non-inferior to that of the radiologists with less experience (1 month and 4 year) on thyroid US, whereas it was inferior to that of the experienced radiologist (7 years). The sensitivity and negative predictive value of the CAD system were significantly higher than those of the less-experienced radiologists, whereas no significant difference was found with the experienced radiologist. A conjunctive combination of grayscale US and the CAD system significantly improved sensitivity and negative predictive value, although specificity and positive predictive value deteriorated for the less-experienced radiologists.

**Conclusion:** The CAD system may offer support for decision-making in the diagnosis of malignant thyroid nodules for operators who have less experience in thyroid US.

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### Integrated thyroid nodule risk stratification using BTA U (ultrasound) and Thy (cytology): outcomes at a large tertiary centre.

**Christopher W Rowe<sup>1,2</sup>, Kristien Boelaert<sup>2</sup>, Steve Colley<sup>3</sup>, Mark Ballard<sup>3</sup>, Paul Pracy<sup>4</sup>, Neil Giblett<sup>4</sup>, Neil Sharma<sup>2,4</sup>**

1. Department of Endocrinology and Diabetes, John Hunter Hospital, Newcastle, NSW, Australia

2. University of Birmingham, Edgbaston, United Kingdom

3. Department of Radiology, Queen Elizabeth Hospital, Birmingham, United Kingdom

4. Department of ENT, Queen Elizabeth Hospital, Birmingham, United Kingdom

**Aim:** This study reports the frequency of malignancy in thyroid nodules sonographically assessed as BTA U3 or greater (Indeterminate, Suspicious or Malignant) at a large tertiary institution, and assesses the synergistic performance of the BTA ultrasound (U) and cytology (Thy) grading in nodule stratification<sup>1</sup>.

**Methods:** Electronic record search at University Hospital Birmingham between 2014 and 2018 identified all thyroid fine needle aspiration cytology (FNAC), matched with corresponding ultrasounds scored as BTA U3 or greater. U2 nodules were excluded. Final nodule diagnosis was determined using a hierarchy of histopathology, FNAC, and clinical follow up.

**Results:** A total of 446 thyroid nodules with U3-5 and Thy1-5 scores were included in the analysis. The frequency of U classification was 69% (U3), 24% (U4) and 7% (U5) with corresponding malignancy rates of 13%, 30% and 67%. Frequency of Thy classification was 24% (Thy1), 31% (Thy1c/2/2c), 28% (Thy3a/3f), 10% (Thy4) and 7% (Thy5), with rates of malignancy 8%, 1%, 30%, 40% and 86%. The following observations are instructive: In U3 nodules, Thy1-2 result have low malignant risk (1.6%), but malignancy rates for other Thy scores (Thy3 (30%), Thy4 (33%) and Thy5 (50%)) are not significantly different. Therefore any U3Thy3+ nodule should be considered of similar malignant risk. In U4+ nodules, a Thy2 result has 8% malignancy risk; but all other Thy scores have a >20% risk of malignancy, including 32% malignancy risk for U4Thy3. Two nodules classified as Thy2 returned malignant histopathology - both were U4 on ultrasound. In Thy3 nodules, U classification does not further predict malignancy risk (U3=28% malignant, U4=33% malignant, p=0.72).

Discussion: Integrated assessment of thyroid nodules using ultrasound and cytology features result in greater diagnostic information than either modality alone. The BTA U/Thy system performs strongly to risk stratify nodules, with comparable data to published studies<sup>2</sup>.

1. Perros P, Boelaert K, Colley S, et al. Guidelines for the management of thyroid cancer. *Clin Endocrinol (Oxf)*. 2014; 81 Suppl 1: 1-122.
2. Persichetti A, Di Stasio E, Guglielmi R, et al. Predictive Value of Malignancy of Thyroid Nodule Ultrasound Classification Systems: A Prospective Study. *The Journal of Clinical Endocrinology & Metabolism*. 2018; 103: 1359-68.

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## Comparison of Three International guidelines of Concordance of Ultrasonography Classified Nodules and Impact of Biopsy Size Thresholds on Diagnostic Performance.

**Youngee Yim<sup>1</sup>, Dong Gyu Na<sup>2</sup>**

1. Kangwon National University Hospital, Chuncheon-si, KANGWON-DO, South Korea

2. Radiology, GangNeung Asan Hospital, University of Ulsan College of Medicine, Gangneung, Kangwon-do, South Korea

**PURPOSE:** To investigate the concordance of ultrasonography (US) classified nodules and the impact of biopsy size thresholds on diagnostic performance for thyroid malignancy among three international guidelines of Korean Thyroid Association/ Korean Society of Thyroid Radiology (KTA/KSThR), American Thyroid Association (ATA), and American College of Radiology (ACR).

**MATERIALS AND METHODS:** A total of 2,586 thyroid nodules ( $\geq 1$  cm) were retrospectively collected from two multicenter study datasets. Thyroid nodules were classified according to US categories of nodules for malignancy risk by the three guidelines. Concordance rate of classified nodules among three different guidelines was calculated. Diagnostic performance of biopsy size criteria was evaluated by using the simulated four different biopsy size criteria.

**RESULT:** Concordance rate of high or intermediate suspicion nodules was high (84.1-100%), however, low or mildly suspicious nodules showed a relatively low concordance rate (63.8-83.8%) among the three guidelines. The differences of sensitivity, specificity, and accuracy among the guidelines were 0.7 - 19.8%, 0 - 40.9%, and 0.1 - 30.5% with original biopsy criteria, which decreased to 0 - 5.9%, 0 - 10.9%, and 0.1 - 8.2% with simulated similar biopsy size criteria, respectively. The difference of unnecessary biopsy rate with original biopsy criteria (0-33.8 %) decreased with simulated biopsy size criteria (0-8.7%).

**CONCLUSION:** Concordance rate of high or intermediate suspicion nodules is high among the three guidelines. The difference in the diagnostic performance of the guidelines is mainly influenced by the different size thresholds for biopsy and partly by the different US categorization system of thyroid nodules.

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## Lymph node metastases detection by serum thyroglobulin and neck ultrasonography, and long term follow up in patients with papillary or follicular thyroid cancer previously treated with radioiodine and/or surgery

**Poupak Fallahi<sup>1</sup>, Silvia Martina Ferrari<sup>2</sup>, Giusy Elia<sup>2</sup>, Ilaria Ruffilli<sup>2</sup>, Francesca Ragusa<sup>2</sup>, Sabrina Rosaria Paparo<sup>2</sup>, Gabriele Materazzi<sup>3</sup>, Paolo Miccoli<sup>3</sup>, Alessandro Antonelli<sup>2</sup>**

1. Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

2. Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

3. Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy

Our purpose was to assess the early detection of metastatic lateral neck lymph nodes (LNL) in patients with papillary or follicular differentiated thyroid cancer (DTC), and long-term follow-up after I<sup>131</sup> and/or surgery treatment. Seventy hundred and ten patients were enrolled in the study and their LNL evaluation was made by thyroglobulin (Tg) measurement and neck ultrasonography (NU). These patients were affected by DTC and had been treated with a near-total thyroidectomy and I<sup>131</sup> ablation of residual tissue, and subjected to follow-up by Tg measurement, NU, diagnostic and post-therapy whole body scan (WBS), alone or in combination. In the LNL detection the TSH-stimulated Tg alone had a sensitivity of 86%, and a negative predictive value (NPV) of 84%; whereas NU had a sensitivity of 70%, and NPV of 68%. The sensitivity reached the 98% by combining TSH-stimulated Tg plus NU, while the NPV reached the 96%.

In patients with LNL uptaking radioiodine and then subject to a I<sup>131</sup> treatment, the following outcomes were reported: 49% responded after a median of 4 cycles (median 354 mCi, cumulative dose; 7.4 years median follow-up) with "disappearance of uptake at post-therapy WBS and Tg<1 ng/ml" (complete remission, CR); 12% of not responding cases had CR following other treatments. A treatment with surgery, and subsequent I<sup>131</sup> readministration was made on LNL patients not uptaking or not responding to I<sup>131</sup> (9%), who showed a CR in 23% of cases (6.5 years median follow-up).

To sum up, an early detection of LNL in DTC-patients could help in the achievement of a CR by I<sup>131</sup> in 49% of them, after 4 or less I<sup>131</sup> treatments. Combining different therapies could help patients not responder/not uptaking I<sup>131</sup> to reach a CR in 24% of cases.

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## Appropriate frequency and interval of follow-up ultrasonography (FUUS) surveillance during the first 10 years after total thyroidectomy (TT) in patients with papillary thyroid carcinoma (PTC)

**Yoo Jin Lee<sup>1</sup>, Dong Wook Kim<sup>1</sup>**

1. Busan Paik Hospital, Inje University College of Medicine, Busan, South Korea

**Objectives:** To determine the appropriate frequency and interval of FUUS during the first 10 years in patients who have undergone TT for PTC.

**Methods:** From January 2006 to December 2007, 272 patients underwent TT for PTC. Nineteen patients were excluded because of lack of US follow-up data for the neck. Follow-up US was performed by one of two radiologists in all patients. Tumor recurrence/persistence (TRP) was confirmed by histopathology.

**Results:** Mean interval between surgery and the last FUUS was  $79.0 \pm 39.2$  months and mean number of FUUS sessions was  $5.9 \pm 2.8$  in the 253 evaluable patients. Eleven patients (4.3%) developed TRP, and mean interval between TT and the first detection of TRP on FUUS was  $23.5 \pm 20.2$  (range 6–60) months. T and N stages were independently associated with TRP ( $p < 0.0001$ ). There was no significant difference in patient age or sex, size or location of primary PTC, multifocality, or interval between surgery and the final FUUS between TRP (-) and TRP (+) groups ( $p > 0.05$ ). The interval between surgery and first suspicion of TRP on FUUS was  $\leq 12$  months in 6 patients (mean 8.2 [range 6–11] months) and 20, 35, 41, 53, and 60 months in remaining 5 patients.

**Conclusions:** For detection of TRP after TT in patients with PTC, one or two sessions of follow-up US during the first 2 years, depending on T and N stages and one session of follow-up US in every second year during the following 8 years may be appropriate.

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## **Malignancy risk assessment using ultrasonography patterns in each cytology categories: Korean-Thyroid Imaging Reporting and Data System (TIRADS) vs American College of Radiology-TIRADS**

**Sunyoung Kang<sup>1</sup>, Young Joo Park<sup>1</sup>, Ka Hee Yi<sup>1</sup>, Do Joon Park<sup>1</sup>, Sun Wook Cho<sup>1</sup>**

1. Seoul National University Hospital, Seoul, SEOUL, South Korea

**Background:** Thyroid nodules have wide ranges of estimated risks of malignancy in each cytology categories. Combined with the cytology results, ultrasonography (US) patterns can help to determine how to manage thyroid nodules. We compared efficacy of Korean Thyroid Imaging Reporting and Data System (K-TIRADS) and American College of Radiology (ACR)-TIRADS in terms of predicting risk of malignancy in each cytology categories.

**Methods:** Among 1153 thyroid fine needle aspiration (FNA) cases performed at Seoul National University Hospital in 2017, ultrasonography images of 225 thyroid nodules, which have been pathologically confirmed by core needle biopsy or surgery, were reviewed by two medical doctors. Binominal test was used to investigate interaction between FNA cytology and US pattern (K-TIRADS and ACR-TIRADS) in assessment of the malignancy risk of thyroid nodules.

**Results:** Among 225 finally diagnosed thyroid nodules, 119 nodules (52.9%) were confirmed as malignancy. Overall, malignancy risks were 20.3%, 28.5%, 75.0%, 98.5% in thyroid nodules of Bethesda category III, IV, V, VI, respectively. Among 119 malignancy nodules, proportion of each categories were similar between K-TIRADS and ACR-TIRADS (Category III, 3.4% vs 3.4%; category IV, 15.1% vs 16.0%; category V, 81.5% vs 80.5%). In benign and suspicious for malignancy nodules, low suspicious US pattern in K-TIRADS significantly decreased the malignancy risk, whereas low suspicious US pattern in ACR-TIRADS did not change the risk of malignancy. Both high suspicious US pattern in K-TIRADS and ACR-TIRADS significantly increased the risk of malignancy in benign, atypia of undetermined significance/follicular lesion of undetermined significance, and suspicious for malignancy nodules.

**Conclusion:** The malignancy risk of thyroid nodules can be more effectively evaluated using combined cytology results and sonographic patterns. K-TIRADS may be more useful to assess malignancy risk in Bethesda category II, V nodules compared to ACR-TIRADS.

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## **Aurora Kinases Regulate Telomerase in Thyroid Cancer**

**Grace Lim<sup>1,2</sup>, Martyn Bullock<sup>1,2</sup>, Eugene Choi<sup>3</sup>, Hilda Pickett<sup>3</sup>, Roger Reddel<sup>4</sup>, Roderick Clifton-Bligh<sup>1,2,5</sup>**

1. Cancer Genetics, Kolling Institute of Medical Research, St Leonards, NSW, Australia

2. University of Sydney, Sydney, NSW, Australia

3. Telomere Length Regulation Unit, Childrens' Medical Research Institute, University of Sydney, Westmead, NSW, Australia

4. Cancer Research Unit, Childrens' Medical Research Institute, University of Sydney, Westmead, NSW, Australia

5. Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

Current Tyrosine Kinase Inhibitor (TKI) therapies for advanced thyroid cancer (TC) only modestly improves progression-free survival and the discovery of novel therapeutic targets is a high clinical priority. Activation of telomere maintenance mechanisms (e.g. Telomerase) is critical for cancer cells to acquire the replicative immortality necessary for sustained tumour growth. Telomerase activation can occur via Telomerase Reverse Transcriptase promoter (TERTp) mutations, which arise frequently in TC and strongly correlate with poor clinical outcome. We recently screened a drug library for TERTp inhibitors and discovered that Aurora Kinases (AURKs), positively regulate the TERTp in TC cells. In this study, we investigated the underlying regulatory mechanism and evaluate the therapeutic potential of AURK inhibitors (AURKi).

The dose- and time-dependent changes in TERTp activity following AURKi versus TKI treatments (Heperadin and ENMD-2076 versus Sorafenib) was assayed in SW1736, C643, TPC1 TC cell-lines transfected with WT/C228T-TERTp luciferase-reporters. TERT expression was quantified by qRT-PCR with RNA harvested from similarly treated cells, or from cells transfected with AURKB-targeting shRNA. Telomerase activity was assessed in TC cells treated for 5 days with the IC<sub>50</sub> dose of AURKi. Phospho-blots (p-WB) of lysates isolated from treated TC cells were performed to assess kinase pathway activity.

AURKi treated TC cells had suppressed WT/C228T-TERTp activity (6-fold  $p < 0.005$ , 4-fold  $p < 0.0001$  respectively). Similarly, AURK inhibition by AURKi and AURKB-shRNA led to a decrease in *TERT* expression (8-fold  $p < 0.001$ , 4-fold respectively), which was not evident in Sorafenib treated cells. Furthermore, telomerase activity was significantly suppressed (6-fold  $p < 0.01$ ) and p-WB revealed a significant inhibition of pERK and pAKT ( $>95\%$ ,  $p < 0.001$ ) but no significant effect on pS6 levels.

We have discovered that AURKi treatment downregulates telomerase in TC cells via TERTp inhibition, and this previously unrealised regulatory action of AURKs identifies them as a potential therapeutic target for treating TERTp positive TC.

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## Disruption of thyroid hormone receptor action in the cerebellar Purkinje cell impaired long-term plasticity

**Noriyuki Koibuchi<sup>1</sup>, Ayane K Ninomiya<sup>1</sup>, Michifumi Kokubo<sup>1</sup>, Izuki Amano<sup>1</sup>, Asahi Haijima<sup>1</sup>, Wataru Miyazaki<sup>1</sup>**

1. Gunma University Graduate School of Medicine, Maebashi, GUNMA, Japan

Thyroid hormone (TH) is essential for normal brain development. Disruption of TH action during perinatal period causes abnormal brain development. However, the precise role of TH in the developing brain has not yet been fully understood. TH action is mainly exerted by binding to the nuclear TH receptor (TR), which is the ligand-regulated transcription factor. To clarify the mechanism of TH action in brain, we have generated a transgenic mouse expressing a human dominant-negative TR $\beta$ 1 (Mf-1) specifically in the cerebellar Purkinje cell (PC) (Yu et al. 2015), which is the only output neuron in the cerebellum to control motor performance. Using this animal model, we further clarified the mechanism. Although cerebellar morphogenesis as well as various gene expressions retarded during perinatal period, these were returned to be normal at pubertal age. Nevertheless, motor performance tests revealed the impairment in motor coordination and motor learning in Mf-1 mice. The electrophysiological study at synapses between parallel fiber (PF) and PC showed that long-term synaptic plasticity expressed as long-term depression (LTD) was postsynaptically impaired in the PC of Mf-1 mice, whereas presynaptic short-term plasticity expressed as paired pulse facilitation was intact. Single-cell RT-qPCR revealed the decrease in the mRNA level of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase 2 (SERCA2), a calcium transporter located in the endoplasmic reticulum, indicating the disruption of Ca<sup>2+</sup> mobilization in PC to produce LTD. Thus, the motor impairment in Mf-1 mice could be attributed to PCs with TR $\beta$  mutation through the disrupted cellular basis. The present study may suggest that the impairment of long-term plasticity in PCs would be one of the contributing factors to motor impairment in resistance to thyroid hormone (RTH) patient.

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## Diallyl trisulfide induces anaplastic thyroid carcinoma 8505C cells apoptosis by ATM-mediated DNA damage

**Huixin Yu<sup>1</sup>, Li Zhang<sup>1</sup>, Xian Cheng<sup>1</sup>, Shichen Xu<sup>1</sup>, Jiandong Bao<sup>1</sup>**

1. Jiangsu Institute of Nuclear Medicine, Wuxi, JIANGSU, China

Anaplastic thyroid cancer (ATC) is the most aggressive thyroid cancer. Current approaches including surgery, chemotherapy and therapeutic drugs provide limited benefit for ATC patients. Diallyl trisulfide (DATS), a garlic-derived organosulfur compound, has been documented for promising anti-cancer effect in various carcinoma. However, it is remained to uncover the role of DATS in ATC tumorigenesis. DATS treatment at 12.5, 25 and 50  $\mu$ M decreased the viability of ATC cell line 8505C cells determined by SRB, colony formation and Hoechst/PI double staining assays. DATS inhibited the proliferation of 8505C cells both in a dose- and time-dependent manner. Furthermore, DATS induced a G2/M cell cycle arrest which lead to a caspase-dependent mitochondrial apoptosis evidenced by PI-cytometry, Annexin V/PI staining and western blot. The phosphorylation of H2A.X, which is a DNA damage marker, was induced by DATS both in a dose- and time-dependent manner. Moreover, the long tails examined by comet assay further confirmed that DATS induced DNA damage in 8505C cells. The phosphorylation of ATM but not ATR was upregulated under the stimulation with DATS, which then resulted into the activation of target genes Chk1 and Chk2. Further results showed that DATS-induced DNA damage in 8505C cells was ROS-dependent but independent of DNA conformational changes. KU-55933, an inhibitor specific to ATM, reversed the phosphorylation of H2A.X and the cleavage of PARP caused by DATS. Taken altogether, our findings demonstrated that DATS induced an ATM-mediated DNA damage in ATC cells 8505C, which shed a light on the development of novel therapeutic agents for ATC treatment.

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## Targeting TERT activation with mTOR inhibitors in thyroid cancer

**Althea Bastian<sup>1,2</sup>, Saskia Fung<sup>1,3</sup>, Farhana Mollah<sup>1,4</sup>, Hilda Pickett<sup>5</sup>, Eugene Choi<sup>5</sup>, Roger Reddel<sup>6</sup>, Deborah Marsh<sup>2,7</sup>, Roderick Clifton-Bligh<sup>1,7,8</sup>, Martyn Bullock<sup>1,7</sup>**

1. Cancer Genetic Laboratory, Kolling Institute, St Leonards, NSW, Australia

2. School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia

3. University of New South Wales, Sydney, NSW, Australia

4. Western Sydney University, Sydney, NSW, Australia

5. Telomere Length Regulation Unit, Children's Medical Research Institute, Sydney, NSW, Australia

6. Cancer Research Unit, Children's Medical Research Institute, Sydney, NSW, Australia

7. University of Sydney, Sydney, NSW, Australia

8. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

New therapies to treat advanced forms of thyroid cancer (TC) are urgently needed. Healthy somatic cells cannot divide beyond a finite number of cell-divisions, as progressive loss of their chromosomal end-structures called telomeres, ultimately triggers replicative senescence or apoptosis. In contrast, cancer cells possess an unlimited replicative capacity, afforded to them by Telomerase activation, which functions to sustain telomere length. Activating mutations within the promoter of *TERT* (TERTp), which encodes the catalytic component of Telomerase, occur at high frequencies in TC. Synergistic interaction between the TERTp and *BRAF* or *RAS* driver mutations promotes tumor progression and aggressiveness. The activation mTOR by upstream *BRAF* or *RAS* signaling drives several thyroid tumorigenic processes and the kinase is a well-established chemotherapeutic target. In this study we investigated whether mTOR plays a role in the regulation of TERTp activity.

Dose-dependent changes in TERTp activity following 24 hr treatment with Everolimus, AZD8055 or Omipalisib were determined using CRISPR-edited SW1736 cells in which the endogenous mutated TERTp controls expression of a luciferase gene. Then, SW1736, C643, 8505C, TPC1 cell-lines were treated for 24-96 hrs with IC<sub>50</sub>-dose mTOR inhibitor versus DMSO control, and *TERT* expression and telomerase activity were measured by qRT-PCR and qTRAP respectively. Phosphoblots were performed to confirm mTOR inhibition.

The inhibitors Everolimus, AZD8055 or Omipalisib potently suppressed TERTp activity with IC<sub>50</sub> doses of 20, 50 and 50 nM respectively. Correspondingly, a 20-50% reduction in *TERT* expression was observed at 24 hrs post-treatment, which conferred a similar down-regulation in telomerase activity. Interestingly, at later time-points there appeared to a compensatory upregulation in *TERT* expression, although the inhibition of telomerase activity was sustained.

This is the first report implicating mTOR in the regulation of the mutated TERTp, and highlights the therapeutic potential of mTOR inhibitors for TERTp harbouring thyroid cancers.

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## Molecular profiling thyroid cancer; targeted therapies and personalised medicine

**Matti Gild**<sup>1,2,3</sup>, **Martyn Bullock**<sup>1</sup>, **Venessa Tsang**<sup>2,3</sup>, **Lyndal Tacon**<sup>2</sup>, **Mark Sywak**<sup>4</sup>, **Vivek Rathi**<sup>5</sup>, **Alexander Guminski**<sup>6</sup>, **Stan Sidhu**<sup>1,3,7</sup>, **Leigh Delbridge**<sup>7</sup>, **Anthony Gill**<sup>8</sup>, **Bruce Robinson**<sup>1,2,3</sup>, **Roderick Clifton-Bligh**<sup>1,2,3</sup>

1. Kolling Institute of Medical Research, St Leonards, NSW, Australia

2. Endocrinology Department, Royal North Shore Hospital, St Leonards, NSW, Australia

3. University of Sydney, Sydney

4. Endocrine Surgery Department, Royal North Shore Hospital, Sydney, NSW

5. Pathology, St Vincents, Melbourne, VIC

6. Oncology Department, Royal North Shore Hospital, Sydney, NSW

7. Endocrine Surgery, Royal North Shore Hospital, Sydney, NSW, Australia

8. Pathology Department, Royal North Shore Hospital, Sydney, NSW

Radioactive iodine is an effective first line therapy for follicular cell neoplasms of the thyroid gland. Around 5% of cases will be at outset, or become over time refractory to radioactive iodine (RAIRD) and these patients have much poorer prognosis (10% 10-year survival). Tyrosine kinase inhibitors (TKIs) have recently become available for this cohort and can show an increase in progression free survival, but selecting which patients will best respond to these drugs is not yet established. Genetic events in thyroid cancer are well characterised in the literature and include *RAS* (H, K, N), *BRAF*, *NTRK1*, or *RET* rearrangements. The Royal North Shore experience, as a quaternary referral centre sees >200 new cases/year. Next generation sequencing has become widespread for molecular profiling of key cancer genes. We have used these techniques and others to profile molecular events in RAIRD patients, and where possible sought targeted therapies to improve patient outcomes. This has led to establishing a practice of precision medicine where these patients with identifiable driver mutations have new options for treatment. In anaplastic thyroid cancer the distinction between BRAF +/- disease is crucial to the response to MEK inhibition. Furthermore in differentiated thyroid cancer, non targeted TKIs (lenvatinib) are used, but the small patient cohort with RET or NTRK1 mutations have more selective options. Lastly, this strategy has led to enrolment of phase I and II trials in targeted therapies including the highly selective RET inhibitor LOXO-292 at our clinical trials unit. We describe our experience of panel testing in identifying practice changing variants in a small (18) but growing number of patients. As we move to this personalised medicine approach, finding funding and accessing these therapies remains a challenge.

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## Punicalagin induces senescence growth arrest in human papillary thyroid carcinoma BCPAP cells via NF-κB signaling pathway

**Jiandong Bao**<sup>1</sup>, **Xian Cheng**<sup>1</sup>, **Huixin Yu**<sup>1</sup>, **Li Zhang**<sup>1</sup>, **Shichen Xu**<sup>1</sup>

1. Jiangsu Institute of Nuclear Medicine, Wuxi, JIANGSU, China

Papillary thyroid carcinoma (PTC) is the most common endocrine carcinoma. Our previous study revealed that punicalagin (PUN), an active component from pomegranate, triggered autophagic cell death and DNA damage response (DDR) in papillary thyroid carcinoma BCPAP cells. But the detailed anti-cancer mechanisms of punicalagin against PTC still remained to be further explored. DDR activation is a proven cause of cellular senescence, which mediates anti-tumor processes under certain circumstance. In this study, we reported that punicalagin treatment generated a senescent phenotype of BCPAP cells characterized as altered morphology, increased cell granularity and senescence-associated β-galactosidase (SA-β-Gal) staining. Senescence induced by punicalagin treatment was further confirmed by cell cycle arrest and upregulation of cyclin-dependent kinase inhibitor p21. Meanwhile, the senescence-associated secretory phenotype (SASP) included high levels secretion of inflammatory cytokines, principally IL-6 and IL-1β. Furthermore, punicalagin exposure caused the



phosphorylation and subsequent degradation of I $\kappa$ B $\alpha$  as well as the nuclear translocation of p65, suggesting the activation of NF- $\kappa$ B signaling pathway. Inhibition of NF- $\kappa$ B by PDTC, a selective inhibitor of NF- $\kappa$ B, partially reversed the cellular senescent phenotype induced by punicalagin in BCPAP cells as evidenced by the decreased fraction of SA- $\beta$ -Gal staining positive cells and blockage of SASP generation. These results collectively showed that punicalagin treatment induced senescence growth arrest and SASP *via* triggering NF- $\kappa$ B activation. These observations elucidated novel anti-cancer mechanisms of punicalagin and might provide new potential targets for PTC therapy.

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## **MKL1 overexpression predicts poor prognosis in patients with papillary thyroid cancer and promotes nodal metastasis through TGF- $\beta$ /Smad3/MMP2 pathway**

**Xian Cheng<sup>1</sup>, Li Zhang<sup>1</sup>, Huixin Yu<sup>1</sup>, Shichen Xu<sup>1</sup>, Jiandong Bao<sup>1</sup>**

*1. Jiangsu Institute of Nuclear Medicine, Wuxi, JIANGSU, China*

Papillary thyroid cancer (PTC) is the most common thyroid malignancy, with a strong propensity for cervical lymph node metastasis (CLNM). CLNM is known to increase the risk of locoregional recurrence and decrease the survival in some high-risk groups. Hence, there is a need for a reliable biomarker for the prediction of LNM in thyroid cancer. In the present study, both the mRNA and protein levels of MKL1 were significantly increased in PTC patients with lymph node metastasis (LNM) compared with those without LNM. Further receiver operating characteristic (ROC) analysis showed that MKL1 had a diagnostic value in the differentiation of LNM in PTC (AUC=0.87,  $P < 0.001$ ). Meanwhile, Kaplan-Meier analysis revealed that high MKL1 expression was associated with a significant worse survival in PTC. Additionally, the biological function of MKL1 in PTC cells was explored. Our study indicated that MKL1 promoted the migration and invasiveness of PTC cells and a requirement for MKL1 in TGF- $\beta$ /Smad3/MMP2 signal transduction. MKL1 interacted and recruited Smad3 to the promoter of MMP2 to activate MMP2 transcription upon TGF- $\beta$ , a prominent pro-malignancy cytokine. Moreover, there were significant co-expression correlations between TGF- $\beta$ , MKL1 and MMP2 in our clinical cohort of PTC specimens. Our results suggest that the detection of MKL1 expression could be used to predict cervical LNM and inform postoperative follow-up in papillary thyroid cancer.

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## **Study on the relationship between hyperthyroidism and vascular endothelial cell damage in human, rat and cell**

**Peng Liu<sup>1</sup>**

*1. Center for endemic disease control, Harbin, Heilongjiang, China*

The aim is to explore the relationship between hyperthyroidism, iodine, anti-thyroid drugs (propylthiouracil) and vascular endothelial injury.

1. Totally, 136 SD rats were randomly allocated into control, the hyperthyroidism, the hyperthyroidism propylthiouracil, the hyperthyroidism low iodine, the high iodine, and the endothelial injury group. Rats were raised for 60 days and determined the von Willebrand Factor, Thrombomodulin, nitric oxide, endothelin 1, and Pselectin. Additional indicators included the plant haemagglutinin in sample type oxidized low density lipoprotein receptor 1 (LOX1) from the aorta, and the number of endothelial progenitor cells (EPCs) in whole blood. The hyperthyroidism group had significantly higher serum values for vWF, TM, NO, ET1, and Pselectin and a higher number of EPCs in whole blood compared with the control group; the abdominal aorta LOX1 expression was also significantly higher in the hyperthyroidism group. The electron microscope showed that hyperthyroidism does cause a certain degree of endothelial injury to the abdominal aorta in rats. Hyperthyroidism can damage the vascular endothelium and is a high risk factor for cardiocerebrovascular disease. Propylthiouracil could be used in the treatment of hyperthyroidism, thus protecting endothelial cells from damage.

2. Through the population experiments, to find the relationship between cardiocerebrovascular disease and hyperthyroidism and autoimmune diseases. Blood samples were collected from clinical diagnosed hyperthyroidism patients, patients with autoimmune thyroid disease, patients with cardiocerebrovascular disease and healthy and euthyroid people through physical examination in hospital, sixty cases in each group, and were tested vascular endothelium injury index (VWF, TM, ET1, Pselectin). Patients with simple hyperthyroidism and with autoimmune thyroid diseases have higher endothelial injury levels in whole blood than control group; abnormal thyroid function exists in patients with cardiocerebrovascular diseases and is higher than normal group. Hyperthyroidism can cause vascular endothelium injury, simple hyperthyroidism and autoimmune thyroid diseases are high risk factors of the cardiocerebrovascular disease.

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## **Inhibin and Activin: From Reproduction to Metabolism**

**Kelly Walton<sup>1</sup>**

*1. Department of Physiology Monash Biomedicine, Discovery Institute Monash University VIC 3800*

Although first postulated to exist nearly 100 years ago, inhibins were only isolated in 1985 from bovine and porcine follicular fluid. Inhibin A and inhibin B (heterodimers of a common  $\alpha$ - and differing  $\beta$ -subunits) act as part of a negative feedback loop to regulate synthesis of follicle stimulating hormone (FSH) by gonadotrope cells of the anterior pituitary. Inhibins modulate gonadotrope function by preventing activins (homodimers of  $\beta$ -subunits) from binding their receptors and activating SMAD 2/3 transcription factors. In the context of the pituitary, this mode of action ensures that inhibins control activin-induced FSH production. Over the past decade, my group has helped define the mechanisms underlying the synthesis, activation and

receptor binding of inhibin and activin. Based on our understanding of these processes, we have generated highly-potent inhibin agonists and specific activin antagonists. My studies have also redefined the physiological roles of these "reproductive" molecules, with increased circulating levels of activin A promoting muscle wasting and cachexia, while loss of inhibin results in a complex metabolic phenotype. Using our detailed structural knowledge, we can now manipulate the inhibins and activins to study loss/gain of function, allowing us to identify new roles for these age old hormones.

### High-throughput screen identifies new hormone alternative contraceptive

**Alaknanda Alaknanda<sup>1</sup>, Rebecca Robker<sup>1</sup>, Krzysztof Mrozik<sup>2</sup>, Andrew Zannettino<sup>2</sup>, Darryl Russell<sup>2</sup>**

1. *The Robinson Research Institute, Adelaide Medical School, The University of Adelaide, Adelaide, South Australia*

2. *South Australia Health and Medical Research Institute, Adelaide, South Australia*

Despite contraceptive availability and refinement, unplanned pregnancy remains a troubling global reproductive health issue. Global estimates suggest that of the 208 million pregnancies per year, roughly half (41%) are unplanned, leading to millions of unsafe abortions and maternal deaths<sup>1</sup>. A major reason for this is the serious side-effects of hormone contraceptives, including cardiovascular and breast cancer risks and increased depression, which lead to their non-use or discontinued use. There is an acute need for new safer contraceptives that can overcome the systemic side-effects of hormone therapy and offer wider contraceptive choice to women.

We developed a high-throughput approach for screening broad classes of drugs for potential ovulation blocking capacity using automated assessment of cumulus oocyte complex (COC) adhesion to ECM *in-vitro*. One "hit" compound from the drug library screen that potently and dose dependently inhibited COC adhesion *in vitro* caused significantly reduced ovulation (11 vs. 26 oocytes/ovary;  $p=5.8 \times 10^{-6}$ ) compared to controls in mice *in-vivo*. There was no difference in the growing follicular count but ovulated follicle structures were significantly reduced in drug treated group. This was not due to LH-pathway downregulation as *Lhcgr* and downstream signalling remained intact. Both ovarian histology and immunofluorescence revealed structural dysgenesis of COCs with an apparent loss of contact between oocyte and cumulus cells. Importantly, no difference in proliferative (Ki-67) or apoptotic (cleaved caspase-3) cell counts was detected between groups, suggesting minimal drug toxicity. Treatment of COCs with this drug during *in vitro* maturation severely inhibited COC expansion and oocyte meiotic resumption. Overall, this study is the first to 1) develop a unique high-throughput model for screening drugs for contraceptive potential; 2) identify, evaluate and validate a new class of drugs with potent *in vitro* and *in vivo* potential; and 3) demonstrate a critical role of oocyte-cumulus signalling by this target during folliculogenesis and ovulation.

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### Epididymal CRISPs regulates efficient flagellar waveform and optimal sperm function

**Avinash S. Gaikwad<sup>1</sup>, Ashwin Nandagiri<sup>2</sup>, David Potter<sup>3</sup>, Ranganathan Prabhakar<sup>4</sup>, Sameer Jadhav<sup>5</sup>, Julio Soria<sup>6</sup>, Moira K. O'Bryan<sup>1</sup>**

1. *School of Biological Sciences, Monash University, Clayton, VIC, Australia*

2. *IITB-Monash Research Academy, Indian Institute of Technology-Bombay, Mumbai, Maharashtra, India*

3. *Monash Micro Imaging - Advanced Optical Microscopy, Monash University, Clayton, Victoria, Australia*

4. *Department of Mechanical & Aerospace Engineering, Monash University, Clayton, Victoria, Australia*

5. *Department of Chemical Engineering, Indian Institute of Technology-Bombay, Mumbai, Maharashtra, India*

6. *Laboratory for Turbulence Research in Aerospace and Combustion (LTRAC), Department of Mechanical & Aerospace Engineering, Monash University, Melbourne, VIC, Australia*

The oscillatory waveform generated by flagella dictates the ability of sperm to achieve fertilization and is a critical determinant of reproductive success. Indeed, even minor deviations away from optimal flagellar waveform are predicted to affect fertility. The Cysteine-Rich Secretory Proteins (CRISPs) are highly expressed in the epididymis and are hypothesized to be involved in establishing optimal functional competence. Previously, it has been shown that CRISPs can regulate ion flow via various sperm ion channels, and thus potentially regulate sperm function, including motility. We use image-analysis and hydrodynamic calculations to show here that epididymal CRISP1 and CRISP4 indeed influence sperm flagellar beating significantly. This was achieved by developing an image-analysis algorithm on MATLAB which utilizes proper orthogonal decomposition to study the complex dynamics and beating pattern of the sperm flagella. Sperm from wildtype (WT) mice exhibited rhythmic and sinusoidal flagella beating. By contrast, the *Crisp1*<sup>-/-</sup> and *Crisp4*<sup>-/-</sup> sperm displayed asymmetric flagellar beating. The changes observed between genotypes were characterized by reconstructing an average beat cycle, which revealed that *Crisp1*<sup>-/-</sup> sperm possessed an inflexible mid-piece and a highly asymmetric waveform at the distal regions of the flagella. By contrast, the *Crisp4*<sup>-/-</sup> sperm was constrained along the entire length of the flagella. We found that *Crisp1*<sup>-/-</sup> and *Crisp4*<sup>-/-</sup> sperm had altered periodicity of flagellar oscillations between cycles and lowered flagellar amplitude compared to WT. The oscillating frequency of the flagella was reduced in *Crisp1*<sup>-/-</sup> and *Crisp4*<sup>-/-</sup> sperm and that loss of CRISP1 or 4 led to reduced rates of energy dissipation along the flagella. Excitingly, these deficits could be largely rescued by the addition of recombinant CRISPs to sperm, thus suggesting that their use may be of benefit in assisted reproductive technologies. Collectively, the data reveal that CRISPs play a significant role in establishing efficient sperm flagella waveform and function and thus, fertility.

## Deliverable transgenics can be used to rescue infertility in Sertoli cell androgen receptor knock-out mice.

**Annalucia Darbey<sup>2,1</sup>, Pamela Brown<sup>3</sup>, Rod T Mitchell<sup>1</sup>, Lee B Smith<sup>2,1</sup>**

1. MRC Centre for Reproductive Health, The University of Edinburgh, Edinburgh, United Kingdom

2. The University of Newcastle, CALLAGHAN, NSW, Australia

3. Biomolecular Core - SURF, The University of Edinburgh, Edinburgh, United Kingdom

Hypogonadism and male infertility are clinically prevalent conditions and have been linked to an increased risk of cardiovascular-metabolic diseases and earlier mortality [1, 2]. For most cases, the causes of impaired male reproduction remain idiopathic, with a genetic component often suggested to be a cause [3]. Consequently, it is critical to increase understanding and to develop new therapeutics for these conditions. The advancements in gene editing and vector delivery technologies have potential to improve diagnosis and to aid the development of treatments for men with reproductive disorders.

To investigate the potential of viral vectors in the adult testis, we first optimised their delivery to the interstitial and seminiferous tubule compartments and characterised their specific targeting of testicular somatic cells (the Leydig and Sertoli cells). Results revealed that adenoviral vectors were most suitable for targeting Leydig cells, as lentiviral targeting resulted in Leydig cell apoptosis. Conversely, rete injected lentiviral vectors were deemed the best strategy for Sertoli cell targeting, with previous studies demonstrating disrupted tubules following adenoviral delivery.

We then exploited the infertile Sertoli cell androgen receptor knock out (SCARKO) mouse to demonstrate the potential of this technology as a treatment for male infertility [4]. To do so, we utilised the specific targeting of lentiviral vectors to deliver a mouse androgen receptor (AR) transgene to adult SCARKO testis. This resulted in the rescue of Sertoli cell AR expression and subsequently the infertility phenotype with presence of mature spermatids observed within rescued seminiferous tubules.

These studies examine the targeting capabilities of viral vectors in testicular somatic cells using optimised techniques. They also demonstrate the rescue of gene expression and infertility in the adult testis. This technology could be further utilised for both the generation of testis specific mouse models and for exploitation for therapeutic purposes for the treatment of male reproductive disorders.

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## Macrophages mediate uterine vascular remodelling required to establish pregnancy in mice

**Holly M Groome<sup>1,2</sup>, Alison S Care<sup>1,2</sup>, Peck Yin Chin<sup>1,2</sup>, Claire T Roberts<sup>1,2</sup>, Sarah A Robertson<sup>1,2</sup>**

1. The Robinson Research Institute, Adelaide, SA, Australia

2. University of Adelaide, Adelaide, SA, Australia

Successful pregnancy requires uterine vessel remodelling during early pregnancy to allow placental, hence fetal, growth. Macrophages are crucial for immune tolerance and embryo implantation yet their role in promoting maternal vascular adaptations remains unclear. We hypothesised that macrophages modulate vascular remodelling in early pregnancy to permit early placental development. We utilised the *CD11b-Dtr* mouse model to transiently deplete macrophages on day 5.5 post coitum (pc) using diphtheria toxin (DT) which binds the transgenic monkey diphtheria toxin receptor (*Dtr*) in CD11B+ macrophages. In wild-type control females, DT administration elicits no adverse effects on macrophages or pregnancy. DT administration to pregnant *CD11b-Dtr* females caused >90% macrophage depletion and substantial fetal loss, partly attributable to decreased serum progesterone and ovarian haemorrhage. Then, in separate cohorts, we either administered progesterone pellets or transferred wild-type bone marrow-derived macrophages (BMDM) to macrophage-depleted mice. Both progesterone and BMDM increased pregnancy rate on day 7.5 pc (81% and 78% vs 43%,  $p < 0.01$ ). At this time maternal vascular remodelling was impaired in macrophage-depleted mice ( $p < 0.05$ ). Progesterone treatment was insufficient to rescue uterine vessels remodelling as assessed by lumen diameter but BMDM restored it to control levels. RNA expression profiling of over 600 genes in the uterus revealed that of the >250 genes dysregulated, BMDM transfer restored normal gene expression of >15% more dysregulated genes than did progesterone treatment, while markers of embryo invasion and endothelial cell function remained aberrant in progesterone-treated mice. Importantly, BMDM rescued genes are involved in the regulation of vasculature development pathway (FDR=2.23e-08). This study demonstrates that macrophages are essential for the maternal vascular adaptations required during early pregnancy. Defining how macrophages drive vascular remodelling and their interaction with other immune cells may contribute to understanding the causes of, and developing effective therapeutics for women with pregnancy complications associated with vascular maladaptations.

### The small non-coding RNA profile of mouse oocytes is modified during ageing

**Bettina P Mihalas**<sup>1,2</sup>, **Nicole J Camlin**<sup>3</sup>, **Miguel J Xavier**<sup>1</sup>, **Alexandra E Peters**<sup>1,2</sup>, **Janet E Holt**<sup>1</sup>, **Jessie M Sutherland**<sup>1,2</sup>, **Eileen A McLaughlin**<sup>4,1,5,2</sup>, **Andrew L Eamens**<sup>1</sup>, **Brett Nixon**<sup>1,2</sup>

1. Priority Research Centre for Reproductive Science, Schools of Environmental and Life Sciences and Biomedical Science and Pharmacy, The University of Newcastle, Callaghan, New South Wales, Australia

2. Pregnancy and Reproduction Program, Hunter Medical Research Institute, New Lambton Heights, New South Wales, Australia

3. Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States

4. School of Science, The University of Canberra, Bruce, Australian Capital Territory, Australia

5. School of Biological Sciences, University of Auckland, Auckland, New Zealand

Oocytes are dependent on messenger RNA (mRNA) stores that accumulate during their follicular development to support the transcriptionally dormant events of meiosis. However, during maternal ageing, oocytes are known to experience alterations in mRNA abundance: a phenomenon that contributes to reduced developmental potential. Here we have investigated whether small non-protein-coding RNA (sRNA) accumulation is similarly altered in aged mouse oocytes. Further, we assessed whether such changes could potentially influence gene expression in aged oocytes. High throughput sRNA sequencing revealed substantial changes to the profile of the global sRNA population of germinal vesicle (GV) stage oocytes from young (4-6 weeks) and aged mice (14-16 months). Among the changes documented, 160 endogenous small-interfering RNAs (endo-siRNAs) and 10 microRNAs were determined to differentially accumulate within young and aged oocytes. We further demonstrated that these differentially accumulated endo-siRNAs putatively targeted 39 unique mRNAs. More specifically, we discovered that the encoding transcripts of two members of the kinesin protein family, *Kifc1* and *Kifc5b*, were selectively targeted for expression regulation by three endo-siRNAs of elevated abundance in aged GV oocytes. Accordingly, we also revealed a reciprocal decrease in *Kifc1* and *Kifc5b* mRNA expression as well as a reduction of their encoded protein, HSET, in aged GV and metaphase I stage oocytes. The implications of a reduction in functional HSET protein was explored using complementary siRNA-mediated knockdown of *Kifc1* and *Kifc5b* and pharmacological inhibition of HSET from the GV stage, both of which led to increased rates of aneuploidy in otherwise healthy young metaphase II oocytes after *in vitro* maturation. Taken together, our data raise the possibility that an altered sRNA profile, specifically an altered endo-siRNA profile, could contribute to the age-related decline in oocyte quality.

### Determining the role of PRC2 in female germline epigenetic programming and offspring health

**Ellen Jarred**<sup>1,2</sup>, **Tesha Tsai**<sup>1,2</sup>, **Heidi Bildsoe**<sup>1,2</sup>, **Stephen Pederson**<sup>3</sup>, **David Adelson**<sup>3</sup>, **Jessica Stringer**<sup>4</sup>, **Patrick Western**<sup>1,2</sup>

1. Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia

3. Bioinformatics and Computational Genetics, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia

4. Biomedical Discovery Institute, Monash University, Clayton, Victoria, Australia

Epigenetic modifications modulate cell differentiation and lineage specification in multicellular organisms in part by regulating transcription of developmental genes. While it has been proposed that epigenetic programming of germ cells is critical for offspring development and post-natal health, the mechanisms involved are poorly understood. As environmental factors, such as drugs or diet, are proposed to alter germline epigenetic programming understanding these mechanisms is essential. Polycomb Repressive Complex 2 (PRC2) is an epigenetic modifier that catalyses the epigenetic modification, H3K27me3 and represses developmental gene expression in many tissues, including the germline. Using genetic models that lack PRC2 function in the oocyte, we are examining how H3K27me3 establishment is regulated in the maturing oocyte. We demonstrate that oocytes are enriched with H3K27me3 during their growth and that genetic deletion of PRC2 activity alters offspring growth and development. Consistent with this, *de novo* germline mutations in PRC2 subunits EZH2 and EED result in Weaver or Cohen-Gibson Syndromes in humans, characterised by overgrowth, skeletal abnormalities and learning deficits. Using genetic and pharmacological approaches to deplete EED and EZH2 in oocytes, we are determining how these critical PRC2 catalytic and structural components epigenetically program oocytes and consequently regulate growth and development in offspring. Transcriptional analysis of oocytes lacking *Eed* demonstrates that a primary function of PRC2 is to repress developmental genes in oocytes. However, comparison of offspring phenotypes from mice lacking *Eed* or *Ezh2* demonstrate that these PRC2 components differentially regulate growth outcomes in offspring. Comparison of oocytes lacking *Eed* or *Ezh2*, or that have been subjected to EZH2 inhibiting drugs is revealing how targeting these different PRC2 proteins results in differential outcomes in offspring development. This is critical for determining how exposure to clinically relevant EZH2 inhibiting drugs impact on oocyte epigenetic programming, and consequent health and development in offspring.

## Real-world experience with lenvatinib for radioactive iodine refractory differentiated thyroid carcinoma with analyses of prognostic biomarkers for survival outcomes: Korean multicenter study

**Eyun Song<sup>1</sup>, Mijin Kim<sup>2</sup>, Eui Young Kim<sup>3</sup>, Bo Hyun Kim<sup>2</sup>, Dong Yeob Shin<sup>4</sup>, Ho-Cheol Kang<sup>5</sup>, Byeong-Cheol Ahn<sup>6</sup>, Won Bae Kim<sup>1</sup>, Young Kee Shong<sup>1</sup>, Min Ji Jeon<sup>1</sup>, Dong Jun Lim<sup>7</sup>**

1. Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

2. Pusan National University Hospital, Busan

3. Dongnam Institute of Radiological and Medical Sciences Cancer Center, Busan

4. Severance Hospital, Yonsei University College of Medicine, Seoul

5. Chonnam national university hwasun hospital, Chonnam

6. Kyungpook national university hospital, Kyungpook

7. Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul

**Background:** Lenvatinib is the latest addition to the treatment options for radioactive iodine (RAI)-refractory differentiated thyroid carcinoma (DTC). This study assessed the efficacy of lenvatinib in real-world practice and identified prognostic biomarkers for survival outcomes.

**Methods:** A multicenter cohort study was conducted in 43 patients receiving lenvatinib, as 1<sup>st</sup>-line or 2<sup>nd</sup>-line after sorafenib, for RAI-refractory DTC. Progression-free survival (PFS) was evaluated according to various clinical factors including thyroglobulin doubling time (TgDT), tumor volume DT (TVDT), and tumor growth slope (TGS, slope of tumor change rate).

**Results:** Thirty-two patients were previously treated with sorafenib. Patients were treated with lenvatinib for 14 months (median) and the median starting dose was 20 mg with reduction to a maintenance dose of 10mg during follow-up. The median PFS was 21.8 months and median OS was not reached. Disease control rate was 97.7% with time to the first objective response of 3.8 months. The PFS according to previous sorafenib treatment, metastatic sites, or maintenance dose did not exhibit any difference. However, TGS measured before (TGS<sub>pre</sub>) and after (TGS<sub>post</sub>) the initiation of lenvatinib were associated with PFS (TGS<sub>pre</sub> p=0.003; TGS<sub>post</sub> p=0.036). Other factors associated with PFS were the sum of the largest diameters of target lesions (p=0.043) and TgDT (p=0.024). However, TVDT calculated before (TVDT<sub>pre</sub>) and after (TVDT<sub>post</sub>) lenvatinib treatment did not portend any impact on PFS (p=0.923 and p=0.966, respectively). Withdrawal of lenvatinib occurred in 21 patients with 7 patients due to lenvatinib-induced adverse events (AEs). AEs of any grade were reported in all patients and AEs of grade 3-4 occurred in 13.9%. The most common AE was hypertension.

**Conclusions:** Our results support the efficacy of lenvatinib for patients with RAI-refractory DTCs. Measuring the TgDT and TGS can assist in predicting the clinical outcomes in these patients. Although AEs occur frequently, most of them were manageable.

## Multimodality treatment improves loco-regional control and overall survival in patients with anaplastic thyroid cancer

**Pascal Jonker<sup>1</sup>, John Turchini<sup>2</sup>, Anthony Gill<sup>3</sup>, Thomas Eade<sup>4</sup>, Roderick Clifton-Bligh<sup>5, 6, 7</sup>, Bruce Robinson<sup>5, 6, 7</sup>, Venessa Tsang<sup>5, 6, 7</sup>, Matti Gild<sup>5, 6, 7</sup>, Diana Learoyd<sup>7</sup>, Ahmad Aniss<sup>1</sup>, Stan Sidhu<sup>1</sup>, Leigh Delbridge<sup>1</sup>, Mark Sywak<sup>1</sup>**

1. Endocrine Surgery, Royal North Shore Hospital, Sydney, NSW, Australia

2. Pathology Department, Royal North Shore Hospital, Sydney, NSW

3. Department of Pathology, Royal North Shore Hospital, Sydney

4. Radiation Oncology, Royal North Shore Hospital, Sydney, NSW

5. Kolling Institute of Medical Research, St Leonards, NSW, Australia

6. Endocrinology Department, Royal North Shore Hospital, St Leonards, NSW, Australia

7. University of Sydney, Sydney

**Background:** Anaplastic thyroid cancer (ATC) is an aggressive form of thyroid cancer with poor overall survival (OS). The ideal treatment for ATC is not clear and benefits of targeted molecular agents/immunotherapy is not well established. Multimodality treatment (MMT) may reduce locoregional tumor progression (LTP) and improve OS when compared to treatment with palliative intention (TPI). Expanding MMT with targeted therapy/immunotherapy may further improve OS. This study aims to compare survival outcomes and morbidity between ATC patients treated with MMT and TPI. Additionally, the effect of MMT +/- targeted therapy/immunotherapy on OS in ATC was assessed.

**Methods:** We performed a retrospective (1979-2018) cohort study of ATC patients within a single tertiary hospital referral centre. Patients were assigned to either TPI or MMT following MDT discussion and their own preference. The primary outcome measure was LTP. Secondary outcome measures were one-year OS and major treatment related morbidity defined as grade IV events.

**Results:** 67 patients had sufficient follow-up data for inclusion. Disease was staged: Stage IVA (n=1), IVB (n=31) or IVC (n=34) ATC. Patients were treated with MMT (n=33) or TPI (n=34). LTP was lower (p=0.02) in patients receiving MMT (46%) when compared to TPI (74%). MMT increased one-year OS to 43% compared to 0% in TPI (p<0.0001). There was no difference in major complications between the MMT (15%) or TPI (23%) cohort (p=0.54). A combination of MMT, targeted therapy and/or immunotherapy (n=5) increased one-year survival in Stage IVC ATC to 60%, compared to 33% or 0% in patients receiving MMT without targeted therapy and/or immunotherapy (n=11) or PI (n=18) respectively (p < 0.0001).

**Conclusion:** MMT reduces LTP and improves OS in selected ATC patients without increasing morbidity. The addition of targeted molecular therapy or immunotherapy to multimodality treatment protocols is associated with improved OS in ATC patients.

## Lenvatinib for radioiodine refractory thyroid cancer: real-world experience in southern Taiwan

**Yen-Hsiang Chang<sup>1</sup>, Pei-Wen Wang<sup>2,1</sup>, He-Jiun Jiang<sup>3</sup>, Pi-Jung Hsiao<sup>3</sup>**

1. Nuclear Medicine Department, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan

2. Division of Endocrinology and Metabolism, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan

3. Division of Endocrinology and Metabolism, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

### Background

Lenvatinib has significant antitumor effect on Radioiodine-refractory differentiated thyroid cancer (RRDTC). In the phase III SELECT trial, Lenvatinib showed a significant improvement in progression free survival (PFS) in patients with RRDTC compared to placebo. However, almost all of the participants experienced adverse effects. In the present study, we retrospectively evaluate the efficacy and safety in patients with RRDTC in the real-world practice.

### Patients and Methods

Chart records and images of 51 consecutive RRDTC patients treated with Lenvatinib from July 2016 to May 2019 in two tertiary medical centers in southern Taiwan were retrospectively reviewed. The primary objective was the progression-free survival (PFS) according to Response Evaluation Criteria In Solid Tumors v1.1 (RECIST 1.1). Disease control was defined as objective tumor response plus stable disease. The safety assessment was regularly performed.

### Results

The median PFS was not reached during follow-up. Partial responses (PR) and stable disease (SD) were achieved in 12 (24%) and 31 (61%) patients, respectively. Disease control (PR+SD) of >6 months was achieved in 31 (61%) patients. Almost all participants experienced adverse events. The mean daily dose of Lenvatinib was 11.1 mg, and dose interruptions/withdrawal due to adverse events in 26 (51%) of patients. The most common adverse events (any grade) in lenvatinib-treated patients were fatigue/asthenia/malaise (67%), hypertension (60%), and proteinuria (58%).

### Conclusion

These results confirmed that Lenvatinib is an effective treatment for patients with RRDTC, even at a dose far lower than the recommended. Adverse events are frequently occurred and should be identified and managed appropriately.

## Redifferentiation therapy for metastatic follicular / poorly differentiated thyroid cancer utilising I124 PET/CT scan

**Michael Mond<sup>1,2</sup>, Ian Jong<sup>1</sup>, Jimmy Shen<sup>1,2</sup>, Jennifer Wong<sup>1</sup>, Navira Samad<sup>1</sup>, Peter J Fuller<sup>1,2</sup>**

1. Monash Health, Clayton, VIC, Australia

2. Hudson Institute, Clayton, VIC, Australia

A 56 year-old Samoan man underwent thyroidectomy in 2015 for a suspicious thyroid nodule. Comorbidities included obesity, diabetes, hypertension, CKD with proteinuria and IgA paraprotein. Histopathology showed a 50mm follicular carcinoma with vascular invasion and areas of poorly differentiated thyroid cancer. Mutation testing revealed NRAS Q61R mutation.

He was treated with 100mCi of radioiodine (RAI) with recombinant TSH stimulation. The post-dose scan showed iodine uptake in the thyroid bed with two 3mm non-avid lung nodules.

Subsequently, he was treated with 148mCi of RAI due to rising thyroglobulin and progressive subcentimetre lung metastases. The post-dose scan showed iodine avidity in the majority of pulmonary nodules. Imaging 6 months post-therapy indicated stability of pulmonary nodules.

However, rapidly rising thyroglobulin was noted 12 months post-therapy. CT-scan showed recurrence underlying the thyroidectomy scar, nodal enlargement and progression of pulmonary metastases. He underwent resection of local recurrence and a large lymph node metastasis. Unstimulated thyroglobulin fell from 503 preoperatively to 220. Subsequent imaging demonstrated further progression of pulmonary metastases.

Taking into account small volume metastases, mutation status and multiple comorbidities, a trial of redifferentiation therapy was initiated with trametinib 2mg daily for 4 weeks. Apart from lethargy and myalgias necessitating brief dose interruption, this was well tolerated. Pre and post redifferentiation therapy I-124 PET/CT scans performed following thyroxine withdrawal demonstrated significantly increased iodine avidity post-therapy in thyroid bed, left nodal and pulmonary metastases. The patient was treated with 192mCi RAI.

Restaging PET/CT scan 6 months post-therapy showed a favourable response with interval reduction in a majority of pulmonary nodules and nodal metastases. Unstimulated Tg fell from 220 to 153.

### Conclusion

RAI following redifferentiation therapy should be considered in appropriately selected thyroid cancer patients with seemingly iodine refractory progressive disease. It can delay disease progression and avoids side effects associated with long-term tyrosine kinase inhibitor therapy.

## A retrospective evaluation of the benefits of radioiodine therapy for metastatic poor differentiated thyroid cancer

**Yasushi Noguchi<sup>1</sup>**

1. Department of Radiology & Nuclear Medicine, Noguchi Thyroid Clinic & Hospital Foundation, Beppu, Oita, Japan

The benefits of radioiodine therapy (RIT) for metastatic poor differentiated thyroid cancer (PDTC) not quite clear, so we made a retrospective evaluation of the outcome of RIT for metastatic PDTC.

Through May 2007 to April 2012, we experienced 11 cases of RIT for metastatic PDTC. 3 males and 8 females with an average age of 67.7 at the point of RIT. In 6 cases metastasis were detected before thyroidectomy and in the remaining 5 cases were detected while follow up. The average age of each group at the time of surgery was 72.2 and 54.9, at the time of RIT 72.2, 62.4. All cases had an administration of 3700MBq (100mCi) of radioiodine for initial RIT. 6 out of the 11 cases had radioiodine avid lesions and 5 cases had non-avid lesions. The mean follow up period after surgery was 113.4 month (10-312), after RIT was 71.0 month (7-139).

During follow up, 6 out of 11 cases died due to thyroid cancer and 4 cases had progressive disease with increasing metastasis or elevating serum Tg. Only 1 case was controlled. 5 out of 6 cases that have died were cases with metastasis at the time of thyroidectomy. In the cases detected while follow up, only one died, although 3 cases had progressive disease. From a RIT point of view, 3 died and 2 had progressive disease in the cases with non-avid lesions, but 3 out of 6 died and 2 had progressive disease in cases with radio iodine avid lesions. Radioiodine avidity did not have any impact to the outcome.

Although RIT is still considered as the first line treatment for metastatic PDTC RIT does not seem to have a large impact for metastatic PDTC.

## Detection of Metastatic Sporadic Medullary Thyroid Carcinoma using PET/CT with Radiolabelled Gastrin Analogue

**Rinky Giri<sup>1</sup>, Chris J Yates<sup>1,2</sup>, Mohammed Haskali<sup>3</sup>, Rod J Hicks<sup>2,3</sup>**

1. Department of Endocrinology and Diabetes, Western Health, St Albans, Victoria, Australia

2. Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

3. Cancer Imaging, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Medullary thyroid carcinoma(MTC) accounts for 2-8% of thyroid carcinoma<sup>1</sup>. The production of calcitonin and carcinoembryonic antigen(CEA) are characteristic features of MTC<sup>1,2</sup>. Conventional radiographic modalities for detecting metastatic MTC have been Computerised Tomography(CT), Magnetic Resonance Imaging, 18-fluoro-2-deoxyglucose positron-emission-tomography(FDG-PET), single-photon-emission-computed-tomography and somatostatin-receptor scintigraphy<sup>3,4</sup>.

We report a case of a 36-year-old female with sporadic MTC found to have a pericardial metastatic lesion on PET/CT using gallium-labelled gastrin analogue. She presented at 29 weeks' gestation with left sided neck mass with no evidence of thyroid dysfunction, pheochromocytoma or hypercalcemia. Fine needle aspirate was suspicious for MTC. Initial calcitonin level was 191pmol/L(reference-range <5) and CEA was 92pmol/L (reference-range <3.4). She underwent a total thyroidectomy and lymph node dissection. Histopathology demonstrated a 40-mm MTC without regional nodal involvement. Gene testing for RET Proto-oncogene was negative. Baseline staging with CT Chest/Abdomen/Pelvis showed no evidence of lymphadenopathy or metastasis. Nadir of calcitonin and CEA three months post thyroidectomy was 21.73pmol/L and 8.9pmol/L respectively. 20 months post thyroidectomy marked elevation was noted in calcitonin to 104pmol/L and CEA to 21pmol/L with no evidence of recurrence on thyroid ultrasound. Repeat staging CT Chest/Abdomen/Pelvis found no local recurrence and a pericardial nodule 11X9mm. Further staging with gallium-68 labelled with minigastrin analogue(CPO4) PET/CT showed positive tracer uptake in this pericardial node. Patient underwent robotic-assisted excision of this internal mammary lymph node. Histology was consistent with metastatic MTC.

Cholecystokinin-2/gastrin(CCK-2) receptor overexpression is found in over 90% of MTC<sup>5,6,7</sup>. The new gastrin analogue CPO4 has shown stability and affinity to CCK-2 receptor in vitro and safety in vivo<sup>7</sup>. CPO4-labelled analogues have demonstrated to visualize all active disease sites in MTC compared to other modalities<sup>8</sup>. Hence CCK-2/gastrin receptor imaging proves to be a valid and efficient diagnostic method in staging and follow up of patients with MTC facilitating early and accurate localization of the disease<sup>5,6,7,8</sup>.

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## Gender- and age- differences of seasonal changes in thyroid function in healthy subjects in Japan

**Sayaka Yamada<sup>1</sup>, Yasuyo Nakajima<sup>2</sup>, Masako Akuzawa<sup>3</sup>, Koji Sakamaki<sup>3</sup>, Emi Ishida<sup>2</sup>, Tsugumichi Saito<sup>2</sup>, Eijiro Yamada<sup>2</sup>, Atsushi Ozawa<sup>2</sup>, Yohnosuke Shimomura<sup>3</sup>, Isao Kobayashi<sup>3</sup>, Yoshitaka Andou<sup>3</sup>, Masanobu Yamada<sup>2</sup>**

1. *Maebashi Red Cross Hospital, Maebashi, Gunma, Japan*

2. *Gunma University School of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Maebashi, Gunma, Japan*

3. *Hidaka Hospital, Takasaki, Gunma, Japan*

**Aim:** Thyroid hormone affects whole-body metabolisms, such as lipids and glucose metabolism. Accurate evaluation of thyroid function is therefore important. In this study, seasonal changes in thyroid function in over 14,000 normal subjects were investigated, and the effects of gender and age, smoking were also examined.

**Subjects and methods:** The subjects were participants of medical checkups at Hidaka Hospital from 2006 to 2013. Exclusion criteria were a past history of thyroid disease, steroid use, renal failure and liver cirrhosis. We examined 8,489 men and 5,534 women whose blood TSH and FT4 levels were within the reference values for monthly changes in thyroid function. All blood TSH and FT4 levels were measured at 8 ~ 9:00 AM after over night fasting.

**Results:** The average age were 49 ± 10 years old in men and 48 ± 10 years old in women. In men the highest median TSH level was 1.6 µU/ml in January, and the lowest was 1.3 µU/ml (p <0.01) in June, July, August and September. These values were significantly different from the yearly median value of 1.4 µU/ml in April and November. On the other hand, the FT4 level of men was significantly lower in August than in the other months except for February. The extents of seasonal changes in TSH levels were reduced in women and in elderly men. Furthermore, in men the median blood TSH level of 2,262 smokers was lower than that of 4,709 non-smokers (median: smoker, 1.2 vs. non-smoker, 1.5), and the seasonal changes in TSH and FT4 levels were reduced in smokers.

**Conclusions:** This is the first report in large healthy cohort demonstrating that there are significant differences on seasonal changes in thyroid function by gender, age and smoking. These need to be taken into account for accurate evaluation of thyroid function.

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## The relationship between population's iodine status, thyroid function and water iodine in China

**Hongmei S Shen<sup>1</sup>, Peng L Liu<sup>1</sup>, Yang D Du<sup>1</sup>**

1. *Harbin Medical University, Harbin, HEILONGJIANG, China*

**Introduction:** In 2017 and 2018, the national investigation of drinking water iodine distribution was carried out in China. Thirty-one provinces, 2,936 counties and 40,325 townships were investigated. There were 33716 (83.6%) townships with median water iodine (MWI) <10µg/L; 5559 (13.8%) townships with MWI between 10µg/L and 100µg/L; and 1050(2.6%) townships with MWI>100.0µg/L. According to the Chinese standard, areas with WI<10µg/L are the endemic areas of iodine deficiency disorders, and areas with WI>100µg/L are the water-borne iodine-excess areas, what about areas of WI between 10µg/L and 100µg/L? An investigation were conducted in six provinces concerning relationship between population iodine nutrition, thyroid function and thyroid disease in different water-iodine regions.

**Methods:** Totally, 4665 adults, 2098 children and 403 pregnant women were enrolled in areas with different water iodine levels, <10µg/L, 10-19µg/L, 20-29µg/L, 30-39µg/L, 40-49µg/L, 50-59µg/L, 60-69µg/L, 70-79µg/L, 80-89µg/L, 90-99µg/L and 100-150µg/L. Urinary iodine (UI), WI, thyroid function and volume were measured.

**Results:** With the increase of WI, the UI of adults, children and pregnant women showed an increasing trend. When WI >100µg/L, the MUI of adults exceeded 300µg/L and the MUI of pregnant women exceeded 250µg/L. After data fitting, u-shaped curves were presented between WI and the abnormal rates of TSH, FT<sub>4</sub>, TgAb and TPOAb, goiter rate and thyroid nodule rate, separately. Comparing to WI in other range, the UI of adults, children and pregnant women were all in the appropriate ranges, abnormal rates of TSH, FT<sub>4</sub>, TPOAb and TgAb, goiter rate, thyroid nodule rate were all lower when the WI was in 40~ 100µg/L by comprehensive analysis.

**Conclusions:** When the WI was in 40 ~100µg/L, the main indexes were basically in suitable range, hence, those areas can be considered as the iodine adequate areas.

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## A unique training model in the management of Thyroid disorders for Primary care Physicians (PCPs) in India

**Deepak Monga<sup>1</sup>, Sandeep Bhalla<sup>1</sup>, Pushkar Kumar<sup>1</sup>, Tanu Soni<sup>1</sup>, Arshit Koundal<sup>1</sup>**

1. *Centre for Chronic Conditions and Injuries, Public Health Foundation of India, Gurgaon, Haryana, India*

**Background:** 42 million Indians are affected by either hypothyroidism or hyperthyroidism. 1/3<sup>rd</sup> of patients with hypothyroidism remain undiagnosed due to lack of clinical awareness. PCPs play an important role in the screening and initiation evaluation in these patients. In India, understaffed and overburdened tertiary care along with ill-equipped and poorly skilled primary care system remains the unaddressed concern. This gap can be bridged through training of PCPs.

**Methods:** PHFI implemented a training initiative CCMTD along with Chellaram Diabetes Institute, with educational grant from GSK Pharmaceuticals and 15 national expert panel. This 4 modular course covered various aspects of management of thyroid disorders, with mix of theory, case studies and videos, and was delivered once a month on weekends by 49 faculty at various centres in India. An integrated monitoring and supervision mechanism was established to monitor and evaluate the program and assure training quality.

**Results:** Around 2500 PCPs from 41 cities were trained over 3 cycles; 45% were post graduates and 31% from government sector. The success rate measured by exam conducted at the end of course was 89.9%. This course has been endorsed by Asia and Oceania Thyroid Association for 10 years (2016-2025). Three State governments have already adopted this course for training of their doctors. Following successful run in India, the model has also been adopted in Nepal, Afghanistan, Myanmar and many more are in pipeline..

**Conclusion:** This course is unique since it is probably the only national level thyroid disease training to be undertaken in India. High enrolment and compliance rate reveal an acceptability by the physicians in education on thyroid management.

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### Free T3 but not Free T4, is associated with brown adipose tissue and metabolic rate in healthy young adults

Lijuan Sun<sup>1</sup>, Hui Jen Goh<sup>1</sup>, Priya Govindharajulu<sup>1</sup>, Christiani Jeyakumar Henry<sup>1</sup>, Melvin Khee-Shing Leow<sup>1</sup>

1. Singapore Institute for Clinical Sciences, A\*STAR, Singapore

Background: The role of thyroid hormones after cold exposure and subsequent BAT activation is largely unknown.

Objective: To investigate the relationship between cold induced brown adipose tissue (BAT) and thyroid hormones at baseline and after 2 hours cold exposure and relationship with body composition.

Design: Nineteen healthy subjects (7 males, 12 females) with mean age of 26 years (range: 21-35), BMI of 21.7 kg/m<sup>2</sup> (range: 18.5-26.0) underwent <sup>18</sup>F-FDG PET and whole-body calorimetry after 2 hours of cold exposure (~14.5°C) in a crossover design. Standardized uptake values (SUV-mean) of the region of interest was calculated. Blood metabolites were measured before and 2 hours after cold exposure. Body composition was determined by dual-energy-x-ray absorptiometry (DXA).

Results: 12 of 19 subjects were BAT positive. Subjects were divided into groups based on the presence (n=12) or absence (n=7) of BAT. Subjects with BAT had higher T3, FT3/FT4 compared with subjects without BAT. TSH was reduced and FT4 level was significant increased after acute cold exposure. There was a significant positive correlation between FT3 and lean body mass and resting energy expenditure (REE). EE change from baseline after cold exposure was significantly positive related with FT3 at baseline and 2 hours after cold exposure.

Conclusion: Circulating FT3 was higher in BAT positive subjects and was a stronger predictor of EE changes after cold exposure in healthy humans.

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### Radiofrequency Ablation of Primary Parathyroid Adenoma: Preliminary Results for Patients Ineligible for Surgery

Eun Ju Ha<sup>1</sup>

1. Ajou University Medical Center, Suwon, Gyeonggi-Do, South Korea

Purpose: To retrospectively evaluate the outcomes of ultrasonography (US)-guided radiofrequency ablation (RFA) of parathyroid adenoma in patients who were ineligible for surgery

Materials and method: Between October 2010 and June 2016, six parathyroid adenomas (mean diameter, 2.0 cm; range, 1.2-3.8 cm) in six patients with primary hyperparathyroidism were treated with US-guided RFA by two radiologists in two hospitals. The inclusion criteria for this study were (1) primary hyperparathyroidism, (2) pathologically confirmed parathyroid adenoma on US-guided fine-needle aspiration, and (3) refusal- or ineligibility- for surgery. RFA was performed using a RF generator and 19-gauge internally cooled electrode. The hydrodissection technique using the 5% dextrose water was applied in all patients. The medical records were reviewed and analysed, focusing on the procedural profiles of RFA, symptoms and complications during and after RFA, and changes in hormone levels on follow-up US.

Results: Before RFA, the mean nodule volume was 1.0 ± 0.5 mL. The mean parathyroid hormone (PTH) level was 210.4 ± 283.9 pg/mL and calcium level was 10.4 ± 0.9 mg/dL. At 1- and 6- month follow-up after RFA, a significant reduction in the mean volume (78.4 ± 3.7% and 89.1 ± 8.4 %, respectively) was noted and five ablation zones (5/6, 83.3%) near completely disappeared (<=0.1mL). The mean PTH level was decreased to the normal range (50.9 ± 6.5 pg/mL) at 1-month follow-up and were progressively decreased at 6-month follow-up in 5 patients (40.1 ± 7.3 pg/mL). The PTH level in one patient was re-increased from 48 pg/mL to the 241 pg/mL at 6-month follow-up. The mean calcium level was decreased to 9.3 ± 0.8 mg/dL at 6-month follow-up. There was no immediate complication during- and after- the procedure.

Conclusion: RFA might represent an effective and a safe alternative for managing parathyroid adenomas, especially in patients ineligible for surgery

## Does thyroglobulin concentration accurately reflect maternal iodine status during pregnancy? A systematic review and meta-analysis

**Pantea Nazeri<sup>1</sup>, Sakineh Shab-Bidar<sup>2</sup>, Elizabeth Pearce<sup>3</sup>, Mamak Shariat<sup>4</sup>**

1. Family Health Institute, Breastfeeding Research Center, Tehran University of Medical Sciences, Tehran, Iran

2. Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

3. Section of Endocrinology, Diabetes and Nutrition, Boston University School of Medicine, Boston, USA, Boston, Massachusetts, United States

4. Family Health Institute, Maternal, Fetal and Neonatal Research Center, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran

**Objective:** Literature to date has been inconclusive regarding the value of thyroglobulin (Tg) as a marker of iodine status in pregnant women. This systematic review and meta-analysis is one of the first to assess whether Tg concentration accurately reflects maternal iodine status during pregnancy.

**Methods:** We searched the MEDLINE, the Web of Science, the Cochrane Library, Scopus, and other relevant databases to identify relevant studies published in the English language, between January 1988 and December 2018. Mean or median maternal urinary iodine concentration (UIC) and Tg level, along with other relevant data, were extracted from eligible studies. Both fixed and random effect models were used in a subgroup analysis. Potential linear and nonlinear associations between maternal UIC and Tg concentration were examined.

**Results:** Of 814 identified studies, 25 were eligible for inclusion in the meta-analysis. Included studies were conducted in Africa, Asia, Europe, South America, and the Southwest Pacific Ocean. Iodine sufficiency was reported in the general population in school-aged children in all aforementioned regions except Sudan. The pooled mean (95% confidence interval [CI]) Tg concentration in iodine-deficient pregnant women was higher than that in iodine-sufficient pregnant women (10.58 µg/L [5.45- 15.70] vs. 7.34 µg/L [2.20- 12.47]), but the difference did not reach statistical significance. An inverse association was found between maternal UIC <100 µg/L and Tg concentration ( $P_{\text{linearity}} = 0.007$ ;  $P_{\text{nonlinearity}} = 0.027$ ); however, higher levels of UIC were not associated with Tg levels during pregnancy.

**Conclusions:** We found that high Tg concentrations accurately reflected iodine deficiency (UIC <100 µg/L) in pregnant women. Our findings reaffirm the Tg cutoff point of 10 µg/L as an appropriate value to define iodine status in pregnant women. Further studies are warranted to determine the sensitivity of Tg at different degrees of iodine deficiency during pregnancy.

## Sonographic Evolution of Benign Nonfunctioning Thyroid Nodules After Ultrasound Guided Microwave Ablation

**Shuhang Xu<sup>1</sup>, Mo Chen<sup>1</sup>, Xiaoqiu Chu<sup>1</sup>, Chao Liu<sup>1</sup>**

1. Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

**Objective:** To investigate the safety and efficacy of ultrasound-guided percutaneously microwave ablation (MWA) on benign nonfunctioning thyroid nodules.

**Methods:** A total of 41 patients with 41 benign thyroid nodules were treated by MWA in Jiangsu Province Academy of Traditional Chinese Medicine from January 2016 to December 2017. The changes of thyroid nodule volume, effective rate, thyroid function and the incidence of complications were observed respectively before treatment as well as at the postoperative 1, 6, 12 months follow-up, respectively.

**Results:** The median volume decreased from 5.88 ml to 0.65 ml. The mean volume reduction rate after 12 months was  $82.76 \pm 12.68\%$ , and the effective rate was eventually 100%. No serious complications were observed. Thyroid function and autoimmune antibody levels were not affected by MWA, however, serum thyroglobulin level was decreased after MWA. Compared with baseline, the level of ACR TI-RADS of thyroid nodules increased from TR3 to TR4 at 6 months follow-up significantly ( $P < 0.001$ ).

**Conclusion:** MWA has a good safety and efficacy in treating benign nonfunctioning thyroid nodules.

## Oral liquid L-thyroxine (L-T4) treatment in patients thyroidectomized for thyroid cancer (without malabsorption)

**Poupak Fallahi<sup>1</sup>, Silvia Martina Ferrari<sup>2</sup>, Ilaria Ruffilli<sup>2</sup>, Francesca Ragusa<sup>2</sup>, Giusy Elia<sup>2</sup>, Sabrina Rosaria Paparo<sup>2</sup>, Gabriele Materazzi<sup>3</sup>, Paolo Miccoli<sup>3</sup>, Alessandro Antonelli<sup>2</sup>**

1. Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

2. Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

3. Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy

In this study we enrolled 160 patients who had been recently subjected to total thyroidectomy, not reporting malabsorption or drug interference issues. Our aim was to study the efficacy of levothyroxine (L-T4) liquid formulation in comparison to L-T4

tablets, therefore 80 patients received liquid L-T4 formulation, whereas 80 took L-T4 tablets using the same dosage (1.5 mcg/kg/day). The treatment with L-T4, administered 30 min before breakfast, started the day after surgery. In both groups were assessed: thyrotropic hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3), after 6 weeks (1st control), and then after 12 weeks (2nd control). We observed significantly lower TSH levels in the liquid L-T4 group with respect to the tablet L-T4 ones, either for the first ( $P < 0.05$ ) and for the second control ( $P < 0.01$ ), while no significant differences arose for FT4 and FT3. Moreover the prevalence of hypothyroid patients (TSH>3.6 mcU/ml) was higher in the L-T4 tablet group with respect to the liquid L-T4 group.

These results indicate a better control of TSH levels by liquid L-T4 (vs. L-T4 tablets) in patients previously subjected to total thyroidectomy for thyroid cancer without issues of malabsorption, drug interference, or gastric disorders.

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## Molecular imaging of endocrine tumours

**David Pattison<sup>1</sup>**

1. Royal Brisbane & Women's Hospital, Herston, QLD, Australia

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## Neuroendocrine Tumours: the role of the endocrinologist

**Roderick Clifton-Bligh<sup>1,2</sup>**

1. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Cancer Genetics Laboratory, Kolling Institute, Royal North Shore Hospital, Sydney, NSW, Australia

Neuroendocrine tumours (NETs) are characterized by the presence of neurosecretory granules and most commonly include tumours of the intestine (carcinoids), endocrine pancreas, lung, thymus, adrenal medulla, paraganglia, thyroid C cells, parathyroid and pituitary gland. Due to the heterogeneous location and behaviour of NETs, they are best managed in a multidisciplinary setting. The role of the endocrinologist within this team includes: 1. diagnosis and treatment of functioning NETs; 2. diagnosis, longitudinal follow-up and appropriate genetic triage of heritable NET syndromes (multiple endocrine neoplasia (MEN) syndromes 1 and 2, von Hippel Lindau, hereditary paraganglioma syndromes); and 3. management of endocrine complications of treatment, in particular diabetes mellitus. This presentation will focus on specific endocrine presentations of NETs including functioning pancreatic NETs, MEN1 and carcinoid crises.

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## Neuroendocrine tumours - systemic treatments past and present

**David Chan<sup>1</sup>**

1. Royal North Shore hospital, St Leonards, NSW, Australia

Neuroendocrine tumours (NETs) are extremely heterogeneous malignancies marked by their variability in primary site, biological behaviour and the potential production of vasoactive hormones. Multiple systemic options have arisen in the past decade after the publication of positive randomized trials, most recently peptide receptor radionuclide therapy (PRRT/Lutate). However, clinical difficulties remain in terms of sequencing available therapies, and identifying suitable biomarkers to guide treatment selection. This talk will attempt to cover the classification of NETs, the evidence to guide various therapies and the increasing role of PET scans in elucidating tumour biology and driving treatment decisions.

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## Linking Imaging Phenotype with Genotype in Neuroendocrine Neoplasia

**Rod Hicks<sup>1</sup>, Andrew Pattison<sup>1</sup>, Shiva Balachander<sup>1</sup>, Alex Caneborg<sup>1</sup>, Kelly Waldeck<sup>1</sup>, Athena Hatzimihalis<sup>1</sup>, Carleen Cullinane<sup>1</sup>, Annette Hogg<sup>1</sup>, Richard Tothill<sup>1</sup>**

1. Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia

Neuroendocrine neoplasia (NEN) represents a diverse group of tumours arising in almost every tissue of the body. Variation in the tissue of origin, grade and mutational signature of these tumours has profound impact on their presentation and prognosis. However, because each subtype is individually rare, standard clinical trial designs are difficult and rarely performed leading a lack of evidence on which to base management decisions. This provides a strong argument for a precision-medicine approach based on in-depth characterization of tumour biology in individual patients. Advances in genomic technologies have demonstrated that NEN commonly has a hereditary basis and that sporadic cases also involve common pathways. While the cost of sequencing tumours has decreased markedly in recent years, the heterogeneity that is common in NEN poses a challenge for use of this technology in isolation. Molecular imaging provides a platform for *in vivo* characterization of tumour biology on a whole-body scale with the ability to also monitor the evolution of tractable therapeutic targets. Our research focuses on drawing a link between imaging phenotype and genotype in various NEN, particularly including pheochromocytoma/paraganglioma (PPGL), Merkel cell carcinoma (MCC) of the skin and pancreatic NEN (panNEN). Understanding the drivers of these diseases in individual patients will aid in prognostic stratification, molecular imaging staging and surveillance paradigms and, hopefully, in selecting optimum choice or sequencing of treatment. In PPGL and panNEN, understanding of the process of dedifferentiation is important, while for MCC, better understanding of the role of the immune

system and the differences between disease arising from sun-exposure and that related to polyoma viral infection are key research objectives. How genomics will complement “theranostics” will be discussed.

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## Endometriosis: Recognising the heterogeneity of disease experience and phenotype

**Jane Girling**<sup>1,2</sup>

1. *Gynaecology Research Centre, University of Melbourne Department of Obstetrics and Gynaecology, Royal Womens Hospital, Parkville, VIC, Australia*

2. *Department of Anatomy, University of Otago, Dunedin, New Zealand*

The launching of the National Action Plan for Endometriosis in Australia, 2018, put the spotlight on a debilitating disease estimated to affect 5-10% of reproductive-aged women, or approximately 176 million women worldwide. The disease is characterised by the presence of endometrial-like tissues in ectopic locations, usually within the pelvic cavity. Individuals present with diverse symptoms, particularly fertility issues, severe menstrual pain and chronic pelvic pain, with resultant impacts on the physical, psychological and social wellbeing of affected individuals and their whānau. Recognising the heterogeneity of disease experience, we are currently exploring the needs and wants expressed by women with endometriosis. Our aim is to develop tools that assist clinicians and researchers to improve the quality of life of those with endometriosis.

In addition to the variability in symptom presentation, there is also considerable heterogeneity in the nature and extent of endometriotic lesions. Using multiple approaches, we are aiming to provide detailed phenotypic information about these lesions. This includes the characterisation of standard pathology-based lesion biopsies stained with haematoxylin and eosin and dual immunostaining to outline inter- and intra-patient variability across a range of cell and tissue characteristics. Further, we are employing cutting-edge Fourier-transform infrared spectroscopy (FT-IR) and mass-spectrometry imaging to characterise patterns of metabolites and proteins in endometriotic lesions. While still preliminary, we hope these studies will contribute to our understanding of disease aetiology by linking phenotype to the diverse genetic and expression quantitative trait loci (eQTL) data already available. Ultimately, our ongoing studies are aimed at enhancing our understanding of pathophysiology, identifying diagnostics and improving therapeutic options for those with endometriosis.

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## Efficacy and safety of long-term universal salt iodization on thyroid disorders: an epidemiological evidence from 31 provinces of mainland China

**Weiping Teng**<sup>1</sup>, **Zhongyan Shan**<sup>1</sup>, **Lei Zhao**<sup>1</sup>, **Xiaoguang Shi**<sup>1</sup>, **Yongze Li**<sup>1</sup>, **D Teng**<sup>1</sup>, **Yanbo Li**<sup>2</sup>, **Haiyi Chi**<sup>3</sup>, **Eryuan Liao**<sup>4</sup>, **Chao Liu**<sup>5</sup>, **Libin Liu**<sup>6</sup>

1. *Endocrinology, Department of Endocrinology and Metabolism and the institute of Endocrinology, The First Hospital of China Medical University, Shenyang, China*

2. *Endocrinology, The First Affiliated Hospital of Harbin Medical University, Harbin*

3. *Endocrinology, Hohhot First Hospital, Hohhot*

4. *Endocrinology and Metabolism, The Second Xiangya Hospital, Central South University, Changsha*

5. *Endocrine and Metabolic, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing*

6. *Endocrinology and Metabolism, Fujian Institute of Endocrinology, Fujian Medical University Union Hospital, Fuzhou*

**Background:** The mandatory universal salt iodization (USI) program has been implemented in China for twenty years. Although iodine deficiency disorders are effectively controlled, the dangers of excess iodine have been debated.

**Methods:** A nationally representative cross-sectional study of 78,470 participants, aged 18 or older, were enrolled from all 31 provincial regions of mainland China. The participants were given a questionnaire and B-mode ultrasonography on the thyroid. Serum concentrations of thyroid hormones, thyroid antibodies and urine iodine concentration (UIC) were measured.

**Results:** The median UIC of school-age children was 199.75µg/L. The weighted prevalence of the thyroid disorders in adults were as follows: 0.78% of overt hyperthyroidism, 0.44% of subclinical hyperthyroidism, 0.53% of Graves' disease, 1.02% of overt hypothyroidism, 12.93% of subclinical hypothyroidism, 14.19% of autoimmune thyroiditis, 10.19% of positive TPOAb, 9.70% of positive TgAb, 1.17% of goiter and 20.43% of thyroid nodules. Iodine excess was only associated with higher odds of overt hyperthyroidism and subclinical hypothyroidism, while iodine deficiency was significantly associated with higher odds of most thyroid disorders. In addition, increased iodine intake was significantly associated with elevated serum TSH levels, but was inversely associated with thyroid antibodies and thyroid nodule. **Conclusions:** The long-term mandatory USI program with timely adjustments is successful in preventing iodine deficiency disorders and it appears to be safe. The benefits outweigh the risk in a population with a stable a median iodine intake

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## Unmet clinical needs in thyroid cancer

**Won Bae Kim**<sup>1</sup>

1. *Asan Medical Center, University of Ulsan Medical College, Seoul, South Korea*

The rise in the incidence of thyroid cancer is a world-wide phenomenon. National Cancer Database showed that the incidence of thyroid cancer increased from 7.1 per 100,000 in 2000 to 17.6 per 100,000 in 2013 in United States. During this period, stage IV disease increased 1 per 100,000. Because early diagnosis and treatment should lead to a decrease in metastatic disease, it is regarded as results of over-diagnosis and that more aggressive disease is not being removed by early detection. However,

the situation in Korea suggest a different scenario. We reported that age-standardized mortality rates from thyroid cancer increased until 2004 (from 0.17 per 100,000 in 1985 to 0.85 per 100,000 in 2004) and then continuously decreased until 2015 to 0.42 per 100,000 suggesting the early detection might be responsible for decrease.

However, early detection of thyroid cancer seems not to be cost-effective. So, risk stratification of thyroid cancers is important. Most prognostic factors known are based on those obtainable after surgery.

So, identifying prognostic indicator(s) before treatment is an unmet clinical need for thyroid cancer. I would like to discuss on those factors which our group have recently discovered.

Another unmet clinical need is management of patients with radioiodine refractory (RAI-R) differentiated thyroid cancer (DTC). Though sorafenib and lenvatinib had been available, we need novel drug(s). We recently found that PHGDH, a critical enzyme for serine biosynthesis, could be a novel therapeutic target in thyroid cancer.

We found that the lymphocyte-to-monocyte ratio, which reflects the tumor infiltrating immune cell status and host immunity, is a prognostic marker in predicting the survival of patients with anaplastic thyroid carcinoma, and of RAI-R DTC patients. So, we need to get deep insight into tumor-host interaction in our future research in thyroid cancer for better clinical outcomes.

## Autophagy in the thyroid

**Yuji Nagayama<sup>1</sup>**

1. Department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

Autophagy is a catabolic process that involves the degradation of cellular components through the lysosomal machinery, re-locating nutrients from unnecessary processes to more pivotal processes required for survival. It has been reported that systemic disruption of *Atg5* gene, a component of autophagy, is lethal, and that its tissue-specific disruption causes tissue degeneration in several organs. However, the functional significance of autophagy in the thyroid glands remained unknown. Therefore, we evaluated (i) hormonal regulation of autophagy in the thyroid, and (ii) the consequence of *Atg5* gene knockout in the thyroid morphology and function. First, TSH-regulation of autophagy was evaluated in rat thyroid PCCL3 cells and mice. We found an increase in LC3-II puncta by TSH stimulation, demonstrating TSH-induction of autophagy. Second, *Atg5<sup>lox/lox</sup>* mice were crossed with *TPO-Cre* mice, yielding the thyroid follicular epithelial cell (thyrocyte)-specific ATG5 deficient mice (*Atg5<sup>thyro-KO/KO</sup>*). *Atg5* gene knockout was confirmed by a lack of ATG5 expression, and disruption of autophagy was demonstrated by a decrease in LC3-II puncta and an increase in p62. *Atg5<sup>thyro-KO/KO</sup>* mice were born normally, and thyroid morphology, thyroid weights, and serum T<sub>4</sub> and TSH levels were almost normal at 4 months. However, at 8 and 12 months, although thyroid function was still normal, a decrease in the number of thyrocytes, and an increase in TUNEL<sup>+</sup>-thyrocytes (*i.e.*, apoptotic cells) were observed in *Atg5<sup>thyro-KO/KO</sup>* mice. Number of irregularly shaped follicles (gourd-shaped) was also increased. Excess oxidative stress was indicated by increased 8-OHdG and 53BP1 foci in *Atg5<sup>thyro-KO/KO</sup>* mice. These data demonstrate that (i) thyrocytes gradually undergo degradation/cell death in the absence of basal levels of autophagy, indicating that autophagy is critical for the quality control of thyrocytes, and (ii) TSH stimulates autophagy, probably by responding to increased nutritional demand required for TSH-induced cell proliferation

## Seminal fluid elicits epigenetic modulation in thymus-derived regulatory T cells after mating in mice

**Sarah A Robertson<sup>1</sup>, Lachlan M Moldenhauer<sup>1</sup>, John E Schjenken<sup>1</sup>, Christopher Hope<sup>1</sup>, Bihong Zhang<sup>1</sup>, Ella S Green<sup>1</sup>, Simon C Barry<sup>1</sup>**

1. Robinson Research Institute, The University of Adelaide, Adelaide, SA, Australia

Regulatory T cells (Tregs) are essential for pregnancy tolerance, and the peri-conception phase is critical for their generation at the outset of pregnancy(1). Treg insufficiency causes implantation failure in mice and is linked with infertility and gestational disorders in women. The thymic Treg (tTreg) compartment has not previously been evaluated in pregnancy tolerance. To investigate how tTreg as well as peripheral Tregs (pTregs) respond to male partner seminal fluid, FOXP3<sup>+</sup>CD4<sup>+</sup>Tregs were examined in the uterus and uterus-draining lymph nodes (dLN) in virgin estrus C57Bl/6 mice, and on 3.5 days post-coitum (dpc) after mating with Balb/c males. Flow cytometric analysis showed that mating elicited 5-fold increases in uterine Tregs accompanied by extensive Treg proliferation in the dLN. These cells comprised 70% tTregs identified by neuropilin-1 (NRP1) expression, and 30% NRP1- pTregs. tTregs exhibited a greater numerical increase and higher induction of proliferation marker Ki67 and suppressive competence markers FOXP3 and CTLA4 than pTregs. Analysis using the unbiased machine learning algorithm, t-stochastic neighbour embedding (tSNE) confirmed phenotypically distinct tTreg and pTreg clusters with dominant responsiveness to seminal fluid in the tTreg cluster. Bisulphite sequencing of FACS-sorted CD4<sup>+</sup> T cells revealed increased demethylation of the *Foxp3* locus Treg-specific demethylation region (TSDR) in tTregs but not pTregs after mating. These data show tTreg and pTregs both respond to seminal fluid priming, but greater phenotype attenuation occurs in tTregs, and the *Foxp3* epigenetic signature is uniquely increased in tTregs. We conclude that tTregs are sensitive to local regulation by seminal fluid. The tTreg compartment therefore warrants evaluation for potential to influence fertility and pregnancy health in women.

1. (1) Robertson SA et al, J Clin Invest 128:4224-35, 2018

## Activin C inhibits ovarian and prostate cancer cell growth and expression is decreased in higher grade tumours

**Karen L Reader<sup>1</sup>, David G Mottershead<sup>2</sup>, Helen D Nicholson<sup>1</sup>**

1. *University of Otago, Dunedin, Otago, New Zealand*

2. *School of Pharmacy, Keele University, Newcastle-under-Lyme, Staffordshire, United Kingdom*

Activins regulate the growth and differentiation of many tissues and expression has been shown to be dysregulated in some cancers. Reports of the effects of activin A on ovarian and prostate cancer cell growth are contradictory and little is known about the response of ovarian and prostate epithelial cells to activins B and C. We examined the effect of purified, full-length recombinant activin C on the growth and migration of ovarian and prostate cancer cell lines. The levels of activin A, B and C protein expression were compared between human epithelial ovarian cancer tumors of different International Federation of Gynecology and Obstetrics (FIGO) stages and between prostate adenocarcinomas with different Gleason scores using a digital immuno-reactive scoring method. The effect of activin A, activin C and their combination on gene expression in Ovar3 cells was assessed using the NanoString nCounter Pan Cancer Pathway panel. Activin C treatment decreased cell number in three ovarian cancer cell lines and three prostate cell lines, but increased cell number in LNCaP cells. It had no effect on the migration of ovarian cancer cells. All three activins exhibited decreasing immuno-reactive scores with increasing FIGO stage in ovarian tumours. The immuno-reactive scores for activin B were generally higher in higher grade prostate tumours while activin C expression appeared to decrease with increasing Gleason pattern. Differential gene expression analysis showed that activin A and activin C have opposing effects on molecular pathways involved in cancer progression, including the DNA repair, chromatin modification, TGF $\beta$  and hedgehog pathways. Activin C increased expression of BNIP3 mRNA and increased activated caspase 3/7 in Ovar3 cells, suggesting this protein can inhibit cancer cell growth by increasing apoptosis. These findings highlight a potential prognostic and therapeutic role for activin C in ovarian and prostate cancer.

## A unique maternal immune response to insemination: Cell-in-cell structures formed in the uterine epithelium following fertilisation

**Chad L Moore<sup>1</sup>, Gerald J Shami<sup>1</sup>, Samson N Dowland<sup>1</sup>, Laura A Lindsay<sup>1</sup>, Filip Braet<sup>1</sup>, Christopher R Murphy<sup>1</sup>**

1. *Discipline of Anatomy and Histology, The University of Sydney, Sydney, NSW, Australia*

Following mating, leukocytes are recruited to the uterine epithelium where they phagocytose spermatozoa and mediate maternal immune tolerance as well as a mild inflammatory response. However, the precise mechanisms of antigen presentation to establish tolerance in the uterus are not yet known. In this study we utilised array tomography, a high-resolution volume scanning electron microscopy technique to 3D reconstruct the cellular relationships formed by leukocytes in the uterus after mating.

We discovered for the first time that 12 h post fertilisation in the rat, recruited neutrophils are internalised within uterine epithelial cells (UECs). We generated a 3D model from 200 serial SEM images which confirmed that neutrophils are internalised within a large vacuole contained wholly within the cytoplasm of UECs. This is the first description of cell-in-cell structures forming in the endometrium after mating. Material from the uterine lumen appears to be transported into these structures via UEC transcytosis, where it is phagocytosed by the neutrophils. This is suggested to represent a key mechanism of antigen presentation to the maternal immune system at the beginning of pregnancy.

While cell-in-cell structures have previously been observed to occur *in vivo* and *in vitro* in normal and pathological conditions, this observation represents the first evidence of the formation of cell-in-cell structures within the uterine epithelium during normal pregnancy. This is also the first confirmed description of heterotypic cell-in-cell interaction between neutrophils and epithelial cells. This novel observation of neutrophils internalised within uterine epithelial cells may represent the site of antigen presentation that occurs as part of the mediated maternal immune response to semen, which is essential for the development of maternal tolerance to paternal antigens and successful pregnancy.

## Elemental metabolomics to identify risk factors and pregnancy outcomes at 18 weeks gestation

**Daniel R McKeating<sup>1</sup>, Vicki L Clifton<sup>2,3</sup>, William W Bennett<sup>4,5</sup>, Joshua J Fisher<sup>1</sup>, Ping Zhang<sup>1</sup>, Tony V Perkins<sup>1,6</sup>**

1. *Griffith, Gold Coast, QLD, Australia*

2. *Pregnancy and Development Group, Mater Research, Brisbane, QLD, Australia*

3. *Translational Research Institute, Brisbane, QLD, Australia*

4. *School of Environment and Science, Griffith University, Gold Coast, Queensland, Australia*

5. *Department of Biology, University of Southern Denmark, Nordcee, Odense, Denmark*

6. *Cambridge University, Cambridge, England*

Elemental nutrition is associated with a range of perinatal outcomes. Fetal outcomes such as birth weight, head circumference, placental weight, and preterm birth have all been associated with proper infant growth and development, the ability to identify risk factors early is vitally important. This project aims to apply elemental metabolomics in plasma and urine to identify

relationships between elemental concentrations and pregnancy risk factors, using this information to determine gestational outcomes.

Plasma and urine samples were obtained from a cohort of 18-week pregnant women from the Lyell McEwin Hospital (Adelaide, Australia). Inductively coupled plasma mass spectrometry was used to measure 29 elements in plasma and urine from 117 patients (69 control, 48 complicated).

Male offspring were found to have higher concentrations of magnesium and strontium in plasma compared to females (5.3% and 10.1% respectively). Babies with birth weights in the lowest quartile had significantly lower urinary titanium, cobalt, copper, and plasma molybdenum than the middle 50<sup>th</sup> percentile (21.1 - 46.0%). Smaller head circumference was associated with lower cobalt, strontium, lead, and plasma barium (28.9 - 55.7%). Placental weight, small for gestational age (SGA), and preterm birth were able to be predicted at 18 weeks using a combination of elements and analysed by receiver operating characteristic curves. Increased selenium and molybdenum in plasma predicted a placenta under 500 g with 89% accuracy. Babies born SGA were identified 88% of the time using the ratio of copper to strontium in plasma. In 80% of cases, urine thallium and iodine could distinguish preterm birth from term pregnancies.

This study is the first to apply elemental metabolomics early in gestation to predict birth outcomes. This approach could be applied as an early predictor of specific gestational outcomes and provide insight into the mechanisms that drive these complex disorders.

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### **Prematuration culture with c-type natriuretic peptide improves cumulus function and oocyte quality during minimal stimulation IVM in mice**

**Dulama Richani<sup>1</sup>, Yiqing Zhao<sup>2,1</sup>, Xiuhua Liao<sup>3,1</sup>, Adriana E Krysta<sup>1</sup>, Michael J Bertoldo<sup>1,4</sup>, Anne Poljak<sup>5</sup>, Robert B Gilchrist<sup>1</sup>**

1. Fertility and Research Centre, School of Women's and Children's Health, University of New South Wales Sydney, Kensington, New South Wales, Australia

2. Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

3. Reproductive Medicine Center, Fujian Provincial Maternity and Children's Hospital, Fuzhou, China

4. Laboratory for Ageing Research, School of Medical Sciences, University of New South Wales Sydney, Kensington, New South Wales, Australia

5. Bioanalytical Mass Spectrometry Facility, University of New South Wales, Kensington, NSW, Australia

Oocyte in vitro maturation (IVM) is an attractive reproductive technology as it brings significant benefits to patients, however it is not practiced widely due to lower success rates than IVF. Oocyte pre-IVM using c-type natriuretic peptide (CNP) is an innovative new IVM system currently undergoing clinical evaluation. This study aimed to determine temporal effects of CNP-mediated pre-IVM on cumulus cell function and oocyte developmental competence in mildly-stimulated mice. Immature cumulus oocyte complexes (COCs) derived from mildly stimulated (23h PMSG) 28-day old mice were cultured for 0-24h in pre-IVM medium (CNP+E2+FSH+insulin), prior to IVM/IVF. Subsequent embryo development and quality post-IVM/IVF was assessed. Day-6 blastocyst rate increased incrementally with increasing pre-IVM duration: 40.6±2.0%, 45.8±1.2%, 52.2±3.5%, 53.3±5.9% and 59.9±2.5% for 0, 2, 6, 12 and 24h pre-IVM, respectively (P<0.01). DNA content/COC, a measure of cumulus cell proliferation, was significantly higher in 24h pre-IVM group compared to 0, 2, and 6, but not the 12h, pre-IVM groups (P<0.001). Pre-IVM for 24h significantly increased cumulus expansion and mRNA expression of matrix genes *Has2* and *Tnfrsf6* relative to no pre-IVM control (P<0.01). Cumulus-oocyte gap-junctional communication (GJC) was maintained throughout 24h pre-IVM (P<0.0001), and GJC loss was slowed during the subsequent IVM phase, whilst meiotic resumption was accelerated (P<0.05). Pre-IVM increased COC ATP and ADP content (P<0.05), but not AMP, ATP/ADP and energy charge. In conclusion, this study demonstrates that CNP-mediated pre-IVM improves the quality of IVM oocytes in a temporally-dependent manner and significantly influences cumulus cell function including increased cell proliferation, cumulus expansion, and prolonged cumulus-oocyte GJC. To identify the protein signatures reflecting enhanced COC developmental competence endowed by CNP pre-IVM, investigation into the oocyte and cumulus cell proteomes using tandem mass spectrometry is currently underway.

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### **Hyperspectral imaging of the early embryo: can it detect aneuploidies?**

**Cheow Yuen (Tiffany) Tan<sup>1,2</sup>, Carl Campugan<sup>1,2</sup>, Jared Campbell<sup>3,4</sup>, Saabah Mahbub<sup>3,4</sup>, Sanam Mustafa<sup>1,2</sup>, Ewa Goldys<sup>3,4</sup>, Jeremy Thompson<sup>1,2</sup>, Kylie Dunning<sup>1,2</sup>**

1. The University of Adelaide, Adelaide, SA, Australia

2. Adelaide Medical School, Robinson Research Institute, ARC Centre of Excellence for Nanoscale of BioPhotonics & Institute of Photonics and Advanced Sensing, University of Adelaide, Adelaide, SA, Australia

3. The Graduate School of Biomedical Engineering, University of New South Wales, Sydney, New South Wales, Australia

4. ARC Centre of Excellence for Nanoscale of BioPhotonics & Institute of Photonics and Advanced Sensing, University of Adelaide, Adelaide, SA, Australia

Despite its wide-spread use, the success rate of assisted reproductive technologies including in vitro fertilization is less than 20%. Most human embryos are mosaic for chromosome abnormalities: containing cells that are euploid (normal) and aneuploid (incorrect number of chromosomes). Mosaicism is thought to account for the high rates of early pregnancy loss in IVF and natural conceptions. Currently, a cell biopsy is used in the IVF clinic to diagnose aneuploidy in the embryo. However, this does not provide an accurate diagnosis of how many cells are aneuploid. Hence, the development of a non-invasive tool to determine the proportion of aneuploid cells would likely improve IVF success. Aneuploidy in human embryos leads to altered



metabolism. Co-factors utilised in cellular metabolism are autofluorescent and can be used to predict the metabolic state of cells. In this study, we utilised a powerful model of embryo mosaicism where mouse embryos were treated with reversine, a reversible spindle assembly checkpoint inhibitor, from the 4- to 8-cell stage. Eight-cell embryos were then dissociated and euploid and aneuploid blastomeres joined to generate euploid, aneuploid and chimeric blastocysts at different ratios. Blastocysts were fixed and assessed by 65-channel hyperspectral microscopy (broad spectral profile) providing an alternative to metabolomics through non-invasively assessing intracellular fluorophores. Using hyperspectral imaging, we were able to identify type I and type II collagen and the co-enzymes NAD(P)H and FAD which together give us the redox ratio (an important measurement of metabolic activity). In euploid embryos, the abundance of NAD(P)H and the redox ratio were significantly higher compared to 1:1 and 1:3 (euploid:aneuploid) chimeric embryos. Using textural-based analysis, euploid, aneuploid and chimeric embryos showed discrete separation by principle component analysis. These data show hyperspectral imaging is able to distinguish cells based on their ploidy status making it a promising tool in assessing embryo mosaicism.

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## Reversing poor gamete quality and protecting embryogenesis in older fathers

**Macarena Gonzalez<sup>1</sup>, Haley Connaughton<sup>1</sup>, Rebecca Robker<sup>1</sup>**

*1. Robinson Research Institute, University of Adelaide, Adelaide, SA, Australia*

Increasing use of ART to treat age-related infertility in men necessitates new therapeutic strategies to improve success rates. Chaperone-inducing drugs improve oocyte quality and embryo development in obese mice, but whether these improve sperm quality has not been investigated. The aim of this study was to determine whether treatment of sperm with a chaperone-inducing drug can improve gamete quality in older fathers.

Sperm from C57BL6 male mice that were either "old" (>14-months-old), or "young" (<8-months-old) was collected and treated in vitro during capacitation. Sperm quality assessments included motility, zona-binding capacity and mitochondrial activity. In parallel, sperm was used for IVF and embryo development was analyzed by time-lapse imaging.

Sperm from older males had reduced motility (N=9-12; P=0.03), lower mitochondrial membrane potential (N=6-11; P=0.04) and impaired zona-binding capacity (N=4-6; P=0.02) compared to younger males. Each of these sperm quality parameters was improved by treatment. When sperm was used for IVF, embryos from old males had delayed time to first cleavage (N=21-27; P=0.01). Sperm from older males gave decreased 2-cell (N=7-12; P=0.04) and blastocyst rates (N=7-12; P=0.003). Drug treatment of 'old' sperm restored embryo development rates to those of sperm from young males.

To test the efficacy of this in vitro treatment in humans, sperm samples from 40 ART patients were treated in vitro for 30 minutes. Sperm from older men (>40 years old) had reduced motility (N=14; P=0.03), as well as increased levels of both mitochondrial ROS (N=10; P<0.0001) and DNA oxidative damage after wash (N=15; P=0.004). Pharmaceutical treatment increased sperm motility by 10% in older men, while DNA damage levels were reduced in all patients (N=33; P=0.002; P=0.03).

These results demonstrate that male age negatively impacts sperm quality in both mice and humans. Further, pharmaceutical treatment in vitro normalizes sperm quality and, in mice, improves embryogenesis following IVF.

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## Supplementation of culture media with nicotinamide mononucleotide improves embryo development from aged but not young female mice

**Michael J Bertoldo<sup>1,2</sup>, Dale Goss<sup>2</sup>, Kirsty A Walters<sup>1</sup>, Lindsay E Wu<sup>1</sup>, Robert B Gilchrist<sup>1</sup>**

*1. Fertility and Research Centre, School of Women's and Children's Health, University of New South Wales, Sydney, NSW, Australia*

*2. Laboratory for Ageing Research, School of Medical Sciences, University of New South Wales Australia, Sydney, NSW, Australia*

Female patient age is the key factor determining IVF success. Therefore, culture media that improve either embryo quality or yield for older patients undergoing IVF would be a significant advance for infertility treatment. Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) is central to the regulation of ageing. We have shown that treatment of aged female mice with the NAD<sup>+</sup> precursor nicotinamide mononucleotide (NMN) improves fertility. This study aimed to assess the effect of supplementing embryo culture media with NMN on mouse embryo development. Mature oocytes were collected from the ampulla of aged (11–12 months) or young (4 weeks) female C57Bl6 mice primed with PMSG and hCG. Following IVF presumptive zygotes were randomly allocated to culture droplets supplemented with 0, 0.1, 1 or 10µM NMN, and cultured for 6 days. After 5 days there were significantly more blastocysts cultured with 1µM NMN (86.9 ± 5.6%) compared to controls (66.1 ± 7.3%; P<0.05), and likewise with 1µM (93.5 ± 4.9%) and 10µM NMN (89.3 ± 1.6%) compared to controls (60.7 ± 6.7%; P<0.01) on day 6. When the same experiment was repeated using 4-week-old mice, there was no effect of NMN on any developmental readouts. To assess embryo quality embryos from aged females were cultured in either 0µM or 1µM NMN, and total cell number and the ratio of inner cell mass (ICM) to trophectoderm (TE) were assessed on day 6. Embryos from young females were used as positive controls. Embryos from young mice (93.1 ± 5.9) and aged embryos treated with NMN (91.0 ± 4.23) had significantly more cells than untreated aged embryos (72.83 ± 4.1; P=0.01). There were no differences in the ratio of ICM:TE between groups. These results suggest that embryos of aged mice are deficient in NAD<sup>+</sup> signalling and support the hypothesis that supplementation with NAD<sup>+</sup> precursors can ameliorate the effects of ageing on fertility.

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## Association between Thyroid cancer & Hashimoto's thyroiditis

**Min Ji Jeon<sup>1</sup>**

1. Asan Medical Center, University of Ulsan College of Medicine, Songpa-gu, Seoul, South Korea

Hashimoto's thyroiditis (HT) is the most common autoimmune disease which causes hypothyroidism. The pathogenesis of HT is based on an abnormal humoral and cellular immune response against thyroid autoantigens which cause chronic inflammation at thyroid gland. Histologically, HT is characterized by diffuse lymphocytic infiltration (DLI) with numerous lymphoid follicles, fibrosis and parenchymal atrophy.

Because chronic inflammation may be strongly associated with various human cancers, it has long been postulated that HT would be associated with an increased risk of thyroid cancer. Indeed, it has been noticed for a long time that DLI is frequently associated with papillary thyroid cancer (PTC). Many retrospective studies also reported that co-occurrence of HT in PTCs is associated with a better outcome with lower recurrence rates. However, the role of HT in the development and progression of PTC is still debated. The observed effect may be partly due to the modulation of the tumor microenvironment, the induction of immune responses, and also the presence of positive prognostic factors such as female sex, younger age, smaller tumors, lack of extrathyroidal extension, lymph node or distant metastases, earlier TNM stage and low frequency of BRAF mutations in PTCs with HT.

In this session, I'll review recent studies evaluated the association between HT and the development and prognosis of thyroid cancer, especially PTC, and discuss many hypothesis regarding their association.

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## Prolonged Antithyroid drug therapy in Graves Disease

JAYAKUMAR R V<sup>1</sup>, [Jayakumar R V<sup>1</sup>](#)

1. Indian Institute of Diabetes, Trivandrum, India

The commonest cause of Thyrotoxicosis is Graves disease, which is an autoimmune disease caused by thyroid stimulating immunoglobulins (TSI). Treatment for Graves disease include Anti-thyroid drugs, Thyroidectomy and Radio-iodine. There is lack of general agreement as to which therapy is the best, as none is ideal. The goal of treatment in Graves disease is early and durable achievement of euthyroid status with out side effects. The Radio-iodine therapy has been commonly used for the treatment of Graves disease and has been considered as effective and safe. It can be given to all patients more than 5 years age. Pregnancy and significant Orbitopathy are the only contraindications. The main side effect of Radio-iodine is the high incidence of Hypothyroidism in the treated patients. It is difficult to accept the concept, that for treating one disease, you are justified to induce another disease. This led many Physicians to try other modes of treatment like, prolonged anti-Thyroid drugs.

In the Indian subcontinent many Physicians and Endocrinologists are having experience with long term anti-Thyroid drugs. Many of the cases are at the request of the patients who are reluctant to undertake, Radio-iodine therapy or undergo surgery. One of the earlier studies of long term continuous methimazole treatment in comparison with radio-iodine, published in European Journal of Endocrinology in 2005, showed the long term continuous treatment of hyperthyroidism with Methimazole is safe, and efficacious. Recently in a study done in South India by Kannan et al, regarding the long term use of antithyroid drugs for Graves disease concluded that long term ATD use is safe for long periods, 4 to 17 years. With doses of carbimazole 5-10 mg per day, more than 80% of patients remain in euthyroid stage.

In my personal series of 124 patients on long term antithyroid drugs for more than 10 years, 90% are in euthyroid state with a dose of 5-10mg (mean 8 mg) daily. Their quality of life is very good and they and their biochemical parameters are within normal range. It will be presented in detail during the presentation.

It may be concluded that Long term anti-Thyroid drug is an acceptable alternative to radio-iodine therapy for Graves disease.

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## Subclinical thyroid disease: a regional perspective

[John Walsh<sup>1,2</sup>](#)

1. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

2. Medical School, University of Western Australia, Crawley, WA, Australia

The terms subclinical hypothyroidism (SubHypo) and subclinical hyperthyroidism (SubHyper) entered the literature in the early 1970s. Remarkably, nearly 50 years later, the health impact of both conditions remains uncertain.

SubHypo is the commonest disorder of thyroid function in iodine-sufficient populations. Diagnosis can be problematic, because of the effects of non-thyroidal illness on thyroid function and because serum TSH levels increase with age. A recent large, randomised controlled trial reported no symptomatic benefit of levothyroxine treatment in older, largely asymptomatic people with mild SubHypo, as did a subsequent meta-analysis, but this is of little relevance to managing younger patients and those with symptomatic ill health. There are no RCTs of levothyroxine on cardiovascular outcomes in SubHypo, but cohort studies (including several from the Asia-Oceania region) and RCTs with surrogate endpoints suggest that SubHypo is an independent cardiovascular risk factor. Cardiovascular risks of SubHypo appear highest in people who are younger (<65-70 y old), with higher TSH levels (>7 mU/L) and who have other cardiovascular risk factors.

The impact of subclinical hyperthyroidism (SubHyper) on health is becoming better defined. As for SubHypo, RCT data is largely lacking, but a consistent body of evidence suggests that endogenous SubHyper is a risk factor for atrial fibrillation, cardiovascular mortality and fracture. SubHyper is also associated with dementia, but the evidence for a causal relationship is less convincing than for CVD and fracture. Recent studies using Mendelian randomization have provided further evidence for a causal relationship between SubHyper and atrial fibrillation, but not for other cardiovascular diseases. The health risks of exogenous SubHyper (in patients on levothyroxine treatment) are poorly defined, but have probably been somewhat exaggerated.

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## Management of Cushing's Syndrome in pregnancy

**David Torpy<sup>1</sup>**

1. *Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA, Australia*

Cushing's Syndrome is rare in pregnancy; the available literature consists of case reports and small case series. The diagnosis of Cushing's in pregnancy can be difficult as some normal phenomena of pregnancy including weight gain, abdominal striae and a tendency to hyperglycaemia and idiopathic hypertension of pregnancy, are all features shared with Cushing's syndrome. Diagnosis and treatment are important as the presence of Cushing's entails considerable maternal and foetal risk. Once suspected, the screening tests for Cushing's, all of which entail confirmation of hypercortisolism, need to be interpreted in the light of the physiologic hypercortisolism of pregnancy. These tests include 24 hour urine free cortisol, the 1mg overnight dexamethasone suppression test and late night salivary cortisol. 24 hour urine free cortisol levels are elevated up to 2 to 3-fold during pregnancy but can be much higher in Cushing's during pregnancy although there is overlap. Cushing's has a predilection towards unilateral adrenal secretory autonomy. This may reflect the tendency to hyperandrogenism in pituitary Cushing's, the commonest cause in non-pregnant cases, where hyperandrogenism may reduce fertility. Once diagnosed, the options are generally for adrenal or pituitary surgery as ectopic Cushing's is very rare in pregnancy. Surgery is generally performed in the second trimester to reduce the risk of premature delivery and other untoward outcomes. Medical treatment of Cushing's is an option and Metyrapone has been used with some success. Many cases of successful management have been reported and application of the general principles of Cushing's management with an appreciation for the altered hypothalamic-pituitary-adrenal axis and particular risks of treatments in pregnancy should optimise outcomes.

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## Adrenal Disorders in Pregnancy

**Jui Ho<sup>1</sup>**

1. *Southern Adelaide Local health Network, Bedford Park, SA, Australia*

Adrenal disorders are relatively rare in pregnancy, but a timely diagnosis and proper treatment are critical because these disorders can cause significant maternal and foetal morbidity and mortality. Making the diagnosis of adrenal disorders in pregnancy is challenging as symptoms associated with pregnancy are also seen in adrenal diseases. In addition, pregnancy is marked by several endocrine changes, including activation of the renin-angiotensin-aldosterone system and the hypothalamic-pituitary-adrenal axis.

Addison's disease is very rare in pregnancy, and the exact prevalence is unknown. Diagnosis may be missed in the first trimester as many clinical symptoms can occur in normal pregnancies such as fatigue, hyperemesis and weight loss. Appropriately treated patients can expect to have uneventful pregnancies of normal duration and without foetal complications. However, adrenal insufficiency during pregnancy is associated with a high incidence of serious foetal and maternal complications, such as suboptimal birth weight, intrauterine growth retardation, preterm delivery and postpartum adrenal crises, if the disorder is not recognized and adequately treated.

Congenital adrenal hyperplasia (CAH) refers to a group of inherited autosomal recessive disorders of adrenal steroid biosynthesis, resulting in altered cortisol and aldosterone secretion. 21-hydroxylase deficiency (21-OHD) accounts for 95% of all affected patients. Traditionally, reduced fertility and pregnancy rates have been reported in women with classic CAH, whereas fertility is only mildly reduced in the non-classic form. However, without glucocorticoid treatment, an increased miscarriage rate has been reported. Progress in female genitalia reconstructive surgery, individualized hormonal therapies, psychosexual evaluation, and assisted reproductive technology have improved fertility and pregnancy outcomes in women with classic CAH. Successful gestational management in CAH patients requires the close coordination of care between endocrinologists and obstetricians.

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## Human placental growth hormone variant in pregnancy and placental function

**Jo Perry<sup>1</sup>**

1. *Liggins Institute, University of Auckland, Auckland, New Zealand*

The growth hormone and insulin-like growth factor 1 (GH/IGF1) axis is a key regulator of postnatal mammalian growth. Humans produce two growth hormone proteins, GH-N and a placental variant GH-V. GH-N is secreted in a pulsatile fashion from the pituitary, and regulates IGF1 release from the liver, while the placental variant of GH is secreted tonically from the placenta during pregnancy. In pregnancy, concentrations of GH-N in the maternal serum decline, while GH-V increases in the circulation from week five, gradually replacing maternally derived GH-N completely. Although the exact role of GH-V is still unclear, this remarkable change in spatial and temporal GH secretion patterns is proposed to play a role in mediating maternal adaptations to pregnancy. GH-V is pro-angiogenic and increases maternal levels of other important growth factors, such as IGF1. It may also enhance nutrient transfer across the placenta.

GH-V is associated with fetal growth, and its circulating concentrations have been investigated across a range of pregnancy complications. Altered maternal serum levels of GH-V have been observed in certain pregnancy complications such as fetal growth restriction and gestational diabetes mellitus. However, progress in this area has been hindered by a lack of readily accessible and reliable assays for measurement of GH-V. This talk will discuss what is known about the role of placental growth hormone and IGF1 in placental function and will describe our recent studies investigating their association with human pregnancy pathologies.

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## **Fetal steroid exposure**

**Sailesh Kumar**<sup>1</sup>

*1. University of Queensland, South Brisbane, QLD, Australia*

Not made available at time of publishing

### BMP15 and GDF9 are aberrant in women with PCOS

**Angelique H Riepsamen<sup>1</sup>, Adriana E Krysta<sup>2,1</sup>, Prudence Sweeten<sup>2,1</sup>, Shelly Lien<sup>1</sup>, David Agapiou<sup>1</sup>, William L Ledger<sup>2,1</sup>, David M Robertson<sup>1</sup>, Robert B Gilchrist<sup>1</sup>**

1. *Fertility and Research Centre, School of Women's and Children's Health, University of New South Wales, Sydney, NSW, Australia*

2. *IVF Australia, Sydney, NSW, Australia*

The oocyte-secreted factors bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are predominantly expressed by oocytes. Relative abundance of GDF9 to BMP15 determine mammalian ovulation rate and fecundity and mutations in these genes are associated with reproductive pathologies. To date, there are no validated assays available to quantitate these proteins in biological samples.

This research program developed and validated ELISAs to measure BMP15 and GDF9 in serum (n=151), follicular fluid (FF) (n=138) and on cumulus cells (CC) (n=120) collected from three cohorts of women treated for infertility. Sera were from the follicular phase of a treatment cycle. FF samples were pooled from all follicles aspirated during oocyte retrieval and normalised to total protein. CC-bound BMP15 was assessed in salt extracts from pooled CC of individual ICSI patients normalised to DNA. Concentrations were compared to clinical data, including age, number of oocytes retrieved, and polycystic ovaries/syndrome (PCO(S)).

BMP15 was detectable in 67% of serum and 76% of FF, while GDF9 was detectable in 29% of serum and 60% of FF samples. BMP15 was detectable in all CC samples. CC-bound BMP15 was higher in PCOS than non-PCO(S) women (p<0.05). Further, an age-related decline in BMP15/CC DNA was significant for non-PCO(S) women (p<0.05) but absent in PCO(S). In the FF cohort, the inverse was seen, as women with PCO(S) had a lower proportion of detectable BMP15 (p=0.06), and higher proportion of detectable GDF9 (p<0.05), suggesting high FF GDF9:BMP15 is associated with PCO(S). Sera GDF9 of non-PCOS patient increased with increasing oocytes, which was significantly different for PCO(S) (p<0.05). The inverse was observed for BMP15 (p<0.05).

These results demonstrate that BMP15 and GDF9 are aberrant in women with PCO(S) and suggest these growth factors are compartmentalised in CC, FF and serum differently in PCO(S) and non-PCO(S) women.

### Inhibin inactivation disrupts ovarian function in mice

**Monica P Goney<sup>1</sup>, Kelly L Walton<sup>1</sup>, Georgia Goodchild<sup>1</sup>, Karen Chan<sup>1</sup>, Jason Liew<sup>1</sup>, Amy Winship<sup>2</sup>, Karla Hutt<sup>2</sup>, Craig A Harrison<sup>1</sup>**

1. *Department of Physiology, Monash University, Clayton, VIC, Australia*

2. *Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia*

Gonadal-derived inhibins are essential factors in mammalian reproduction, negatively regulating follicle stimulating hormone (FSH) production by gonadotrope cells of the anterior pituitary. Inhibins ( $\alpha/\beta$ -heterodimers) modulate gonadotrope function by antagonizing the actions of the related proteins, activins ( $\beta/\beta$  homodimers), at the level of the receptor. Expression of the inhibin  $\alpha$ -subunit is essential in restricting both the production and activity of activin  $\beta/\beta$ -dimers, as genetic deletion of the inhibin  $\alpha$ -subunit in mice results in a pathological increase in activins. Indeed, activin levels are elevated as much as 500-fold in inhibin  $\alpha$ -subunit deficient mice, inducing gonadal tumour formation and lethal cachectic wasting. Consequently, inhibin knockout mice, which have been the only model available for the past 25 years, do not actually reflect a loss of inhibin activity, but rather a pathological increase in activin levels. Here, we describe a new inhibin mutant mouse model to study inhibin physiology. We used the CRISPR/Cas-9 system to introduce a single inactivating mutation into inhibin in C57/Bl6 mice. This point mutation ablated inhibin bioactivity, without the accompanied pathological increase in activin production. Consequently, the mice did not develop gonadal tumours, nor the cachectic wasting that compromised inhibin  $\alpha$ -subunit knock-out mice. In response to inhibin inactivation, activin activity was unopposed, resulting in elevated levels of circulating FSH in both male and female mice. Elevated levels of FSH in female mutant inhibin mice resulted in a doubling in ovarian weights, and histological analysis indicated that this was due to increased numbers of corpora lutea and large antral follicles. In contrast, testis weights and spermatogenesis appeared to be normal in mutant male inhibin mice. Current studies aim to determine the impact of inhibin inactivation on the establishment and maintenance of pregnancy. Our new inhibin mouse will allow for the first accurate characterisation of the physiological roles of inhibins.

### Chlamydia infects the ovary, elicits an immune response and depletes the ovarian reserve in mice

**Urooza C Sarma<sup>1</sup>, Alison Carey<sup>2</sup>, Ken Beagley<sup>2</sup>, Karla Hutt<sup>3</sup>**

1. *Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia*

2. *Institute of Health & Biomedical Innovation (IHBI) and School of Biomedical Sciences, Queensland University of Technology (QUT), Brisbane, Queensland, Australia*

3. Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia

*Chlamydia trachomatis* is the most common sexually transmitted infection worldwide and can cause severe damage to the Fallopian tubes, often resulting in complete infertility. Recent studies indicate significantly increased miscarriage rates and time to natural conception, along with poor IVF outcomes in women seropositive for *Chlamydia* but in the absence of tubal pathology, suggesting that that fertility may be compromised by mechanisms that extend beyond fallopian tube scarring. In this study, we used a well-characterised mouse model to investigate the hypothesis that *Chlamydia* can infect and damage the ovary. Chlamydial DNA was detected in ovaries at 2 and 35 days post infection (pi) using qPCR and inclusion bodies were localised within macrophages in the ovarian stroma using immunofluorescence. Chlamydial infection was associated with an increase in the expression of mRNA for CXCL16 and IFN $\gamma$ , suggesting the induction of a pro-inflammatory immune response within the ovary. Strikingly, the number of ovarian follicles was significantly reduced 35 days following a single infection compared to uninfected controls ( $p < 0.05$ ,  $n = 4-5$  mice/group) and the extent of follicle depletion was greater following a second infection ( $p < 0.05$ ,  $n = 4-5$  mice/group). Two infections was also associated with changes to the overall ovarian morphology and increased apoptosis and fibrosis in the ovary ( $p < 0.05$ ,  $n = 5$ /group), consistent with activation of a prolonged inflammatory response. Collectively, these observations demonstrate that *Chlamydia* can penetrate the ovary, deplete the ovarian reserve and compromise ovarian function, and suggest that the ovary may act as a potential reservoir of infection. Ovarian follicles are essential for female fertility because they secrete hormones and contain oocytes. Follicles cannot be replaced once lost from the ovary. Thus, our data suggests that damage to the ovary caused by *Chlamydia* is permanent and may underlie some cases of unexplained infertility and poor IVF outcome in women.

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### Interferon-Tau Exerts Direct Prosurvival and Antiapoptotic Actions in Luteinized Bovine Granulosa Cells

Raghavendra Basavaraja<sup>1</sup>, Senasige Thilina Madusanka<sup>1</sup>, Jessica N Drum<sup>2</sup>, Ketan Shrestha<sup>1</sup>, Svetlana Farberov<sup>1</sup>, Milo C Wiltbank<sup>3</sup>, Roberto Sartori<sup>2</sup>, Rina Meidan<sup>1</sup>

1. Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

2. Department of Animal Science, University of São Paulo, Piracicaba, Brazil

3. Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, United States

Interferon-tau (IFNT), a multifunctional type I interferon, serves as a signal to maintain the corpus luteum (CL) during early pregnancy in domestic ruminants. Here we aimed to study whether IFNT directly affects the function of luteinized bovine granulosa cells (LGCs), used as a model for large luteal cells. Recombinant ovine IFNT (roIFNT) stimulated signal transducer and activator of transcription-1 (STAT1) and IFN-stimulated genes (ISGs; *MX2*, *ISG15* and *OAS1Y*) in LGCs. The LGC also had high expression of IFN receptors (IFNAR1) and displayed a rapid and transient phosphorylation of STAT1 as well as an elevation in total STAT1 protein after longer incubation times (24-48h). These results indicate that IFNT activates type-1 interferon pathways in LGCs. In addition, IFNT treatment increased viable LGCs numbers and reduced dead and apoptotic cell counts in flow cytometry analyses using Annexin V staining. Consistent with these effects on cell viability, IFNT upregulated cell survival proteins (MCL1, BCLxL and XIAP) and decreased the levels of proteins implicated in apoptosis, gamma-H2AX, cleaved caspase 3 and thrombospondin-2 (THBS2). Notably, IFNT reversed the actions of thrombospondin-1, a potent luteal apoptotic factor, on cell viability as well as on XIAP and cleaved caspase 3 protein levels. Furthermore, roIFNT stimulated the mRNA concentrations for a series of proangiogenic genes such as *FGF2*, *PDGFB* and *PDGFAR*. In support of the in vitro observations, we found that CL tissue collected from day 18 pregnant cows had higher ISGs along with elevated levels of *FGF2*, *PDGFB*, *THBS2* and *XIAP* as compared to CL from non-pregnant cows on day 18 of the estrous cycle. These findings show that IFNT activates diverse pathways in LGCs, promoting survival and blood vessel stabilization, while suppressing cell death signals. These mechanisms might contribute to CL maintenance during early pregnancy.

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### Sperm proteins outside the blood-testis barrier

Liza O'Donnell<sup>1,2</sup>, Diane Rebourcet<sup>3</sup>, Laura Dagley<sup>4</sup>, Giuseppe Infusini<sup>4</sup>, Peter J O'Shaughnessy<sup>5</sup>, Frederick Chalmel<sup>6</sup>, Adrian Pilatz<sup>7</sup>, Thorsten Diemer<sup>7</sup>, Wolfgang Weidner<sup>7</sup>, Robert I McLachlan<sup>8,2</sup>, Andrew I Webb<sup>4</sup>, Lee B Smith<sup>9,3</sup>, Peter G Stanton<sup>1,2</sup>

1. Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, Victoria, Australia

2. Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, Australia

3. Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia

4. Walter and Eliza Hall Institute, Melbourne, Victoria, Australia

5. University of Glasgow, Glasgow, UK

6. University of Rennes, Rennes, France

7. Justus Liebig University, Giessen, Germany

8. Hudson Institute of Medical Research, Clayton, VIC, Australia

9. MRC Centre for Reproductive Health, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom

Sperm develop within the seminiferous tubules of the testis. Outside the tubules, the interstitium contains Leydig and immune cells, and blood and lymphatic vessels. Sperm first appear at puberty after the development of immune tolerance and must be protected from the immune system to prevent recognition as "non-self". A major protective component is the blood-testis barrier (BTB), comprised of tight junctions between Sertoli cells which prevent free passage of proteins and create a

specialised milieu for sperm development. Sperm proteins in healthy men are widely assumed to be sequestered inside the BTB, and physically prevented from interacting with the immune system or entering the circulation. To address this unproven assumption, we performed the first in-depth proteomic analysis of testicular interstitial fluid (TIF) in mice and humans, and defined the impact of the seminiferous epithelium on the TIF proteome. We show that, in both mice and men, TIF contains hundreds of proteins predominantly or specifically expressed by developing sperm inside the BTB, and many of these are reduced in mouse TIF during seminiferous epithelial disruption. *In silico* analysis revealed some sperm-specific TIF proteins are detectable in human plasma, contradicting the dogma that these proteins are absent from the circulation. TIF also contains numerous cancer-testis antigens (CTAs); sperm-specific proteins aberrantly expressed in cancers. CTAs are thought to be neoantigens and absent from the circulation, hence are considered ideal targets for cancer diagnosis and/or immunotherapy. However our data suggests certain CTAs are present in the circulation and in contact with the immune system. In conclusion, we reveal a conserved feature of the testis is the deposition of sperm proteins into TIF that can then enter the circulation. These findings have major implications for the development of CTA-based diagnostics and therapeutics, and provide hitherto unrecognised opportunities for new diagnostics to monitor testis function.

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### **Cep76 is a centriole-related gene with an essential role in sperm development**

**Brendan J Houston<sup>1</sup>, Liina Nagirnaja<sup>2</sup>, Don Conrad<sup>2</sup>, Moira K O'Bryan<sup>1</sup>**

1. Monash University, Clayton, VIC, Australia

2. Oregon National Primate Research Center, Hillsboro, Oregon, USA

Male infertility is a common disease that affects approximately 7% of men in the Western world and its aetiology is unknown in the majority of cases. It is, however, estimated that at least 50% of male infertility cases are genetic in origin. To identify novel male infertility mutations, we performed whole exome sequencing on infertile men. In one man, we have identified a homozygous SNP mutation in the centriole-related protein gene *CEP76*. Although *CEP76* has been implicated in the prevention of over-duplication of centrioles in cell lines, its role in germ cells and male fertility is largely unknown. In order to define this role, we generated a knockout mouse model for *Cep76*. *Cep76* knockout males mate normally, but are sterile, due to a combination of sperm structural defects within the sperm head and tail, and an almost complete absence of sperm motility. Knockout sperm exhibit minimal twitching motility and are unable to manifest forward progressive motility ( $p < 0.0001$ ). Approximately 40% of the sperm also contain head shape defects ( $p < 0.0001$ ), and on average the sperm tail length is 15% shorter than their wild-type counterparts ( $p < 0.001$ ). These data indicate an essential role of *CEP76* in the processes of sperm head shaping and tail formation during spermiogenesis. Collectively these data strongly support that *CEP76* is an essential regulator of male fertility in humans and mice.

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### **TBC1D21 is vital to maintain the integrity of mitochondria sheath and flagellum in murine sperm**

**Ying-Hung Lin<sup>1</sup>, Yayun Wang<sup>2</sup>**

1. Graduate Institute of Biomedical and Pharmaceutical Science, Fu-Jen Catholic University, New Taipei City, Taiwan

2. Department of Chemistry, Fu-Jen Catholic University, New Taipei City, N/A, Taiwan

Male infertility is a global public health issue and contributes to nearly half of all infertility cases. Small GTP-binding proteins are essential for numerous cellular processes including spermatogenesis. We have identified that *TBC1D21* gene (also named as Male Germ Cell RAB GTPase-activating protein, MGCRABGAP) is a novel germ cell specific GTPase-activating proteins and down-regulated in testicular tissues of the infertile men. Until now, the spermatogenic functions of *TBC1D21* are still known. In present study, we demonstrate that *TBC1D21* is critical for multiple aspects of sperm tail integrity, such as the arrangement and morphology of mitochondria and the integrity of axoneme microtubules. In the *Tbc1d21*<sup>-/-</sup> mice, the sperm number is only slightly lower than in wild-type mice but most of the sperm from *Tbc1d21*<sup>-/-</sup> mice display the severe impaired tail structure and shorter mitochondria sheath in the midpiece. Ultrastructure examination reveals the disordered arrangement and irregular morphology of mitochondria and disorganized microtubules in sperm flagellum. We also identified the interacting partners of *TBC1D21* through co-IP and following by LC-MS/MS. Among these interactors, translocase of outer membrane 22 (TOM22) and dynein heavy chain 7 (DNAH7) are the components of mitochondria and axoneme, respectively. In mature sperm, *TBC1D21* interacts with TOM22 and colocalizes with TOM22 and DNAH7 in the region of midpiece. In addition, loss of *TBC1D21* cause the dispersed localization of TOM22 and DNAH7 from the midpiece. Based on these results, we suggest that *TBC1D21* is a key regulator for maintaining the integration of sperm tail through its interactors.

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### **Germ cells improve blood-testis barrier function**

**Nahal Atashkadeh<sup>\*1</sup>, Elizabeth Murray<sup>\*1</sup>, Medina Pell<sup>1</sup>, Justine Olcorn<sup>1</sup>, Rob McLachlan<sup>1</sup>, Liza O'Donnell<sup>1</sup>, Peter Stanton<sup>1,2</sup>**

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Dept of Molecular and Translational Sciences, Monash University, Melbourne, Victoria, Australia

In males, germ cells undergo meiosis and post-meiotic differentiation within a protective environment inside the seminiferous tubules. This environment is created by the blood-testis barrier (BTB), of which tight junctions (TJ) between Sertoli cells are a major structural component. The BTB forms at puberty and remains essential for male fertility as loss of BTB function causes infertility. Our current understanding is that TJ formation is driven by hormones (FSH, testosterone) and local factors but a role

for germ cells is not clear. However, we demonstrated that BTB function *in vivo* was significantly improved when round spermatids (rSTs) were present (1). Hence the aim of this study was to define the impact of rSTs on Sertoli cell TJs *in vitro*. 20-day old rat Sertoli cells were cultured to form a functional monolayer onto which isolated adult rST were added. TJ function was measured by transepithelial electrical resistance (TER) and a tracer diffusion assay measured changes in molecular weight permeability.

Sertoli cells alone formed stable TJs within 3 days, and FSH and testosterone stimulated TER >2-fold compared to control. Stimulated cells also showed a decreased (~2-fold) permeability to small- (3-5kD, 10kD) and medium- (70kD) sized tracers, but no change for a large (500kD) tracer. Importantly, the addition of rSTs significantly enhanced TJ and barrier function of the Sertoli cell monolayer; TER increased further and the cells acquired the ability to restrict the passage of higher molecular weight molecules (70 & 500kD), thus forming a more functionally mature barrier. This stimulatory effect of rSTs was time-, contact-, and cell number- dependent, and was not observed in an unrelated HEK cell control. We conclude that rSTs contribute to the establishment of a functional barrier in Sertoli cells that creates a specialised milieu necessary for sperm production.

\* equal contributions

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## Pre-receptor regulation of therapeutic glucocorticoid action

**Mark Cooper<sup>1</sup>**

1. *University of Sydney, Sydney, NSW, Australia*

The action of glucocorticoids at a tissue level is dependent on both the level of steroid in the circulation/interstitial space and intracellular metabolism of glucocorticoids by 'pre-receptor' enzymes. For glucocorticoids the best studied pre-receptor enzymes are the 11b-hydroxysteroid dehydrogenases (11b-HSDs) which interconvert cortisone and cortisol. 11b-HSD1 is an activator of glucocorticoids (converting inactive cortisone to active cortisol) whereas 11b-HSD2 is an inactivator. These enzymes also metabolise prednisone (inactive) and prednisolone (active) in a similar manner.

Much attention has focussed on the role of 11b-HSDs in normal physiology and clinical studies have evaluated the effect of drugs which inhibit these enzymes. Less attention has been paid to the role that these enzymes play in mediating the effects of therapeutic glucocorticoids. In a series of studies it has been shown that 11b-HSD activity is critical to the action of therapeutic glucocorticoids. In the absence of 11b-HSD1 many tissues are almost entirely protected against the action of commonly used glucocorticoids. Increased expression of 11b-HSD1 in specific cells and tissues during inflammation may be a key factor underpinning the effectiveness of current glucocorticoid therapy for inflammatory diseases.

These factors need to be considered in the evaluation of novel anti-inflammatory agents particularly when comparisons are made to current glucocorticoids. This knowledge also opens up the possibility of developing novel therapeutics with superior ability to selectively regulate glucocorticoid receptor action in target tissues.

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## The regulation of placental inflammation by glucocorticoid receptor isoforms

**Vicki L Clifton<sup>1</sup>**

1. *The Mater Research Institute - UQ, Brisbane, QLD, Australia*

In pregnancy, glucocorticoids derived from the maternal system and acting via the placenta are essential for the maturation, development and survival of the fetus in all mammalian species. However, *in utero* exposure to maternal stress and excess glucocorticoids, either endogenous or synthetic, can be detrimental to fetal growth, development and survival. Pregnancy complications where the fetus is exposed to excess glucocorticoids or inflammation can increase the risk of fetal growth restriction and have long term consequences for the health of live offspring. The physiological mechanisms that confer different outcomes in morbidity and mortality of the fetus exposed to stressful environments may be driven by differences in the expression pattern of placental glucocorticoid receptor (GR) isoforms. Our team has identified the presence of multiple GR protein isoforms in the placenta of the human, guinea pig and the mouse that vary in relation to gestational age at delivery, sex, glucocorticoid exposure, fetal growth and maternal health. We propose that some GR isoform patterns are protective against an adverse outcome for the fetus while others may be detrimental to fetal growth and survival. We have recently discovered that in small for gestational age (SGA) pregnancies there is increased GR $\alpha$ -D1 expression in both the cytosolic and nuclear compartments of the placenta when compared to appropriate for gestational age pregnancies (AGA). These placentae also have GR $\alpha$ -A isoform expressed in both cellular compartments but increased expression of inflammatory genes that would normally be suppressed by the presence of GR $\alpha$ -A. These data suggest GR $\alpha$ -D1 may play a central role in activating proinflammatory mechanisms in the placenta and provides a mechanism for the dichotomy observed in SGA pregnancies where there are increased concentrations of glucocorticoids and high circulating levels of pro-inflammatory cytokines.

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## The role of growth hormone signalling in insulin sensitivity, obesity, and longevity in mice

**Andrew Brooks<sup>1</sup>**

1. *Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia*



Growth hormone (GH) has an important function in regulating post-natal growth, metabolism, and lifespan and its aberrant signalling has been shown to promote cancer and diabetes. GH acts as an insulin antagonist with elevated insulin sensitivity evident in humans and mice lacking a functional GH receptor (GHR). By utilising a panel of mice possessing specific deletions of GHR signalling pathway we have identified that insulin sensitivity and glucose disappearance rate were enhanced in the muscle and adipose tissue of mice lacking the ability to activate STAT5 via the GHR (*Ghr-391<sup>-/-</sup>*) as for GHR null mice (*Ghr<sup>-/-</sup>*). These changes were associated with a striking inhibition of hepatic glucose output associated with altered glycogen metabolism and elevated fasting hepatic glycogen content. The enhanced hepatic insulin sensitivity was associated with increased insulin receptor  $\beta$  and IRS1 activation along with activated downstream AKT signalling cascades. While *Pck1* expression was unchanged, its inhibitory acetylation was elevated due to decreased SIRT2 expression thereby promoting loss of PCK1. Loss of GH-STAT5 signalling to hepatic ChIP targets would further increase lipogenesis, supporting hepatosteatosis while lowering glucose output. Finally, upregulation of IL-15 expression in muscle, with increased secretion of adiponectin and FGF1 from adipose tissue further promoted this insulin sensitivity. While the insulin sensitivity was also evident in female mouse, the lifespan extension in *Ghr* mouse models was sexually dimorphic. However, in mice with targeted GHR-JAK-STAT disruption (GHR Box1-motif disruption), allowing Src but not JAK2 activation, life extension was evident in both genders. Our study does not support a universal role for IGF1-deficiency and insulin sensitivity in longevity but indicates towards gender-independent effect of GH-induced Src signalling in promoting longevity.

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## Androgen Receptor: A Soloist in a Breast Cancer Symphony

Iza Denis<sup>1</sup>

1. Dame Roma Mitchell Cancer Research Laboratories, Adelaide Health and Medical School, University of Adelaide, Adelaide, South Australia, Australia

Estrogen receptor negative (ER-) breast cancer remains the major therapeutic challenge for breast cancer treatment and a better understanding of the mechanisms underlying the progression of this disease is necessary to develop efficient therapeutic strategies. The molecular apocrine breast cancer subtype lacks ER expression but is characterised by the expression of the androgen receptor (AR). In prostate cancer, the forkhead transcription factor FoxA1 is a key determinant of AR cistrome but is not essential for AR binding to DNA. In breast cancer, FoxA1 is also a key determinant of ER cistrome but is essential for ER to bind DNA. In the molecular apocrine breast cancer context, AR and FoxA1 have been shown to maintain an ER-like breast cancer phenotype. Whether FoxA1 is required for AR to bind DNA in molecular apocrine breast cancer cells is unknown and has not been previously explored. We show that FoxA1 was required for cell growth but not for AR binding to chromatin in a molecular apocrine breast cancer context. In the absence of FoxA1, AR cistrome was expanded in the MDA-MB-453 model of molecular apocrine breast cancer, with a gain of new AR binding events. Proteomic analysis showed a significant change in AR chromatin protein-bound complex in the absence of FoxA1. AP2a motifs were enriched at AR binding sites gained without FoxA1 and quantitative proteomic comparison of the AR chromatin-bound complex confirmed an increased interaction between AP2a and AR in the absence of FoxA1.

Our findings indicate that FoxA1 is not required for AR to bind DNA in molecular apocrine breast cancer cells. In the absence of FoxA1, AR interacts with AP2a to induce a new phenotype with features of a secretory cell while maintaining luminal characteristics. The deeper understanding of AR signaling in ER- breast cancer could open avenues for new therapeutic strategies.

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## Antiphospholipid antibodies induce endoplasmic reticulum stress in the syncytiotrophoblast and cause the extrusion of dangerous extracellular vesicles

Larry Chamley<sup>1</sup>, Qi Chen<sup>1</sup>, Tony Hickey<sup>2</sup>, Katie Groom<sup>3,4</sup>

1. Department of Obstetrics and Gynaecology, University of Auckland, Auckland, , New Zealand

2. School of Biological Sciences, University of Auckland, Auckland, New Zealand

3. Maternal-Fetal Medicine, National Women's Health, Auckland District Health Board, Auckland, New Zealand

4. Liggins Institute, University of Auckland, Auckland, New Zealand

The human placenta is covered by the multinucleated syncytiotrophoblast, which extrudes large quantities of extracellular vesicles (EVs) into the maternal circulation. Antiphospholipid autoantibodies increase a woman's risk of preeclampsia ten-fold. These antibodies are internalised by the syncytiotrophoblast where they bind intracellular vesicular structures<sup>1</sup>. Subsequently, EVs extruded from this cell are altered such that, they cause maternal endothelial cell activation. The mechanism driving this change in syncytiotrophoblast-derived EVs is unknown. We investigated whether endoplasmic reticulum stress was involved in the changes in EVs induced by antiphospholipid antibodies.

Normal first trimester placental explants were treated with two antiphospholipid antibodies, ID2 or IIC5 (25 ug/ml) overnight. Micro- and nano-EVs were harvested by differentiation ultracentrifugation. Misfolded proteins were quantified following staining with ThT. Specific proteins were quantified by western blot or ELISA. All experiments were repeated a minimum of three times.

Treating placental explants with antiphospholipid antibodies increased the amount of misfolded proteins in the syncytiotrophoblast and in both the micro-EVs (p=0.028) and nano-EVs (p=0.026) extruded from the explants. Levels of HSP70, a marker of endoplasmic reticulum stress, as well as secretion of TNF $\alpha$ , were also increased. The syncytiotrophoblast of a placenta from a pregnancy complicated by antiphospholipid antibodies also demonstrated increased misfolded proteins.

Excess misfolded proteins may cause cell death. To avoid death, cells can export the misfolded proteins. Micro- and nano-EVs are usually considered to have different biogenesis pathways and consequently to carry different cargos. However, to avoid accumulation of misfolded proteins induced by antiphospholipid antibodies, the syncytiotrophoblast exported these misfolded proteins in both micro- and nano-EVs. This may preserve the syncytiotrophoblast but it is possible the misfolded proteins then

inflict damage on the maternal cells that clear the EVs. This is a potential mechanism by which antiphospholipid antibodies contribute to the dysfunction of maternal endothelial cells in preeclampsia.

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## The functional role of Dynamin in the Human Placenta and its putative dysregulation in Preeclampsia

**Jacinta H Martin<sup>1</sup>, Prathama Mukherjee<sup>1</sup>, Saije Morosin<sup>1</sup>, Sarah Delforce<sup>1</sup>, Brett Nixon<sup>1</sup>, Kirsty Pringle<sup>1</sup>**

1. *Priority Research Centre for Reproductive Sciences, University of Newcastle and The Hunter Medical Research Institute, Newcastle, NSW, Australia*

The placenta functions as a conduit between the mother and the developing fetus and is essential for fetal growth. Unfortunately, a large proportion of pregnancies are affected by complications arising from inadequate placentation. Requisite to understanding these pathologies is first understanding healthy placental development. In this vein, we aimed to determine the role of the Dynamin isoforms (DNM 1, 2 & 3), a superfamily of mechanoenzymes classically involved in endocytotic and exocytotic processes, in the placenta.

Q-PCR and western blotting approaches were used to confirm the expression of DNM at the mRNA and protein level in both first trimester and term human placentae, while complimentary immunocytochemical and immunohistochemical analyses provided crucial information relative to the localization of DNM. These analyses revealed that all 3 DNM isoforms were expressed at the mRNA level, however only DNM2 and DNM3 were expressed at the protein level. DNM2 protein expression was significantly more abundant in term placentae ( $P < 0.0001$ ) compared to first trimester placentae. DNM2 was uniformly distributed in the syncytiotrophoblast, with enrichment toward the apical margin. Whereas DNM3 was distributed throughout the cytosol of both cytotrophoblast and syncytiotrophoblast cells. In addition, western blotting indicated that DNM2 and 3 were significantly downregulated in placentae from women with preeclampsia ( $P < 0.05$ ).

In an effort to understand the functional role of placental DNM, the BeWo choriocarcinoma cell line was cultured with the dynamin inhibitor 'Dynasore'. Dynamin inhibition results in an overall reduction in the secretome of BeWo cells. Furthermore, analysis of the individual bands detected by silver stain demonstrated that the secretion of a protein at ~29kDa was significantly reduced ( $P < 0.01$ ), putatively corresponding to the soluble (pro)renin receptor (s(P)RR). Since women with preeclampsia are known to exhibit increased maternal levels of s(P)RR, future analyses will focus on unraveling the contribution of DNM to the pathogenesis of preeclampsia.

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## Proteomic and metabolic analysis of placental mitochondria following trophoblast differentiation.

**Joshua J Fisher<sup>1</sup>, Daniel R McKeating<sup>1</sup>, James S Cuffe<sup>2</sup>, Olivia J Holland<sup>1</sup>, Tony V Perkins<sup>1</sup>**

1. *Griffith University, Labrador, QLD, Australia*

2. *School of Biomedical Science, University of Queensland, Brisbane, QLD, Australia*

Differentiation of trophoblast cells from mononuclear cytotrophoblast to a multinucleated syncytiotrophoblast is paralleled by alterations in the organelles which reside within these cell lineages. The most drastic alteration is seen in mitochondria, cytotrophoblast mitochondria remain stereotypical with large well-defined cristae while syncytiotrophoblast mitochondria are much smaller, spherical, with a less defined cristae structure. However, the molecular mechanisms which drive this process remains unknown. This study aimed to identify key protein differences between trophoblast mitochondrial subpopulation and associate these to functional and morphological characteristics.

Mitochondria were isolated from the cytotrophoblast and syncytiotrophoblast of healthy term placenta with LC-MS to generate proteomic profiles. To evaluate and confirm the finding western blotting and plate based assays were used in conjunction with O2k Oroboros respirometry to assess the metabolic and bioenergetic capacity of the subpopulations.

Proteomics identified several proteins involved in complexes of the electron transport chain that were decreased in the syncytiotrophoblast mitochondria including NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 ( $p < 0.05$ ) (Complex I), Succinate dehydrogenase ( $p < 0.05$ ) (complex II) and ATP synthase subunit A1 ( $p < 0.01$ ) and B ( $P = < 0.0001$ ) (complex V). Furthermore, decreases in proteins involved with carbohydrate metabolism, fatty acid metabolism and amino acid utilisation were observed in syncytiotrophoblast mitochondria including: pyruvate dehydrogenase phosphatase ( $p < 0.05$ ), phosphoenolpyruvate carboxykinase ( $p < 0.002$ ), very long chain acyl-CoA dehydrogenase ( $p < 0.007$ ), Mitochondrial 2-oxoglutarate/malate carrier protein ( $p < 0.05$ ). Protein expression was validated using western blotting, respirometry and enzyme assays confirming pyruvate dehydrogenase activity decreased significantly ( $p < 0.05$ ), there was a decreased maximum respiratory capacity ( $p < 0.001$ ) decreased ATP production in syncytiotrophoblast mitochondria.

This research highlights multiple metabolic pathways which appear altered between the two mitochondrial subpopulations following trophoblast differentiation. These changes appear to have a direct effect on the bioenergetic capacity of the mitochondria present in syncytiotrophoblast with many of the identified proteins linked to the cristae structure and morphology.

## Reduction in regulatory T cell number in early pregnancy impairs decidual artery remodeling and leads to fetal growth restriction

Alison S Care<sup>3, 2, 1</sup>, Holly M Groome<sup>1</sup>, Sandra T Davidge<sup>3, 2</sup>, Sarah A Robertson<sup>1</sup>

1. Robinson Research Institute and Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia

2. Women and Children's Health Research Institute, Edmonton, AB, Canada

3. Department of Obstetrics and Gynaecology, University of Alberta, Edmonton, AB, Canada

**INTRODUCTION:** Preeclampsia is an important cause of maternal and perinatal morbidity and mortality, and increases the susceptibility of the mother and offspring to cardiovascular disease later in life. In preeclampsia, a deficiency in regulatory T (Treg) cells has been observed. Treg cells prevent maternal immune rejection of the fetus, and suppress inflammatory activation. We have shown they also contribute to uterine vascular function in pregnant mice (Care et al Hypertension 2018), consistent with emerging roles in systemic vascular homeostasis. In particular, Treg cell deficiency causes impaired uterine artery function in mid-pregnancy. We hypothesise that a reduced Treg cell population will alter uteroplacental haemodynamics in late gestation, affecting fetal and placental development.

**METHODS:** Transgenic *Foxp3-DTR* mice have FOXP3 promoter-driven expression of the human diphtheria toxin (DT) receptor to enable selective depletion of FOXP3+ (Treg) cells. DT was injected (37.5ng/g) on gestational day (GD)3.5 and GD5.5 to selectively deplete FOXP3+ cells; vehicle-treated *Foxp3-DTR* mice served as controls. Morphometric analysis of decidual spiral arteries was conducted on day GD10.5, ultrasound biomicroscopy and fetal biometrics were assessed on GD18.5.

**RESULTS:** Following DT-treatment to deplete Treg cells, decidual spiral artery remodeling was impaired on GD10.5, with a 20% smaller diameter compared to control mice ( $P < 0.05$ ). In late pregnancy (GD18.5) uterine artery hemodynamics were perturbed, with the pulsatility index increased by 20% ( $P < 0.05$ ). Furthermore, male and female fetuses were growth restricted, being 15-18% lighter ( $P < 0.05$ ), and female fetuses had a shorter abdominal girth. The fetal:placental weight ratio, a surrogate measure of placental efficiency, was reduced in placentas from male and female fetuses.

**CONCLUSION:** We demonstrate an essential role for Treg cells in fetal growth and uteroplacental vascular function. Given the severe implications of preeclampsia on the future health of the mother and her baby, investigation of therapeutic strategies targeting Treg cells offers a promising intervention.

## Histone variant H2A.B3 is important for male fertility.

Nur Anuar<sup>1</sup>, Matt Field<sup>2</sup>, Sebastian Kurscheid<sup>1</sup>, Josephine Bowles<sup>3</sup>, Peter Koopman<sup>3</sup>, Philip Gregory<sup>4</sup>, Lei Zhang<sup>4</sup>, David J Tremethick<sup>1</sup>, Tanya Soboleva<sup>1</sup>

1. JCSMR, ANU, Canberra, ACT, Australia

2. James Cook University, Cairns, QLD, Australia

3. School of Biomedical Sciences, Faculty of Medicine, UQ, Brisbane, Queensland, Australia

4. Sangamo BioSciences Inc, Richmond, California, USA

Epigenetic-based mechanisms are essential for cell differentiation and function but how exactly epigenetic control is transmitted and interpreted is poorly understood. One of the most crucial and dramatic examples of epigenetic regulation is during spermatogenesis. Male germ cells undergo sequential differentiation steps within seminiferous tubules all tightly epigenetically controlled, to ensure that chromatin is remodeled in a tight spatio-temporal manner, in order to produce one of the most specialised cell types: mature spermatozoa.

Our work is focused on deciphering a role of a spermatid-specific histone variant, H2A.B3, in spermatid differentiation. We have previously shown that histone variant H2A.B3 is expressed in haploid round spermatids and participates in activation of the male germ cell expression program<sup>1</sup>. Uniquely, this histone variant can bind directly to both DNA and RNA and participate in RNA processing<sup>2</sup>. To investigate the function of H2A.B3 in spermiogenesis further, we have generated a knock-out (KO) H2A.B3 mouse. We have found that H2A.B3 KO male mice are sub-fertile, that is likely due to deregulation of RNA Pol II transcription and down-regulation of expression of key spermiogenesis-specific genes<sup>3</sup>. The H2A.B3 KO also results in the production of defective sperm. Unexpectedly, we have found that absence of H2A.B3 in haploid germ cells affects histone-protamine exchange and even more surprisingly, the function of somatic Sertoli cells.

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## Proteomic markers in seminal plasma as a non-invasive alternative for the differential diagnosis of azoospermia

**Raouda Sgaier<sup>1,2,3</sup>, Daniela Fietz<sup>3</sup>, Martin Bergmann<sup>3</sup>, Liza O'Donnell<sup>1</sup>, Peter Stanton<sup>1</sup>, Laura Dagley<sup>4</sup>, Andrew I Webb<sup>4</sup>, Hans-Christian Schuppe<sup>2</sup>, Adrian Pilatz<sup>2</sup>, Thorsten Diemer<sup>2</sup>**

1. Hudson Institute for Medical Research, Clayton, VIC, Australia

2. Department of Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Hessen, Germany

3. Department of Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Giessen, Hessen, Germany

4. The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

Infertility affects approximately 15% of couples of reproductive age with almost equivalent male and female contribution. Azoospermia, characterized by non-measurable levels of sperm in semen, is responsible for 5-20% of male infertility cases. With the advent of ARTs, an exact diagnosis of male factor infertility is of prime importance in infertility management. The aim of this study was to discover biomarkers for non-invasive differential diagnosis of azoospermia. Using label-free LC-MS/MS, we compared proteomic profiles of seminal plasma from fertile men (healthy controls, HC) and men diagnosed with three different forms of azoospermia: obstructive (OA), hypospermatogenesis (HS) and Sertoli cell only syndrome (SCO) (all groups n=8). Proteins significantly down-regulated in SCO and OA compared to the control included testis-specific LDHC ( $FC_{HC/OA} = 5.24$ ;  $p=0.02$ ,  $FC_{HC/SCO} = 5.38$ ;  $p=0.01$ ) and testis-enhanced TSN ( $FC_{HC/OA} = 2.96$ ;  $p=0.04$ ,  $FC_{HC/SCO} = 3.24$ ;  $p=0.02$ ) and HSP90AA1 ( $FC_{HC/OA} = 2.25$ ;  $p=0.04$ ,  $FC_{HC/SCO} = 2.29$ ;  $p=0.02$ ). This decrease is caused by two distinct mechanisms: the physical obstruction of the male reproductive tract in OA, and testicular failure in SCO. Results also showed a lower abundance of a number of epididymal-enriched proteins in seminal plasma from the OA group compared to the non-obstructive azoospermia (SCO, HS) and control groups. ELSPBP1 ( $FC_{HC/OA} = 9.78$ ,  $p=0.02$ ), and MGAM ( $FC_{HC/OA} = 6.27$ ,  $p=0.04$ ) were selected as candidate markers of vas deferens obstruction. Quantitation of these proteins in seminal plasma using antibody-based assays will be undertaken as a primary marker validation step. Further analysis of sample distribution demonstrated the potential of seminal plasma proteomics to distinguish between hypospermatogenesis and SCO, and to classify OA cases according to the site of obstruction. Developing tools for the accurate, non-invasive diagnosis of azoospermia can inform clinical decisions and help speed up infertility treatment. Proteomic markers measured in seminal plasma, in combination with conventional clinical tests, can offer a good alternative to testicular biopsy.

## Sperm function in metabolically-healthy obese mice

**Angela Crean<sup>1</sup>, Kelsey Pool<sup>1</sup>, Andries Van Woerkom<sup>1</sup>, Matthew Hooke<sup>1</sup>, Jinfeng Lin<sup>1</sup>, Gregory Cooney<sup>1</sup>, Amanda Brandon<sup>1</sup>**

1. The University of Sydney, Camperdown, NSW, Australia

Sperm counts are declining in step with global increases in rates of obesity, suggesting the two factors may be linked. However, surprisingly, meta-analyses consistently fail to find evidence for a relationship between male body mass index (BMI) and semen quality. While BMI is the standard measure used to categorise obesity, not all obese (BMI > 30) individuals show signs of metabolic disease. Experiments using the Geometric Framework for Nutrition have revealed that mice fed a high carbohydrate/low fat/low protein diet become obese, yet do not develop impaired glucose tolerance. Hence, to test whether sperm function is related to body mass or metabolic health, we fed male mice one of 3 diets – high fat, high starch, or chow – for 12 weeks, and then assessed metabolic health and sperm quality. Both high fat and high starch males gained significantly more weight, driven by increases in fat mass, than chow-fed males. Yet, despite similar amounts of adiposity, high starch males had better glucose tolerance than high fat males. High starch males had smaller testes, but larger seminal vesicles, than either high fat or chow males. However, high starch males were able to compensate for their smaller testes, with no difference in sperm concentration found among diet treatments. Sperm motility and velocity was reduced in high fat, but not high starch males. The sperm of high starch males showed lower levels of oxidative stress than either high fat or chow males. No effect of diet treatment on sperm viability or DNA fragmentation was found. These results indicate that sperm quality is related to metabolic health, not fat mass. Hence, it appears that the lack of a correlation between BMI and male fertility is because obese, yet metabolically-healthy, males have normal sperm function.

## Alternative splicing: A major key to poor sperm morphology

**Mark A Baker<sup>1</sup>, Jacob K Netherton<sup>1</sup>, Rachel A Ogle<sup>1</sup>, Gary Hime<sup>2</sup>**

1. University of Newcastle, Newcastle, NSW, Australia

2. Anatomy and Neuroscience Reserach, University of Melbourne, Melbourne, VIC, Australia

Male infertility is a very common condition, with reports suggesting that one in 15-20 men of reproductive age are affected. Understanding why or how men produce defective sperm is a question that has remained elusive. We have used proteomic screens to identify mechanisms responsible for building defective sperm in men. Significantly, we have found regulators of alternate splicing appear to be a major key; being more abundant within infertile spermatozoa. To understand this, we overexpressed specific alternate-splicing regulators within *Drosophila*. Amazingly, our data show that sperm overexpression RNA-splicing regulators produced typical patterns of "male-factor" infertility, including (i) decreased amounts of sperm production, (ii) head morphology defects and (iii) poor sperm motility. Furthermore, fertility data demonstrate changes to

alternate splicing have dramatic consequence. Fly strains ranged from completely infertile to extremely subfertile. This data strongly suggest that aberrant alternate splicing is likely to play a major role when it comes to the production of poor quality spermatozoa, and male factor infertility.

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## Iodine nutrition in pregnant women in China

**Zhongyan Shan**<sup>1</sup>

1. *First Hospital of China Medical University, Heping District, Shenyang, China*

Not made available at time of publishing

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## Iodine deficiency during pregnancy: the implications and challenges for Australian women and their offspring

**Kristen Hynes**<sup>1</sup>

1. *University of Tasmania, Hobart, Tasmania, Australia*

The re-emergence of iodine deficiency (ID) in Australia, particularly in the south-eastern states of New South Wales, Victoria and Tasmania, in the late 1990s/early 2000s resulted in two national public health responses. The first, involving fortification of commercially baked bread with iodised salt, was begun in Tasmania in 2001 and became mandatory across Australia and New Zealand in 2009. This initiative has seen the general population in Tasmania return to a status of iodine sufficiency.

Recognition that the increased iodine requirements of pregnant and breastfeeding women would be difficult to attain by bread fortification alone, the second initiative was introduced in 2010 with the National Health and Medical Research Council (NHMRC) recommending daily iodine supplementation for women planning pregnancy, and for the duration of gestation and lactation. Adequacy is vital during pregnancy, as even mild ID has been shown by our team and others to be associated with deficits in neuro-cognitive development manifesting as persistent poorer educational outcomes and lower IQ.

While supplementation improves the iodine status of pregnant and breastfeeding women, studies conducted in different Australian regions indicate that the optimal level and timing of supplementation requires further investigation. Our research suggests that non-pregnant/non-breastfeeding women of child-bearing age in Tasmania remain iodine deficient despite bread fortification and as such may have insufficient thyroid stores prior to conception to maintain iodine sufficiency throughout gestation, even if they begin supplementing once pregnant. Only women who began supplementation prior to conception and continued throughout pregnancy, at the recommended dose, were able to maintain an iodine status within current World Health Organization recommendations. Other Australian research suggests that iodine supplementation may not be warranted and even detrimental in regions without ID.

Given the potential for vast regional, and indeed individual, variation in the underlying iodine status of women in Australia, a national survey of women of reproductive age and of pregnant and breastfeeding women is warranted to inform the appropriateness of the current NHMRC supplementation recommendations. Development of methods to determine individual iodine status, in addition to existing reliance on epidemiological-based population assessment, is also merited.

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## Iodine Supplementation in Pregnancy and Breastfeeding

**Fereidoun Azizi**<sup>1</sup>

1. *Endocrine Research Center and Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Iodine requirement increases during pregnancy and breastfeeding. Inadequate iodine intake during pregnancy and breastfeeding may impair thyroid hormone synthesis in both mother and fetus. Iodine supplementation may help to meet the increased iodine demand during this critical period and prevent or correct iodine deficiency and its consequences. The aim of this symposium lecture is to assess trials available on iodine supplementation and its effects on short-and long-term outcomes in pregnant and breastfed women and their children.

**Pregnancy:** Nine randomized clinical trials and 2 intervention studies reported the effects of maternal supplementation on maternal and neonatal iodine status and thyroid function. Four RCTs and one intervention study addressed the impact of gestational iodine supplementation on neonatal anthropometric indices at birth and infant neurocognitive development. In nearly all studies, there was improvement in maternal and fetal iodine status, however, studies on the thyroid function of pregnant women and their infants showed inconsistent results. None of the RCTs reported beneficial effects of iodine supplementation on neonatal anthropometric indices. No improvements were found in infant cognitive, language, and motor development, although there was an improvement in some motor function. Iodine supplementation before conception was more effective than during pregnancy.

**Breastfeeding:** A meta-analysis revealed that the iodine concentrations detected in colostrums and breast-milk samples from iodine-sufficient countries indicated the provision of adequate iodine to breast-fed infants residing in these countries. Studies have shown that supplementation of breast-feeding mothers with either 300 or 150 µg/day iodine improved maternal UIC compared to the placebo and the formula-feeding groups. However, it has been demonstrated that in iodine-sufficient areas with effective salt iodization, lactating mothers and infants have no need for iodine supplements.

**Conclusions:** Salt iodization is a safe, cost-effective, widely accepted and sustainable strategy for the prevention and control of iodine deficiency. Countries with well established universal salt iodization programs report great success in eliminating iodine deficiency among general populations, though this is not reflected in the most susceptible groups, namely pregnant women and

lactating mothers. Although major societies have recommended iodine supplementation of 150 µg daily during pregnancy and lactation, this recommendation is effective only in areas with severe or moderate iodine deficiency. No definitive conclusion has been yet reached regarding the beneficial effects of iodine supplementation in areas of mild iodine deficiency and those with universal salt iodization program, in particular on infant growth and neurocognitive development. Further high quality RCTs with larger sample sizes are still required to gain a better understanding of these issues.

## Key drivers of embryo-endometrial crosstalk to establish pregnancy

**Evdokia Dimitriadis**<sup>1</sup>

1. *The University of Melbourne, Melbourne, VIC, Australia*

Human embryo implantation requires an activated blastocyst, a receptive endometrium and communication between the two. Implantation is initiated following apposition and firm adhesion to the endometrium where abnormalities in firm adhesion results in implantation failure and infertility. Abnormal implantation can result in disease associated with placental insufficiency. Very little is known of blastocyst-endometrial interactions in humans. Recently non-coding RNA have been identified to be dysregulated in implantation disorders including implantation failure/infertility and miscarriage and preeclampsia suggesting they may be useful as biomarkers and treatments for these conditions. Using a unique model to study human embryo – endometrial interactions we demonstrate that cellular and extracellular non-coding RNA regulate embryo implantation. We also demonstrate that microRNA processing machinery in the endometrium is abnormal during receptivity in women with unexplained infertility implying an important role in the endometrial tissue preparation for implantation. As each microRNA has the ability to alter the levels of many genes and proteins they are master regulators and may be useful as treatment targets for disorders of implantation.

## Endometrial receptivity failure: origins and prediction

**Tracey Edgell**<sup>1</sup>

1. *Hudson Institute, Clayton, VIC, Australia*

For successful embryo implantation a synchronous and receptive endometrium must be developed within the uterus. Failure to achieve endometrial receptivity means pregnancy cannot occur regardless of embryo quality. The secretions from the endometrium into the uterine cavity reflect the hospitable or otherwise nature of the endometrium and form the microenvironment of implantation.

Our work is protein focussed utilising cytokine/chemokine analysis, proteomic and glycoproteomic analysis to identify changes to the uterine fluid associated with receptivity and infertility. Our approach has been to both understand and predict receptivity failure; studying both the vital implantation window but also the regenerative proliferative phase; examining for clues as to where and when receptivity failure originated. Our belief is that identifying these dysregulated factors will open up a new era in the development of novel therapeutic options for treating women impacted by poor endometrial receptivity.

Our uterine fluid studies have been complimented with development of a serum based multivariate assay to predict receptivity with the goal of predicting likelihood of successful implantation following embryo transfer. An initial retrospective trial of 283 women, testing serum collected at hCG+2, was successful in predicting >80% of transfer outcomes. A multicentre validation trial is now in progress for completion 2020.

## The legacy of nutrient availability: lessons from pluripotent stem cells

**Alexandra Harvey**<sup>1</sup>

1. *School of BioSciences, The University of Melbourne, Parkville, VIC, Australia*

While the preimplantation embryo possesses a degree of developmental plasticity in response to changes in the extracellular nutrient environment, enabling short term adaptation, this plasticity comes at a cost to embryo viability and subsequent long-term offspring health. However, the mechanisms by which the extracellular environment elicits persistent changes have remained unclear.

Beyond their role in generating ATP, metabolites and cofactors have recently been shown to induce long-term cellular changes through the regulation of the epigenome, a phenomenon referred to as metaboloepigenetics. Using Pluripotent Stem Cells (PSC), as an *in vitro* representative of the blastocyst stage inner cell mass, our data reveal links between metabolic activity and stem cell health, differentiation kinetics, maintenance of pluripotency and reprogramming of adult cells to induced Pluripotent Stem Cells (iPSC).

Cell culture conditions can elicit permanent changes that perturb embryonic stem cell responses to physiological stimuli and interfere with differentiation timing, and highlight the significance of assessing cell physiology in *in vitro* culture models. The relative availability of several key metabolites alters not only stem cell physiology but also the epigenetic landscape and impacts subsequent differentiation. Furthermore, we have identified the retention of somatic metabolic memory in iPSC, which can be modulated by altered nutrient availability during the reprogramming process to affect cell physiology, cell integrity and genetic stability.

Metabolic regulation of the epigenome is therefore a plausible mechanism underpinning how the extracellular microenvironment induces persistent modifications during early embryonic programming.

## Use of machine learning tools to define immune cell changes in recurrent miscarriage women

**Lachlan Moldenhauer<sup>1</sup>, Jasmine Wilson<sup>1</sup>, Ella S Green<sup>1</sup>, M. Louise Hull<sup>1</sup>, Sarah A Robertson<sup>1</sup>**

*1. Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia*

Recurrent miscarriage (RM), defined as two or more pregnancy losses before gestational week 24, affects ~5% of women. The underlying causes are not known, although emerging evidence suggests immune dysregulation is a common cause of idiopathic RM. Immune cells and factors are key determinants of endometrial receptivity and capacity to establish healthy pregnancy, particularly CD4+ regulatory T (Treg) cells which control inflammation and assist in vascular remodelling. Suboptimal Treg cell responses are irrefutably shown to cause pregnancy loss in animal models and in women, are associated with infertility and a range of pregnancy complications. Here we aimed to elucidate phenotypically distinct peripheral blood T cell populations between RM patients and healthy control women by assessing multi-coloured flow cytometry data using the machine learning, dimensionality reducing algorithm t-distributed stochastic neighbour embedding (t-SNE). Peripheral blood samples were collected from n=27 RM patients and n=15 control women with no history of RM at the mid-luteal phase of the menstrual cycle. CD3, CD4 and CD8 lineages were assessed using 18 colour flow cytometry panels. Only CD4 T cells showed significant changes between groups. To dissect the specific CD4+ T cell changes in RM Th1, Th2, Th17 and Treg cell abundance and phenotype was evaluated. The mean proportion of CD25+FOXP3+ Treg cells within the CD4+ T cell compartment was reduced by 44% in RM compared to control women (P<0.01). Additionally, fewer terminally differentiated effector memory Treg cells and a subset of highly suppressive and proliferative Treg cells was identified in RM patients, with greater expression of Ki67, HLD-DR and CTLA4. This data suggests that women experiencing idiopathic RM have reduced Treg cells and specifically, exhibit a memory Treg cell deficiency consistent with premature Treg cell exhaustion. This analysis gives new insight on the nature and underlying causes of immune dysfunction in some RM women.

## Have we been thinking about subfertility in men who are obese wrong all along?

**Nicole McPherson<sup>1,2</sup>**

*1. Freemasons Center for Mens Health, University of Adelaide, Adelaide, SA, Australia*

*2. Robinson Research Institute, School of Medicine, University of Adelaide, Adelaide, SA, Australia*

Worldwide sperm counts are declining at a rate of 0.70million/ml per year, with some western countries experiencing up to a 72.6% decrease in sperm counts over the past 50 years. Coinciding with the decreased sperm counts is the epidemic increased incidence of metabolic disorders encapsulating; obesity, type II diabetes, high blood pressure, hyperlipidaemia, fatty liver disease, etc. among men of reproductive age, with rates of diagnosis increased by 35% from 1988–1994 to 2007–2012 in many western nations. Obese men in the general population have higher odds ratio of experiencing infertility, while those receiving assisted reproductive treatment for their infertility have lower rates of live birth. There is still conflicting findings in the literature about obesity effects on sperm quality. Our extensive research in animal models of obesity, suggests that increased fat mass is not the driver for the changes in sperm function seen and why conflicting reports still exist in the current literature. It seems that a combination of obesity related co-morbidities (such as, hyperglycaemia, poor nutrition, pro-inflammatory state etc.) are the main culprits. In humans, obese men who present with azoospermia/oligospermia are more likely to have an additional underlying medical condition (i.e. fatty liver disease or hyperglycaemia) than just increased fat mass. Interestingly, in our animal obesity model, when we target just one of these comorbidities i.e. altered glucose control with metformin, or poor nutrition with micronutrient supplementation we can restore sperm function, sperm oxidative DNA damage and fetal growth without a need to reduce adiposity. Therefore, moving forward we must assess obesity related subfertility as a syndrome ensuring we determine the real underlying reasons for their changes in sperm quality, as restoration may require more or less than just telling a man to lose weight.

## An Unexpected Role for Endogenous Estrogen Signaling in Penis Development

**Andrew Pask<sup>1</sup>, Luke Govers<sup>1</sup>, Tiffany Phillips<sup>1</sup>, Sam Cripps<sup>1</sup>, Deidre Mattiske<sup>1</sup>, Gail Risbridger<sup>2</sup>**

*1. School of BioSciences, The University of Melbourne, Parkville, VIC, Australia*

*2. Department of Anatomy & Developmental Biology, Monash University, Clayton, Vic, Australia*

Hypospadias, a developmental defect of the penis, is one of the most common congenital malformations in humans. Its incidence has rapidly increased over recent decades, and this has been largely attributed to our increased exposure to endocrine disrupting chemicals. Penis development is primarily an androgen driven process, however estrogen and xenoestrogens are known to affect penis development in both humans and mice. Here, we investigated the role of estrogen in the developing penis. Using a novel penis culture system and transcriptomics we showed that exogenous estrogen directly targets the developing penis altering gene expression profiles resulting in hypospadias. In addition, we also uncovered an unexpected endogenous role for estrogen in normal penis development and showed that a loss of estrogen signaling results in a mild hypospadias phenotype, the most common manifestation of this disease in humans. Our findings demonstrate that a delicate balance of androgen and estrogen signaling is intrinsically required for normal gene expression and urethral closure. These findings broaden our understanding of the impact of endocrine disruptors on early development and demonstrate that penis development is not an entirely androgen driven process, but one in which endogenous estrogen signaling also plays a critical role.

## Sex in a changing world: behavioural, ecological and evolutionary impacts of anthropogenic change

**Bob Wong<sup>1</sup>**

1. School of Biological Sciences, Monash University, Vic 3800, Australia

The pursuit of mating opportunities, even at the best of times, can pose a significant challenge to sexually reproducing organisms. Charles Darwin in the *Origin of Species* described it as a 'sexual struggle' arising from intense competition among individuals for the opportunity to mate. This struggle can be so powerful that it can shape the course of evolution itself – a process known as sexual selection. In most animals, reproduction is finely attuned to the environment. So, what happens when environmental conditions are disturbed due to anthropogenic activities? One particularly insidious form of anthropogenic disturbance is contamination of the environment by a myriad of human and veterinary pharmaceuticals that can affect the behaviour, morphology and physiology of non-target organisms. In this talk, I will consider the pivotal role that reproductive behaviour plays in determining the fate of individuals, species and populations under human-induced environmental change, and discuss recent research investigating the ecological and evolutionary impacts of pharmaceutical contaminants on reproduction and mechanisms of sexual selection in fish.

## The effects of heat stress on sheep reproduction - Review

**Gordon Refshauge<sup>1</sup>, J P Rickard<sup>2</sup>, F Ataollahi<sup>3</sup>, S P de Graaf<sup>2</sup>**

1. New South Wales Department of Primary Industries, Cowra Agricultural Research Station, Cowra, NSW, Australia

2. Faculty of Science, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia

3. New South Wales Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia

Reproduction is reported to be the most sensitive component of sheep production to heat stress. Reports from the 1940s suggest Australian sheep producers were aware that summer-mated flocks had lower reproduction rates, with lay observations reporting an association between high ambient temperatures and lower flock reproduction. Recent anecdotal evidence of low pregnancy scanning percentages following mating during heat wave conditions supports these observations. However, large-scale scientifically robust on-farm data that describes the mechanisms and extent to which heat stress impacts sheep reproduction and subsequent farm profitability are lacking. This is a significant issue given the context of a rapidly changing Australian climate.

Potential drivers of reproductive failure from heat stress include alterations to behaviour and nutrition as the animal attempts to reduce its body temperature as well as direct effects on reproductive cells and tissues due to the increase in body temperature. The metabolic consequence of heat stress in the mammal is increased production of reactive oxygen species (ROS). When the production of oxygen radicals exceeds antioxidant capacity, damage is incurred. Sperm, oocytes, corpora lutea, early embryos, placental blood flow, neonatal survival and milk production are affected.

Little research has examined solutions for the seasonal problem of heat stress impairing sheep reproduction. As animals become more productive, their set-point body temperature increases, narrowing the gap between the sheep's normally high body temperature and hyperthermia. Breeding for tolerance is not directly possible because there are no genetic correlations estimated between productivity traits and heat tolerance for Australian sheep. The industry currently operates independently of the potential effects of selection for production and a rapidly changing climate and a future offering more intense and frequent heat stress events.

This review examines the impacts of heat stress on sheep reproduction and discusses the modifications that need to be considered in the context of hotter and longer summers.

## Experience of the Glucagon Stimulation Test for the Assessment of Growth Hormone Deficiency for Subsidised Growth Hormone Treatment in Australia

**Reetu Gogna<sup>1</sup>, Caroline Jung<sup>1</sup>, Kylie McLachlan<sup>1</sup>, Richard Maclsaac<sup>1,2</sup>, Balasubramanian Krishnamurthy<sup>1</sup>, Maresa Derbyshire<sup>1</sup>, Nirupa Sachithanandan<sup>1</sup>, Carmela Caputo<sup>1</sup>**

1. Department of Endocrinology and Diabetes, St Vincent's Hospital, Fitzroy, Melbourne, Victoria, Australia

2. The University of Melbourne, Melbourne, Victoria, Australia

### Objective:

In December 2018, the Pharmaceutical Benefits Scheme subsidised growth hormone (GH) for the treatment of severe adult GH deficiency (GHD). The eligibility criteria requires documented GHD by one of three dynamic tests; therefore, the number of referrals for glucagon stimulation tests (GSTs) have increased significantly, preferred for its relative safety compared to the insulin tolerance test. This study aims to review the safety and adverse events (AEs) of the GST.

### Research Methods:

A retrospective analysis was performed on all GSTs (n=30) undertaken at St Vincent's Hospital, Melbourne from January–June 2019. All patients received a fixed dose of intravenous 1mg glucagon, and GH levels were measured every 30 minutes for 240 minutes. GHD was defined as peak GH level <3.0mcg/L. Due to protocol changes throughout the year, 20 cases had either



venous or capillary blood glucose levels (BGL) checked throughout testing. Blood pressure (BP) was checked twice during the test and if cases were symptomatic. AEs were documented for each patient including significant hypotension (systolic BP (SBP) <90mmHg or >20mmHg decrease in SBP); hypoglycaemia (venous or capillary BGL  $\leq$ 3.5mmol/L) and; nausea/vomiting.

**Results:**

Participants were aged between 20–66 years, 63% were female and 53% were previously on GH replacement. The GSTs confirmed GHD in 90% of cases. In total, 40% experienced one and 17% more than one AEs. Nausea/vomiting occurred in 6/30 (20%) and hypoglycaemia in 10/20 (50%) patients. Significant hypotension occurred in 8/30 (27%) patients, with hypotension occurring in 7/30 (23%) patients despite taking their usual cortisol replacement prior to testing.

**Conclusion:**

This audit demonstrates that AEs are common during the GST. As a result of these findings, protocol changes have been made at this centre to prevent these AEs occurring. Larger, prospective studies need to be conducted with a consistent protocol to examine these risks further.

**Diagnostic value of copeptin in central diabetes insipidus**

**Emma Boehm<sup>1</sup>, Julie Sherfan<sup>2</sup>, Joel Smith<sup>3</sup>, James King<sup>4</sup>, John Wentworth<sup>1</sup>, Cherie Chiang<sup>1,3</sup>**

1. Dept Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, VIC, Australia

2. Dept Clinical Pathology, NSW Health Pathology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

3. Dept Pathology, Royal Melbourne Hospital, Melbourne, VIC, Australia

4. Dept Neurosurgery, Royal Melbourne Hospital, Melbourne, VIC, Australia

**Background:**The diagnosis of diabetes insipidus (DI) relies on indirect measurement of serum and urine sodium and osmolality. Since the diagnosis can only be made when an inappropriately dilute urine is paired with a significantly concentrated serum, the process is tedious for the clinician and uncomfortable for the patient. Copeptin is the C-terminal portion of the anti-diuretic hormone (ADH) prohormone which correlates with the less stable ADH, therefore providing a direct measurement of posterior pituitary response to hyperosmolar stress. (1,2)

**Aim:** This study aims to assess the diagnostic accuracy of copeptin in patients with central DI compared with subjects who underwent pituitary surgery without DI.

**Methods:** Serum samples from subjects with central DI, control subjects post pituitary surgery with no DI (NDI) and control subjects with SIADH were collected and analysed on the BRAHMS KRYPTOR copeptin assay. Groups were compared using unpaired T-test and Levene's test for equal variance.

**Results:** 56 samples from 22 subjects (13 females, 9 males, mean age 53.9  $\pm$  15.5 y.o.) were analysed. Two subjects had resolved DI (RDI) after copeptin analysis and were successfully weaned off DDAVP and reclassified as NDI. Of the DI subjects, 1 had acute and 5 had chronic DI. Copeptin was lower in DI compared to NDI group ( $p = 0.013$ ), while serum sodium, osmolality, urine osmolality were similar. Copeptin did not differentiate between the SIADH and NDI groups. After exclusion of NDI samples with serum sodium  $\leq$  140 mmol/L, the area under the curve was 0.97 (95% CI 0.9 to 1.0), a copeptin cut-off of 2.9 pmol/L predicts DI with a sensitivity of 92% and a specificity of 90%.

**Conclusion:**Copeptin concentration of < 3.0 pmol/L concurrently with serum sodium concentration of > 140mmol/L predicted central DI when using post pituitary surgery subjects without DI as controls.

**Table: Biochemical parameters between groups.**

	DI	NDI	SIADH
No.	6	12	4
Age (years)	42 $\pm$ 16 #	56 $\pm$ 13	67 $\pm$ 14
Serum Na (mmol/L)	142 $\pm$ 9 #	139 $\pm$ 2	122 $\pm$ 4 *
Serum Osmol (mOsm/kg)	289 $\pm$ 15 #	290 $\pm$ 5	253 $\pm$ 0.7 *
Urine Osmol (mOsm/kg)	371 $\pm$ 203	635 $\pm$ 219	445 $\pm$ 216
Copeptin (pmol/L)	1.89 $\pm$ 0.59 *	12.98 $\pm$ 13	22.68 $\pm$ 34.45

Data presented as mean  $\pm$  SD. \* P < 0.05 vs NDI, # p < 0.05 vs SIADH

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**Effect of variability in hypothalamic-pituitary-adrenal axis activity on vascular function, insulin sensitivity and lipids**

**Anjana Radhakutty<sup>1,2,3</sup>, Tien Lee<sup>1,3</sup>, Brenda Mangelsdorf<sup>1</sup>, Sophie Drake<sup>1</sup>, Morton Burt<sup>1,3</sup>**

1. Southern Adelaide Diabetes and Endocrine Services, Repatriation General Hospital, Adelaide, SA, Australia

2. *Endocrinology, Lyell McEwin Hospital, Adelaide, SA, Australia*

3. *School of Medicine, Flinders University, Adelaide, SA, Australia*

**Background:** Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis with resultant cortisol hypersecretion in response to stress has been proposed to underlie an association between low birth weight and increased cardiovascular disease. However, the mechanisms underlying this association are not fully clarified. As glucocorticoid therapy can cause vascular dysfunction, insulin resistance and dyslipidaemia we have investigated whether HPA hyperactivity affects these variables.

**Methods:** Thirty healthy adults (age =  $63 \pm 8$  years, BMI =  $25 \pm 4$  kg/m<sup>2</sup>) were studied. Serum cortisol 30 minutes after 1 µg Synacthen (ACTH<sub>1-24</sub>, Novartis Pharmaceuticals, Australia) was used to define HPA activity. Subjects with a cortisol above (n=15) and below (n=15) the median were categorized as high responders and low responders respectively. Reactive hyperaemia index (RHI) was measured by peripheral arterial tonometry to measure endothelial function and augmentation index (AIx) by applanation tonometry to quantify arterial stiffness. Lipids were measured on a fasting blood sample and the Matsuda index was calculated from glucose and insulin concentrations collected fasting and 30 minutes for two hours after a mixed meal (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat) to estimate insulin sensitivity. Analyses were corrected for age, sex and smoking status using analysis of covariance.

**Results:** High responders had a lower RHI than low responders ( $2.1 \pm 0.2$  vs  $2.6 \pm 0.2$ ,  $p=0.04$ ) indicative of worse endothelial function. High responders had a higher AIx than low responders that approached statistical significance ( $26.3 \pm 1.4$  vs  $22.6 \pm 1.4$  %,  $p=0.08$ ) suggesting greater arterial stiffness. Age ( $p=0.02$ ) and sex ( $p<0.001$ ) were also independent predictors of AIx. There were no significant differences in LDL cholesterol ( $2.7 \pm 0.3$  vs  $3.0 \pm 0.3$  mmol/L,  $p=0.47$ ), triglycerides ( $1.2 \pm 0.3$  vs  $1.0 \pm 0.3$  mmol/L,  $p=0.66$ ) or Matsuda index ( $15.9 \pm 4.4$  vs  $21.8 \pm 4.4$ ,  $p=0.73$ ) between high and low responders.

**Conclusion:** Endothelial dysfunction and increased arterial stiffness are likely to contribute to the association between HPA hyperactivity and an increased risk of cardiovascular disease.

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### Delayed hyponatraemia following transsphenoidal pituitary surgery: predictive factors and the impact of routine day 7 post-operative sodium measurement on readmission rate

**Michelle Isaacs**<sup>1,2</sup>, **Ann McCormack**<sup>1,3,2</sup>

1. *Endocrinology, St Vincent's Hospital, Darlinghurst, NSW, Australia*

2. *St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia*

3. *Hormones and Cancer Group, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia*

#### Background:

Hyponatraemia is the most common reason for readmission after transsphenoidal pituitary surgery (TSS). Risk factors for delayed post-operative hyponatraemia (DPH) remain unclear. We hypothesised that routine measurement of Day 7 post-operative sodium (D7Na) would facilitate outpatient fluid restriction (FR) for DPH and reduce the readmission rate.

#### Aims:

To evaluate the impact of D7Na measurement on the incidence and severity of DPH in patients who had TSS at St Vincent's and St Vincent's Private Hospitals, Sydney. We also aimed to identify pre-operative and early post-operative risk factors for DPH.

#### Methods:

Retrospective audit of all TSS between March 2016 and August 2017 ( $n=71$ ). Measurement of D7Na commenced in March 2017 ( $n=36$ ).

#### Results:

Median age was 48 (20-67) years; 51% were female. Fifty-eight (82%) had a pituitary adenoma. DPH occurred in 12 cases (17%): 6 severe, 3 moderate and 3 mild. Eight patients (11%) required readmission. There was no difference in age, gender, body mass index, previous TSS, pre-operative sodium, lesion size, presence of cavernous sinus invasion, surgical pathology or incidence of preceding DI between the 8 patients who required readmission and the 63 who did not. Day 4 post-operative sodium (D4Na) was lower in those who required readmission ( $140$  [139-140] vs  $142$  [140-143] mmol/L;  $p=0.008$ ). Results were similar when patients with any severity DPH were compared with those who maintained normal sodium. D4Na  $\leq 140$  mmol/L was 88% sensitive and 71% specific for readmission with DPH.

Measurement of D7Na increased detection of DPH (22% vs 11%) but FR at that stage did not impact readmission rate. Readmitted patients already had significant symptoms by D7 or had worsening DPH despite FR.

#### Conclusion:

Routine measurement of D7Na results in increased detection of milder DPH but does not reduce readmission. FR when D4Na  $\leq 140$  mmol/L may reduce readmission for DPH and warrants further investigation.

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### Diagnosis Of Primary Aldosteronism By Seated Saline Suppression Test - Analysis Using Immunoassay

**Moe Thuzar**<sup>1,2</sup>, **Karen Young**<sup>3</sup>, **Ashraf H Ahmed**<sup>2</sup>, **Greg Ward**<sup>3</sup>, **Martin Wolley**<sup>2</sup>, **Zeng Guo**<sup>2</sup>, **Richard D Gordon**<sup>2</sup>, **Brett C McWhinney**<sup>4</sup>, **Jacobus P Ungerer**<sup>4</sup>, **Michael Stowasser**<sup>2</sup>

1. *Department of Endocrinology & Diabetes, Princess Alexandra Hospital, Brisbane, Queensland, Australia*

2. *Endocrine Hypertension Research Centre, University of Queensland Diamantina Institute, Princess Alexandra Hospital, Brisbane, Queensland, Australia*

3. *Department of Chemical Pathology, Sullivan Nicolaides Laboratories, Brisbane, Queensland, Australia*

4. Department of Chemical Pathology, Pathology Queensland, Brisbane, Queensland, Australia

**Background:** Primary aldosteronism (PA) is characterized by excessive, autonomous secretion of aldosterone which fails to suppress upon administration of fludrocortisone and/or salt loading. Seated saline suppression testing (SSST) was recently reported to be superior to recumbent.<sup>1</sup> The recommended diagnostic cut-off 4-hour plasma aldosterone concentration (PAC; 162 pmol/L) for SSST was based on that measured by HPLC-MS/MS. Most diagnostic laboratories, however, use immunoassays to measure PAC.

**Objective:** To define the optimal cut-off PAC measured by immunoassay for the diagnosis of PA using SSST.

**Methods:** This study involved 80 of the 85 SSST studies whose data were used to originally define the optimal HPLC-MS/MS cut-off. PA was confirmed in 65 (23 unilateral, 34 bilateral and 8 subtype yet to be determined) by positive fludrocortisone suppression testing (FST) and/or lateralization on adrenal venous sampling and excluded in 15 (12 cured of PA post-adrenalectomy and 3 non-PA) by negative FST. PAC was measured by a chemiluminescence immunoassay (PAC<sub>IA</sub>) using the DiaSorin Liaison XL analyzer.

**Results:** Receiver operating characteristics analysis revealed good performance (AUC = 0.893; P<0.001) of the 4-hour PAC<sub>IA</sub> for the diagnosis of PA and an optimal diagnostic cut-off of 171 pmol/L, with sensitivity and specificity of 95.4% and 80.0% respectively (Youden index = 0.754). Using this cut-off, none of the three subjects with false-negative SSST had unilateral PA. The cut-off with the next highest Youden index (0.728) was 217 pmol/L and demonstrated lower sensitivity (86.2%) but higher specificity (86.7%). PAC<sub>IA</sub> measurements strongly correlated with PAC measured by HPLC-MS (Pearson correlation coefficient r = 0.94, P<0.001).

**Conclusions:** A higher diagnostic cut-off level for SSST should be employed when PAC is analyzed by immunoassay compared to that by HPLC-MS/MS.

**Reference.** 1. Stowasser M, et al. Comparison of seated with recumbent saline suppression testing for the diagnosis of primary aldosteronism. *J Clin Endocrinol Metab* 2018;103:4113-24.

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## Impact of Victoria's first dedicated Endocrine Hypertension Service on the pattern of primary aldosteronism diagnoses

**Yin Yu Lim**<sup>1,2</sup>, **Renata Libianto**<sup>1,3</sup>, **Jimmy Shen**<sup>1,3</sup>, **Peter J Fuller**<sup>1,3</sup>, **Jun Yang**<sup>1,2,3</sup>

1. Department of Endocrinology Unit, Monash Health, Clayton, Victoria, Australia

2. School of Clinical Sciences, Monash University, Clayton, Victoria, Australia

3. Hudson Institute of Medical Research, Clayton, Victoria, Australia

**Background:** Primary aldosteronism (PA) accounts for 3.2-12.7% of hypertension in primary care but is rarely diagnosed. The Endocrine Hypertension Service (EHS) was established in July 2016 to address the low diagnostic rate by offering education and a streamlined diagnostic pathway to primary care clinicians.

**Aims:** To analyse the impact of Victoria's first dedicated EHS on PA diagnoses.

**Methods:** Clinical data from all patients who attended the EHS since July 2016 (N=242) was collected prospectively. Sociodemographic information was obtained from patient questionnaires, while medical information was obtained from hospital records. Patients were divided into Year 1 (Y1), Year 2 (Y2), and Year 3 (Y3), based on the date of their first EHS visit.

**Results:** Following the establishment of the EHS, the proportion of referrals from primary care increased (20% in Y1, 47% in Y2, 54% in Y3) with more referrals being made for treatment-naïve hypertension (2 in Y1, 10 in Y2, 22 in Y3). Amongst PA patients, the median duration of hypertension prior to the first EHS visit decreased (11 years in Y1, 10 years in Y2, 9 years in Y3), and the prevalence of end-organ damage decreased (44% in Y1, 42% in Y2, 23% in Y3). Targeted management of PA improved clinical and biochemical outcomes. The average reduction in blood pressure following targeted management increased from 15/11mmHg in Y1 to 18/13mmHg in Y2 and 30/22mmHg in Y3.

**Conclusion:** The EHS together with PA-related education programs led to increased primary care referrals and detection of PA earlier in the course of hypertension in patients who otherwise would have had a missed or delayed diagnosis. Referred patients were on fewer antihypertensives and had less end-organ damage which simplified the PA diagnostic process, allowing targeted treatment to be commenced earlier and patient outcomes optimised.

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## Variation in the aldosterone/renin ratio indicates the need for age- and sex-specific reference ranges when screening for primary aldosteronism

**Pravik Solanki**<sup>1,2</sup>, **Stella Gwini**<sup>3,4</sup>, **Peter J Fuller**<sup>2,5</sup>, **Jimmy Shen**<sup>2,5</sup>, **James CG Doery**<sup>1,6</sup>, **Kay Weng Choy**<sup>6</sup>, **Jun Yang**<sup>1,2,5</sup>

1. Department of Medicine, Monash University, Clayton, VIC, Australia

2. Endocrinology, Monash Health, Clayton, Victoria, Australia

3. Department of Epidemiology and Preventive Medicine, Monash University, Clayton, Victoria, Australia

4. University Hospital Geelong, Barwon Health, Geelong, Victoria, Australia

5. Hudson Institute of Medical Research, Clayton, Victoria, Australia

6. Pathology, Monash Health, Clayton, Victoria, Australia

**Background:** The aldosterone/renin ratio (ARR) is the standard screening test for primary aldosteronism (PA), a treatable disease causing ~10 – 20% of hypertension. Current guidelines define ARR>70 (pmol/L)/(mIU/L) as positive regardless of age or sex. However, research suggest that fluctuations in female hormones over the menstrual cycle influence the ARR.

**Objective:** To characterise variations in the ARR according to age and sex.

**Methods:** A retrospective analysis of 466 clinically indicated ARR at Monash Health from December 2016 – June 2018 was conducted. Patients who were on spironolactone, oral contraceptive pill, pregnant or had a known adrenal condition (including untreated PA) were excluded.

**Results:** Among patients aged 20-39 years (N=74), females had significantly higher median aldosterone (373.5 vs 231 pmol/L,  $p=0.017$ ), lower median renin (16.5 vs 23.5 mIU/L,  $p=0.004$ ), and higher median ARR (20.75 vs 10.49,  $p<0.001$ ) than males. However, females had lower median systolic (135 vs 145 mmHg,  $p=0.021$ ) and diastolic (89 vs 96.5 mmHg,  $p=0.007$ ) blood pressure (BP) than males. These sex differences were not observed in the 40 – 59 years ( $n=161$ ) or 60 – 79 years ( $n=157$ ) age groups.

Females were then divided into pre- and post-menopausal groups with an arbitrary cut-off at age 45. Women  $\leq 45$  years had significantly higher median aldosterone levels (364 vs 273 pmol/L,  $p=0.047$ ), lower systolic BP (139 vs 148 mmHg,  $p=0.002$ ), but higher diastolic BP (89.5 vs 80 mmHg,  $p=0.001$ ) than those aged  $>45$ .

**Conclusion:** The ARR is significantly higher in pre-menopausal women but not associated with higher systolic BP, suggesting the potential for false positive results if a single ARR reference range is applied to both sexes at all ages. Our findings highlight the need for age- and sex-specific ARR reference ranges, particularly for premenopausal women, to increase the specificity of the ARR when screening for PA.

## Predictors of mortality in patients with Multiple Endocrine Neoplasia Type 1

Jasmine J Zhu<sup>1</sup>, John R Burgess<sup>1,2</sup>

1. Department of Endocrinology, Royal Hobart Hospital, Hobart

2. School of Medicine, University of Tasmania, Hobart, TAS

**Background:** Multiple Endocrine Neoplasia Type 1 (MEN 1) is an autosomal dominant disease predisposing to hyperplasia and neoplasia of parathyroid, pancreatic and pituitary tissue. Patients typically present prior to the age of 30 years and are subject to reduced life expectancy.

**Aim:** To determine predictors of mortality in MEN 1.

**Method:** Retrospective cohort study of 170 patients with a common *MEN1* genotype. Information was extracted from hospital records. Cox proportional hazards analysis was used to assess median life expectancy (MLE).

**Results:** Cohort MLE was 65.3 years. Compared with female sex, male sex was associated with decreased survival (MLE 70.4 vs 62.7 years,  $p = 0.03$ ). The presence of adrenal neoplasia diagnosed prior to, compared with after, age 45 years (MLE 51.8 vs 70.8 years,  $p < 0.01$ ) and liver metastases diagnosed prior to, compared with after, age 45 years (MLE 38.6 vs 68.9 years,  $p < 0.01$ ) also predicted survival. However, diagnosis of liver metastases beyond the age of 45 years (MLE 68.9 years) was not associated with a statistically significant difference in survival compared with those without liver metastases (MLE 65.6 years,  $p = 0.66$ ).

The presence of the following disease manifestations diagnosed prior to, compared with after, age 45 years, was not associated with a statistically significant difference in MLE: primary hyperparathyroidism (MLE 68.9 vs 70.8 years,  $p = 0.15$ ); pancreatic neuroendocrine tumour (MLE 68.9 vs 66.2 years,  $p = 0.70$ ); gastrinoma (MLE 60.5 vs 70.4 years,  $p = 0.17$ ); pituitary adenoma (MLE 72.6 vs 70.8 years,  $p = 0.32$ ).

**Conclusion:** Among patients with MEN 1, males and those who are diagnosed with either adrenal neoplasia or liver metastases prior to the age of 45 have a poorer prognosis.

## The rising incidence of endocrine toxicity in the era of combination immunotherapy

Anna Galligan<sup>1,2</sup>, Cherie Chiang<sup>3,1</sup>, Nirupa Sachithanandan<sup>1,4</sup>, Shahneen Sandhu<sup>1</sup>, Richard MacIsaac<sup>4</sup>, Thomas Kay<sup>2</sup>, Bala Krishnamurthy<sup>2</sup>

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

2. St Vincent's Institute of Medical Research, Melbourne, Australia

3. Department of Diabetes and Endocrinology, Melbourne Health, Melbourne, VIC, Australia

4. St Vincent's Hospital, Fitzroy, VIC, Australia

**Background:** Immune checkpoint blockade is now established as standard of care in several malignancies. Combined cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) blockade results in improved tumour responses in melanoma but is associated with grade 3-4 immune related adverse events (irAEs) in 55% of patients [1]. Immune-mediated damage to endocrine glands can be a diagnostic and management challenge. We aimed to review the incidence, biochemical evolution and imaging findings of endocrine toxicity related to combined anti CTLA-4 and anti-PD-1 therapy in patients with advanced melanoma.

**Methods:** A retrospective chart review of patients who received combined ipilimumab and nivolumab for metastatic melanoma at Victorian Comprehensive Cancer Centre (VCCC) between 2016-2019 was undertaken. Onset and duration of abnormal biochemistry in endocrine irAEs were recorded.

**Results:** 162 patients received combination ipilimumab and nivolumab. At least one irAE was recorded in 135 (83%) of patients, 100 (62%) required glucocorticoids, and 84 (52%) had an unplanned hospital presentation due to irAEs. Thyroiditis occurred in 30.9%, with a median time to onset of 30.9 days (range 1-234 days). 35/50 cases were identified with routine biochemistry

performed every 4-6 weeks. TSH receptor antibody was measured in 13 patients and all were negative. 58% of patients with thyroiditis developed permanent hypothyroidism. Cortisol deficiency occurred in 18.5% with a median time to diagnosis of 67.5 days (range 5-286). 4/30 cases of hypophysitis were diagnosed on routine biochemistry while 26/30 cases presented with symptoms prompting investigation. Raised pancreatic enzymes were noted in 6 patients but type 1 diabetes did not occur.

Conclusion: The incidence of thyroiditis and hypophysitis are increasing in the era of combination immunotherapy. Routine thyroid function and cortisol testing leads to the detection of some but not all cases. Early recognition of cortisol deficiency and avoidance of unplanned presentations remains a challenge.

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## Utility of Androstenedione and 17-hydroxyprogesterone for monitoring androgen excess in Congenital Adrenal Hyperplasia due to 21-hydroxylase deficiency – a local experience

**Timothy Lin<sup>1</sup>, Ee Mun Lim<sup>1,2</sup>, Bronwyn Stuckey<sup>1,3,4</sup>, Jennifer Ng<sup>4</sup>, Simon Carrivick<sup>1,2</sup>**

1. Sir Charles Gairdner Hospital, Nedlands, WA, Australia

2. Pathwest, Perth

3. University of Western Australia, Perth, Western Australia

4. Keogh Institute, Perth

### Background

Serum 17-hydroxyprogesterone (17-OHP) and Androstenedione are markers of adrenal androgen excess in patients with congenital adrenal hyperplasia (CAH). 17-OHP has considerably greater diurnal variation than Androstenedione, which may limit its clinical utility as a marker of overall androgen exposure.

### Objective

To examine variability of longitudinal serum 17-OHP and Androstenedione measurements in patients with CAH.

### Method

We conducted a retrospective case series on a small cohort of young adults with CAH requiring glucocorticoid replacement. We reviewed longitudinal measurements serum 17-OHP and Androstenedione by LCMS and correlated them with clinical records to evaluate variability of 17-OHP and Androstenedione.

### Results

We analysed the results of 6 patients (1 male, 5 female) with CAH with a minimum of three synchronous measurements of serum 17-OHP and Androstenedione by LCMS over 16 to 58 months. All patients required glucocorticoid replacement with oral hydrocortisone or cortisone acetate.

Inter and intra patient serum 17-OHP was highly variable with peak levels exceeding the upper reference limit by a factor of 50 or more in four patients. Nadir serum 17-OHP in one patient fell within the reference range, whereas the remaining 5 patients had nadir levels which exceeded the upper reference limit by a factor of 1.5 to 31.

Serum Androstenedione levels were less variable. One patient had peak androstenedione levels exceeding 10x the upper reference limit. Nadir Androstenedione levels were within the reference range in four patients, and approximately twice the upper reference limit in the remaining two.

### Conclusion

Our results demonstrate marked variability of serum 17-OHP results in a small cohort of patients with CAH. This likely reflects diurnal variation in response to glucocorticoid therapy. Clinical utility of intermittent measurements of serum 17-OHP is limited in the absence of standardised timing of sample collection in relation to glucocorticoid dosing.

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## Genomic analysis of premature ovarian insufficiency: new genes, pleiotropic genes and clinical implication of diagnosis

**Elena J Tucker<sup>1,2</sup>, Sonia R Grover<sup>3,1,2</sup>, Sylvie Jaillard<sup>1,4,5</sup>, Gorjana Robevska<sup>1</sup>, Jocelyn Van Den Bergen<sup>1</sup>, Chloe Hanna<sup>3,1</sup>, Katrina M Bell<sup>6</sup>, Simon Sadedin<sup>6</sup>, Jérôme Dulon<sup>3,5</sup>, Philippe Touraine<sup>5,7</sup>, Andrew H Sinclair<sup>1,2</sup>**

1. Reproductive Development, Murdoch Children's Research Institute, Parkville, Australia

2. Department of Paediatrics, University of Melbourne, Parkville, VIC

3. Department of Paediatric and Adolescent Gynaecology, Royal Children's Hospital, Melbourne, VIC, Australia

4. Service de Cytogénétique et Biologie Cellulaire, CHU Rennes, Rennes, France

5. Frances Pathologies Gynécologiques Rares, INSERM, Rennes, France

6. Bioinformatics, Murdoch Children's Research Institute, Parkville, VIC

7. Department of Endocrinology and Reproductive Medicine, Sorbonne University Medicine, Rennes, France

Premature ovarian insufficiency (POI), affecting up to 1 in 100 women, is defined by amenorrhea and elevated follicle stimulating hormone before the age of 40. Causative variants have been described in more than 50 genes, but these explain only a minority, suggesting the involvement of many unidentified genes. POI-related genes affect various processes such as gonadal development, DNA replication/repair, hormonal signalling, immune function and metabolism. We have used massively parallel sequencing (MPS) to investigate the genetic basis of POI. In two individuals, we identified C-terminal truncating variants in *TP63* as a new genetic cause of POI. This established a new genotype:phenotype correlation for the *TP63*-related syndromes. We also discovered a surprising cause of "isolated" POI in two cases. In one patient, a homozygous nonsense variant in *NBN* was causative. Recessive pathogenic *NBN* variants typically cause POI in the context of Nijmegen Breakage Syndrome, characterized by microcephaly, cancer predisposition and immunodeficiency, none of which were evident. At a cellular level, however, we found evidence of chromosomal instability, suggesting an elevated cancer risk. In the second case, compound heterozygous variants in *EIF2B2* were causative. Recessive pathogenic *EIF2B2* variants can cause POI in addition to progressive leukoencephalopathy. MRI revealed sub-clinical neurological abnormalities, implicating likely future decline. These diagnoses demonstrate that causative variants in pleiotropic POI genes can be identified before full clinical manifestation. As MPS is increasingly used in research and the clinic, similar cases are likely to arise at an accelerating rate. Clinicians and researchers offering MPS to patients with POI, need to understand and communicate the possible implications of diagnosis, and ideally involve genetic counsellors in patient care. Analysis of our MPS data is ongoing but includes additional diagnoses in known POI genes, as well as many variants of interest in candidate POI genes.

## A new model of urethral closure in the developing penis; the role of discrete hedgehog signalling

**Gerard A Tarulli<sup>1</sup>, Yu Chen<sup>1</sup>, Asao Fujiyama<sup>2</sup>, Andrew J Pask<sup>1</sup>, Geoff Shaw<sup>1</sup>, Marilyn B Renfree<sup>1</sup>**

1. University of Melbourne, Melbourne, VIC, Australia

2. National Institute of Genetics, Mishima, Shizuoka, Japan

Hypospadias is a failure of appropriate urethral positioning within the phallus (penis) during fetal development, and represents the second most common birth defect in Victoria. While modifications in androgen, oestrogen and hedgehog signalling are all implicated in the aetiology of hypospadias, the underlying tissue and molecular mechanisms controlling normal urethral closure are yet to be fully revealed. This study aimed to identify the fundamental signalling networks that regulate urethral closure, and develop a new model for this developmental process.

Marsupials give birth to altricial young, with penis development and urethral closure occurring postnatally. This provides a unique system to directly manipulate the mechanisms controlling urethral closure. Male and female wallaby pouch young were treated orally with estrogens or androgens, respectively, during a critical period of urethral closure. Samples were subjected to RNAseq for differential gene expression and gene-ontology analyses. The distribution of the hedgehog proteins, Sonic Hedgehog (SHH) and Indian Hedgehog (IHH), as well as the transcription factor SOX9, was assessed in normal wallaby phallus tissue using immunofluorescence. Lastly, normal phallus tissue culture explants were treated with SHH or IHH and analysed for *AR*, *ESR1*, *SOX9*, *IHH* and *SHH* gene expression by qPCR.

Gene ontology showed enrichment for chondrocyte proliferation/differentiation in male samples compared with either female samples, or samples from males that received oestradiol. The expression of SHH and IHH localised to discrete regions of the phallus during normal development similar to their independent, compartmentalised expression in developing cartilage. Treatment of phallus explants induced the expression of genes associated with chondrocyte proliferation/differentiation in response to SHH or IHH. Results of this study reveal a new developmental interaction involved in urethral development that mimics chondrocyte differentiation, providing insight into the potential causes of hypospadias.

## The effect of preconception metabolic optimisation on end organ inflammation in late gestation mice.

**Natassia Rodrigo**<sup>1,2</sup>, **Jasmin Russell**<sup>3</sup>, **Hui Chen**<sup>3</sup>, **Carol Pollock**<sup>2,4</sup>, **Sarah Glastras**<sup>1,2</sup>

1. Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

2. University of Sydney, Sydney, NSW, Australia

3. University of Technology, Sydney, Sydney, NSW, Australia

4. Renal Department, Royal North Shore Hospital, Sydney, NSW, Australia

**Background and aims:** Maternal obesity affects 20% of pregnant women and negatively impacts metabolic health in mothers. Maternal complications include gestational diabetes, preeclampsia, fatty liver disease and increased rates of cardiovascular disease. Whilst the mechanisms underlying these complications are not fully elucidated, metabolic inflammation is emerging as a crucial factor. To date, no studies have addressed whether pre-conception maternal weight loss improves inflammatory markers in obese mothers. We aimed to determine if weight loss prior to pregnancy, either with diet modification or liraglutide treatment, improves maternal metabolic outcomes and reduces inflammation in maternal blood, placenta and liver.

**Materials and Methods:** Maternal obesity was modelled in C57BL/6 mice; with dams fed a high fat diet (HFD) versus chow diet for 8 weeks and compared to lean chow-fed controls. In obese dams, liraglutide (0.3mg/kg, s.c., for 4 weeks) or diet modification (switch to chow) was utilised to induce pre-conception weight loss. Pregnancy rates were observed after mating. Maternal anthropometric measures, glucose tolerance and metabolic markers were measured before and 1 week after intervention, and at late gestation. Pregnant dams were sacrificed at gestational Day 18-20 and maternal blood, liver and placenta were collected. Immunohistochemistry, western blotting and real-time PCR were used to measure tissue-specific metabolic profiles together with inflammatory markers including TGF- $\beta$ , IL-6 and hs-CRP.

**Results:** HFD-fed dams had greater weights and reduced glucose tolerance compared to chow-fed dams. Following intervention with liraglutide or diet modification, insulin resistance and body weight were reduced. Weight intervention with either liraglutide or diet improved conception rates and normalised foetal number in HFD-fed dams. Liver and placental inflammatory markers and metabolic markers were improved in the intervention groups compared to the non-treated HFD-fed group.

**Conclusions:** Preconception weight loss can improve maternal weight leading into pregnancy. It further improves maternal insulin resistance, organ inflammation and metabolic markers.

## Gonocyte transformation into spermatogonial stem cells (SSC): The key to understand infertility and malignancy of cryptorchidism

**Ruili Li**<sup>1,2</sup>, **Amanda Vannitamby**<sup>1</sup>, **Sarah, S.K. Yue**<sup>1</sup>, **Jorien Meijer**<sup>1</sup>, **Sanduni A. Abeydeera**<sup>1</sup>, **Melissa Y. Tien**<sup>1</sup>, **Emily C. Burton**<sup>1</sup>, **Gulcan Sarila**<sup>1</sup>, **John Hutson**<sup>1,3,2</sup>

1. F. Douglas Stephens Surgical Research Laboratory, Murdoch Children's Research Institute, Parkville,, Victoria, Australia

2. Department of Paediatrics, University of Melbourne, Parkville,, Victoria, Australia

3. Urology Department, Royal Children's Hospital, Parkville,, Victoria, Australia

### Introduction

Undescended testis (UDT) is a major health problem, affecting over 2% of new-born boys with increased infertility (30-60%) and testicular cancer (5-10 fold > normal males) later in life. We have studied animal models in conjunction with human biopsies of UDT in order to understand the process of gonocyte transformation into SSC to elucidate how to prevent infertility and testicular cancer in cryptorchidism.

### Methods

We used testes from Oct4-promoter-driving GFP transgenic mice, androgen receptor knockout (ARKO) mice, hypogonadal (*hpg*) mice, Bax knockout (BaxKO) mice and human biopsies for gene expression, immunohistochemistry and confocal imaging analysis. Serum and testes were collected for hormone analysis.

### Results

We have found that mouse gonocytes transformed into SSC between postnatal days 2-6 during minipuberty when testosterone, FSH receptor and Oct4 peaked. There was no difference for number of gonocytes transformed into SSC/tubule between ARKO mice and wild type (WT) littermates. Germ cells/tubule were significantly less in *hpg* mice comparing to WT. There were persisting gonocytes in BaxKO testicular tubules which were not present in WT. UDT biopsies showed empty tubules without germ cell significantly increased and number of germ cells decreased with increasing age of orchidopexy. There were persisting gonocytes in testicular tubules of congenital UDT after gonocyte transformation.

### Conclusion

In conclusion, we found that gonocytes transform into SSC at 2-6 days of age in mouse. Like human minipuberty does exist in mouse and coincides with gonocyte transformation into SSC. Gonocyte transformation in mouse is independent from androgen but gonadotrophin deficiency caused germ cell death. Disruption of apoptosis regulator, Bax, caused persisting gonocytes. Orchidopexy at older age showed significant germ cell depletion. These suggest that FSH may be important in gonocyte transformation and persisting gonocytes in congenital UDT could be due to disruption of apoptosis during gonocyte transformation, which could cause testicular cancer.

## Interferon-Tau Exerts Direct Prosurvival and Antiapoptotic Actions in Luteinized Bovine Granulosa Cells

**Raghavendra Basavaraja<sup>1</sup>, Senasige Thilina Madusanka<sup>1</sup>, Jessica N Drum<sup>2</sup>, Ketan Shrestha<sup>1</sup>, Svetlana Farberov<sup>1</sup>, Milo C Wiltbank<sup>3</sup>, Roberto Sartori<sup>2</sup>, Rina Meidan<sup>1</sup>**

1. Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

2. Department of Animal Science, University of São Paulo, Piracicaba, Brazil

3. Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, United States

Interferon-tau (IFNT), a multifunctional type I interferon, serves as a signal to maintain the corpus luteum (CL) during early pregnancy in domestic ruminants. Here we aimed to study whether IFNT directly affects the function of luteinized bovine granulosa cells (LGCs), used as a model for large luteal cells. Recombinant ovine IFNT (roIFNT) stimulated signal transducer and activator of transcription-1 (STAT1) and IFN-stimulated genes (ISGs; *MX2*, *ISG15* and *OAS1Y*) in LGCs. The LGC also had high expression of IFN receptors (IFNAR1) and displayed a rapid and transient phosphorylation of STAT1 as well as an elevation in total STAT1 protein after longer incubation times (24-48h). These results indicate that IFNT activates type-1 interferon pathways in LGCs. In addition, IFNT treatment increased viable LGCs numbers and reduced dead and apoptotic cell counts in flow cytometry analyses using Annexin V staining. Consistent with these effects on cell viability, IFNT upregulated cell survival proteins (MCL1, BCLxL and XIAP) and decreased the levels of proteins implicated in apoptosis, gamma-H2AX, cleaved caspase 3 and thrombospondin-2 (THBS2). Notably, IFNT reversed the actions of thrombospondin-1, a potent luteal apoptotic factor, on cell viability as well as on XIAP and cleaved caspase 3 protein levels. Furthermore, roIFNT stimulated the mRNA concentrations for a series of proangiogenic genes such as *FGF2*, *PDGFB* and *PDGFAR*. In support of the in vitro observations, we found that CL tissue collected from day 18 pregnant cows had higher ISGs along with elevated levels of *FGF2*, *PDGFB*, *THBS2* and *XIAP* as compared to CL from non-pregnant cows on day 18 of the estrous cycle. These findings show that IFNT activates diverse pathways in LGCs, promoting survival and blood vessel stabilization, while suppressing cell death signals. These mechanisms might contribute to CL maintenance during early pregnancy.

## Maternal adipose tissue derived exosomes modulates glucose and fatty acid uptake in human primary trophoblast cells

**Nanthini Jayabalan<sup>2,1</sup>, Martha Lappas<sup>2,3</sup>, Carlos Salomon<sup>4,1</sup>**

1. Exosomes Biology Laboratory (EBL), Centre for Clinical Research (CCR), The University of Queensland, Herston, QLD, Australia

2. Obstetrics, Nutrition and Endocrinology Group, Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Victoria, Australia

3. Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, Victoria, Australia

4. Department of Clinical Biochemistry and Immunology, Faculty of Pharmacy, University of Concepción, Concepción, Chile

Maternal obesity and gestational diabetes (GDM) are conditions associated with increased placental glucose uptake and excessive fat accumulation in the fetus causing fetal overgrowth. We have previously shown that adipose tissue from GDM women secretes higher number of exosomes and positively correlates with fetal birthweight. These findings led us to hypothesise that adipose tissue derived exosomes (AT-exo) from GDM women may influence placenta nutrient uptake and be responsible for fetal overgrowth. In this study, we aimed to determine the effect of AT-exo on placental glucose and fatty acid uptake.

Human omental adipose tissue was obtained from women with GDM (n=9 lean; n=6 obese) and BMI-matched normal glucose tolerant (NGT) controls (n=9 lean; n=6 obese) at the time of term Caesarean section. Adipose tissue explants were performed and exosomes were isolated from conditioned media by differential centrifugation and characterised based on size distribution, protein markers and morphology. To determine the effect of AT-exo on placenta, trophoblast cells were isolated from fresh placenta (n= 6 patients). The effect of AT-exo on glucose and fatty acid uptake in human primary placental trophoblast cells was evaluated using 2-NBDG and BODIPY, respectively. Statistical analyses between the groups were conducted using one-way ANOVA and  $p < 0.05$  was considered significant.

AT-exo from both lean and obese women with GDM significantly increased placental glucose uptake compared to BMI-matched NGT women. There was no difference in placental glucose uptake between AT-exo obtained from lean or obese women in both NGT or GDM women. Interestingly, AT-exo from obese GDM and NGT women significantly reduced fatty acid uptake compared to AT-exo from lean GDM and NGT women, respectively.

In conclusion, maternal AT-exo involves in regulating placental nutrient uptake, which can have an impact on fetal growth.



## The impact of surgical menopause on body Composition, bone and metabolic parameters: a cross sectional analysis

**Kathy Fu<sup>1,2</sup>, Christopher White<sup>3</sup>, Leon Botes<sup>4</sup>, Lesley Andrews<sup>4</sup>, Angela Sheu<sup>2,5,6</sup>**

1. Prince of Wales Hospital, Randwick, New South Wales, Australia

2. St Vincent's Hospital, Darlinghurst, NSW, Australia

3. Department of Endocrinology, Prince of Wales Hospital, Randwick, NSW, Australia

4. Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick, NSW, Australia

5. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

6. St Vincent's Clinical School, UNSW Medicine, Randwick, NSW, Australia

**BACKGROUND:** Natural menopause induces adverse body composition<sup>1</sup>, bone and metabolic changes. We aimed to examine whether similar changes occur after surgical menopause (SM), which differs by earlier age and the lack of perimenopausal transition. SM women have increased risk of bone loss<sup>2</sup>; higher rates of cardiovascular disease are also observed<sup>3</sup> with conflicting data about underlying mechanisms<sup>4,5</sup>.

**METHODS:** 85 women with previous risk-reducing salpingo-oophorectomy (RRSO) for high oncology risk were compared with 30 age-matched friends. Dual Energy Xray Absorptiometry (DXA) was performed measuring bone density and body composition. Fasting blood samples for bone turnover, biochemical menopausal status, and glucose homeostasis were measured. RRSO women on HRT were excluded. Statistical analysis was conducted with Anova, Spearman's correlations and backwards linear regression.

**RESULTS:** As a cohort, RRSO women had higher total fat mass ( $p=0.047$ ) and fasting insulin levels ( $p=0.014$ ) and trended towards higher BMI ( $p=0.064$ ) and truncal fat mass ( $p=0.079$ ) than age matched controls.

On subgroup analysis, RRSO women <2.5 years post-operatively had higher weight ( $p=0.040$ ) and lean mass ( $p=0.042$ ) than controls and trended towards higher total fat mass ( $p=0.077$ ), truncal fat ( $p=0.099$ ) and BMI ( $p=0.081$ ). No differences with increasing duration since RRSO were seen (Fig 1).

Women undergoing RRSO within 2.5 years also exhibited higher P1NP levels ( $p=0.003$ ) and trend towards higher alkaline phosphatase ( $p=0.058$ ) (Table 1). This was associated with lower PTH ( $p=0.02$ ) despite similar calcium, phosphate and vitamin D levels. These parameters returned to baseline >2.5 years post-RRSO.

There was significant correlation between total and trunk fat mass and bone markers (Vitamin D, P1NP and ALP) after controlling for age in both groups.

**CONCLUSION:** Fat mass, weight and bone turnover changes are correlated in women undergoing SM compared to controls, and all increase detrimentally within 2.5 years after RRSO. These changes appear to attenuate with increasing duration since

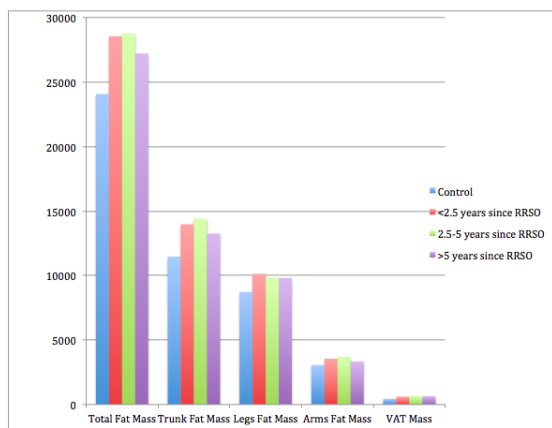


Figure 1: Trends in mean fat mass changes as measured by DXA

RRSO.

Table 1: Selected Results.

	CONTROLS	RRSO GROUP			Total Group
		0 - 2.5yrs	2.5-5yrs	>5yrs	
<b>N</b>	30	42	18	15	75
<b>Age (years)</b>	48.6 (4.8)	45.4 <sup>a</sup> (4.1)	47.8 <sup>a</sup> (3.2)	53.0 <sup>a</sup> (3.5)	47.5 (4.8)
<b>Weight (kg)</b>	65.0 <sup>b,c</sup> (13.0)	72.3 <sup>b</sup> (15.6)	70.7 (14.4)	68.2 (15.4)	71.1 <sup>c</sup> (15.2)
<b>BMI (kg/m<sup>2</sup>)</b>	24.7 (4.8)	27.0 (6.1)	27.0 (5.5)	26.0 (5.5)	26.8 (5.7)
<b>Total Fat Mass (g)</b>	24064 (9117)	28551 (11448)	28783 (9815)	27210 (11175)	28339 (10895)
<b>Total Lean Mass (g)</b>	38198 <sup>d</sup> (5598)	40757 <sup>e</sup> (4548)	39127 (5567)	38071 (5650)	39829 (5085)
<b>Trunk Fat Mass (g)</b>	11470 (4947)	13970 (6936)	14410 (6375)	13248 (6584)	13931 (6659)
<b>VAT Mass (g)</b>	435 (362)	607 (575)	662 (636)	653 (594)	630 (586)
<b>HOMA2-IR</b>	0.684 (0.181)	0.652 (0.069)	0.816 (0.635)	0.714 (0.090)	0.704 (0.320)
<b>P1NP (ug/L)</b>	44.8 <sup>e</sup> (23.0)	64.1 <sup>c,f</sup> (30.9)	41.1 <sup>f</sup> (18.2)	44.1 <sup>f</sup> (23.7)	54.6 (28.8)
<b>ALP (U/L)</b>	60.79 (19.48)	69.48 (21.73)	60.89 (12.87)	61.47 (12.91)	65.8 (18.7)
<b>PTH (pmol/L)</b>	4.26 <sup>g</sup> (1.81)	3.34 <sup>g</sup> (1.61)	3.41 (1.30)	3.96 (1.36)	3.48 (1.50)

<sup>a</sup>Values in each group are displayed as the mean (SD)

Superscripts a,b,c,d,e,f,g denote  $p<0.05$

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### Psychiatric comorbidities in women with polycystic ovary syndrome.

**Chau Thien Tay<sup>1,2</sup>, Helena Teede<sup>1,2</sup>, Deborah Loxton<sup>3</sup>, Jayashri Kulkarni<sup>4</sup>, Anju Joham<sup>1,2</sup>**

1. Monash Centre for Health Research and Implementation, Clayton, VIC, Australia

2. Department of Diabetes and Vascular Medicine, Monash Health, Clayton, VIC, Australia

3. Research Centre for Generational Health and Ageing, University of Newcastle, Callaghan, New South Wales, Australia

4. The Monash Alfred Psychiatry Research Centre, Monash University and The Alfred Hospital, Melbourne, Victoria, Australia

**Background:** PCOS is associated with increased risk for depression and anxiety but its association with other psychiatric disorders is less clear. We aimed to investigate the prevalence of psychiatric disorders in women with PCOS, clarify the relationship between PCOS and psychiatric disorders and identify important correlates of psychiatric disorders in women with PCOS.

**Methods:** A cross-sectional study was conducted in the cohort of community-recruited women born 1989-95 from the Australian Longitudinal Study of Women's Health (ALSWH). Survey data was collected online in 2015. 760 and 7910 women with and without self-reported PCOS were included. The main outcomes were self-reported psychiatric diagnoses including depression, anxiety, post-traumatic stress disorder, bipolar affective disorder, obsessive compulsive disorder, borderline personality disorder and others. The main explanatory variables examined was self-reported PCOS status. Other factors examined included adverse childhood events, social support, perceived stress, sociodemographic and lifestyle factors.  $\chi^2$  tests were used to examine the differences in prevalence between groups. Logistic regression analyses were performed to assess factors associated with psychiatric disorders and the relationship between PCOS and psychiatric disorders.

**Results:** Compared to women not reporting PCOS, women reporting PCOS had significantly higher prevalence of anxiety (44.7% vs 32.3%), depression (53.2% vs 37.1%), post-traumatic stress disorder (11.7% vs 5.5%), bipolar affective disorder (3.4% vs 2.2%), obsessive compulsive disorder (6.3% vs 3.0%) and borderline personality disorder (6.8% vs 2.7%). Adjusted analyses showed that self-reported PCOS was significantly associated with increased odds for all these psychiatric disorders. Adverse childhood experience was the strongest factors associated with psychiatric disorders (ACES  $\geq 4$ : adjusted OR 2.9, 95% CI 2.4-3.5) and this is much more commonly reported in women with PCOS.

**Conclusions:** Women with PCOS have high prevalence of psychiatric comorbidities other than anxiety and depression which require consideration. An assessment of psychological wellbeing in women with PCOS is warranted.

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### BMI trajectories in women with and without polycystic ovary syndrome

**Chau Thien Tay<sup>1,2</sup>, Helena Teede<sup>1,2</sup>, Roger Hart<sup>3</sup>, Arul Earnest<sup>1</sup>, Dorota Doherty<sup>4</sup>, Martha Hickey<sup>5</sup>, Lisa Moran<sup>1</sup>**

1. Monash Centre for Health Research and Implementation, Clayton, VIC, Australia

2. Department of Diabetes and Vascular Medicine, Monash Health, Clayton, VIC, Australia

3. Division of Obstetrics and Gynaecology, University of Western Australia, King Edward Memorial Hospital, Perth, Western Australia, Australia

4. Faculty of Health and Medical Sciences, University of Western Australia, Perth, Western Australia, Australia

5. Department of Obstetrics and Gynaecology, University of Melbourne and the Royal Women's Hospital, Melbourne, Victoria, Australia

**Background:** Over 60% of women with polycystic ovary syndrome (PCOS) are either overweight or obese but the natural history of weight gain in women with PCOS is not well understood. We aim to examine the natural history of weight gain in women with and without diagnosed PCOS from birth until adulthood.

**Methods:** We performed a longitudinal analysis on 227 females of the Western Australian Pregnancy Cohort (Raine) Study where 66 females were diagnosed with PCOS using the Rotterdam Criteria. Anthropometric measurements were collected by trained researchers at birth and ages 1, 2, 3, 5, 8, 10, 14, 16, 20 and 22. The primary outcome was body mass index (BMI). T-tests and  $\chi^2$  tests were used to examine the differences between groups. Longitudinal analysis of BMI was performed using Generalized Estimating Equations using PCOS on time and hyperandrogenism on time as interaction terms. Regression models were adjusted for parental BMI, family income, age of menarche, employment history, smoking and relationship status.

**Results:** Cross-sectional analysis showed that compared to women without PCOS, women with PCOS had higher BMI from age 14 onwards. Compared to women with non-hyperandrogenic PCOS, women with hyperandrogenic PCOS had higher BMI from year 8 onwards. In longitudinal analysis, significant interaction was detected between PCOS and time (Wald test <0.001) in the overall population and between hyperandrogenism and time (Wald test <0.001) in women with PCOS. Adjusted regression showed that women with and without PCOS had similar trend for BMI gain but significantly higher BMI gain occurred in women with PCOS from age 14 onwards. In women with PCOS, adjusted regression showed that women with hyperandrogenism had higher BMI gain from age 14 onwards.

**Conclusion and relevance:** Excessive weight gain in women with PCOS occurred after puberty and hyperandrogenism status is predictive of excessive weight gain.

## The relationship between maternal osteocalcin measurements and glucose metabolism in pregnancy

Angela McPhee<sup>1</sup>, Constance Yap<sup>2,3</sup>, Gideon Meyerowitz-Katz<sup>4,5</sup>, SA Paul Chubb<sup>6,7</sup>, Vita Birzniece<sup>8</sup>, Bu B Yeap<sup>9,10</sup>, N Wah Cheung<sup>2,3</sup>, Mark Mclean<sup>1,11</sup>, Sue Lynn Lau<sup>2,1,3,11</sup>

1. Department of Diabetes and Endocrinology, Blacktown Hospital, Sydney, NSW, Australia
2. Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia
3. Faculty of Medicine, Western Clinical School, University of Sydney, Sydney, NSW, Australia
4. Western Sydney Diabetes, Western Sydney Local Health District, Sydney, NSW, Australia
5. University of Wollongong, Wollongong, NSW, Australia
6. PathWest Laboratory Medicine, Fiona Stanley Hospital, Perth, WA, Australia
7. School of Pathology and Laboratory Medicine, University of Western Australia, Perth, WA, Australia
8. University of NSW, Kensington
9. The Medical School, University of Western Australia, Perth, WA, Australia
10. Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, WA, Australia
11. School of Medicine, University of Western Sydney, Sydney, NSW, Australia

### Introduction

Total osteocalcin (TOC) comprises of undercarboxylated (ucOC) and carboxylated (cOC) forms. In non-pregnant populations, low ucOC and cOC levels predict incident type 2 diabetes<sup>(1-4)</sup>. Studies in pregnancy, however, demonstrate higher TOC in gestational diabetes (GDM) compared to controls<sup>(5,6)</sup>.

### Aims/Methods

We evaluated the relationship between glucose and osteocalcin levels, using data from a prior randomised controlled trial of vitamin D supplementation in pregnancy. 209 women (gestational age <20 weeks) commenced vitamin D, 5000 or 400 IU daily, at a mean first visit gestation of 14.7 wks. 179 underwent OGTT at 26-28 weeks, 19 developed GDM. TOC, ucOC, proportion ucOC (%ucOC), P1NP and C-telopeptide (CTX) were measured in 205 stored serum samples collected at first visit and 174 samples at 26-28 weeks. We assessed correlation between baseline OC levels, glucose measures at OGTT, and bone turnover markers. Logistic regression evaluated baseline %ucOC as a predictor of subsequent GDM, adjusting for GDM risk factors.

### Results

Table 1 lists baseline characteristics and biochemistry. %ucOC but not ucOC was correlated with 2-hr glucose ( $r=0.22$ ,  $p=0.003$ ). Both ucOC and %ucOC correlated with BMI. ucOC positively correlated with CTX and P1NP ( $r=0.48$ ,  $r=0.61$  respectively,  $p<0.001$ ), whereas %ucOC was negatively correlated with CTX and P1NP ( $r=-0.21$ ,  $r=-0.34$ ,  $p<0.01$ ). OC levels did not change significantly between baseline and 26-28 weeks. The odds ratio (OR) for GDM for subjects with %ucOC values above the median ( $\geq 48.5\%$  compared to  $<48.5\%$ ) was 4.07 (1.28-12.90,  $p=0.017$ ). Higher %ucOC remained significantly associated with GDM after adjusting for parity, BMI, ethnicity, family history of diabetes, maternal age, and 25OHvitD level at 26-28 weeks, OR 3.47 (1.02-11.81,  $p=0.046$ ).

### Conclusion

Higher %ucOC before 20 weeks' gestation was associated with higher rates of GDM and higher 2-hr OGTT values. Data support a relationship between bone and glucose metabolism. Cause or direction of effect remains unknown.

Table 1 - Baseline characteristics and biochemistry at visit 1 and visit 2.

	N	Mean (SD)
Maternal age at visit 1	-	29.4 (4.9)
Gestation at visit 1	-	14.7 (2.6)
Pre-pregnancy BMI (kg/m <sup>2</sup> )	-	25.5 (5.4)
Ethnicity		
Caucasian	69	-
Non-Caucasian	140	-
Family history of diabetes	59	-
Parity		
n = 0	98	-
n = 1	66	-
n = 2	27	-
n ≥ 3	18	-
Visit 1 OC measurements and bone turnover markers	205	
TOC (µg/L)	-	14.13 (5.24)
ucOC (µg/L)	-	6.89 (2.35)
P1NP (µg/L)	-	51.04 (19.16)
CTX (ng/L)	-	239.22 (109.63)
ucOC:TOC (%)	-	50.15 (10.76)
Gestation at visit 2	-	27.1 (0.9)
Fasting BGL at OGTT (mmol/L)	-	4.1 (0.4)
Two-hour BGL at OGTT (mmol/L)	-	5.9 (1.5)
Fasting insulin at OGTT (mIU/L)	-	6.9 (7.1)
HOMA-IR	-	0.9 (1.4)*
Vitamin D at visit 2 (nmol/L)	-	77 (29)
Visit 2 OC measurements and bone turnover markers	174	
TOC (µg/L)	-	13.32 (4.78)
ucOC (µg/L)	-	7.25 (2.76)
P1NP (µg/L)	-	50.49 (25.12)
CTX (ng/L)	-	429.92 (182.21)
ucOC:TOC (%)	-	54.87 (9.95)

\*median (IQR)

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## Estradiol method evaluation: DiaSorin Liaison compared to liquid chromatography tandem mass spectrometry (LC-MS/MS)

**Caroline Bachmeier<sup>1</sup>, Brett McWhinney<sup>1</sup>, Matthew Burke<sup>1</sup>, Melanie Coogan<sup>1</sup>, Rebecca Swenson<sup>1</sup>, Carel Pretorius<sup>1</sup>**

*1. Chemical Pathology, Pathology Queensland, Brisbane, QLD, Australia*

### Background

Estradiol (E2) is a commonly requested test. The ability to measure very low concentrations is relevant when assessing gonadal function in children, men, postmenopausal women on hormone replacement therapy and breast cancer patients treated with aromatase-inhibitors. Accuracy, specificity, sensitivity and reproducibility are critical. Various methods for measuring E2 exist, however, the gold standard is isotope-dilution liquid-chromatography-tandem mass spectrometry (LC-MS/MS).<sup>1,2</sup>

### Methods

At Pathology Queensland E2 is measured via a chemiluminescent immunoassay on the Diasorin-Liaison instrument. We performed a comparison of 356 samples submitted for routine E2 analysis with both LC-MS/MS and immunoassay to determine whether they meet the analytical quality specifications at low concentrations. A total allowable error of 27% based on biological variation is the minimum acceptable quality specification, allowing differentiation between 100 and 50pmol/L.

### Results

356 samples were analysed. Of these, 274 had a Liaison result above the Liaison's lower reporting limit (36pmol/L). Despite the acceptable correlation and regression slope of 1 for the entire group a significant constant bias of 30pmol/L was detected. Numerous Liaison results were outside the allowable difference limits, particularly at low concentrations.

Results between the Liaison's lower reportable limit and 300pmol/L (n=215) were examined separately and the allowable error tolerance was further relaxed to ±25pmol/L below 100pmol/L. Liaison demonstrated a positive bias of 31pmol/L and many results remained outside the extended allowable tolerance limits. We mathematically corrected the constant bias of the Liaison

E2 results and repeated the statistical analysis of differences. The number of discordant results below 150pmol/L remained significantly elevated ( $p < 0.001$ ). After correcting for bias a result of 100pmol/L will have a 95% confidence distribution of  $\pm 60$ pmol/L, which is more than double the minimum acceptable  $\pm 27$ pmol/L.

#### Conclusion

Although automated immunoassay methodology has improved with lower detection levels, E2 can only be reliably measured by LC-MS/MS at very low concentrations.

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## Lipoprotein X

**Caroline Bachmeier<sup>1</sup>, Carel Pretorius<sup>1</sup>**

1. *Chemical Pathology, Pathology Queensland, Brisbane, QLD, Australia*

Hyponatraemia can be caused by a variety of reasons. Serum lipoprotein X is an abnormal lipoprotein rich in phospholipid and unesterified cholesterol. It can occur in patients with cholestasis resulting in pseudohyponatraemia when sodium is measured using a direct ion-specific electrode (ISE) method.

We present the case of a 60 year-old man who presented to hospital with painless jaundice. Bilirubin was 239 $\mu$ mol/L ( $<20$ ), conjugated bilirubin 148 $\mu$ mol/L ( $<4$ ), ALP 436U/L (30–100), GGT 1480U/L ( $<55$ ), ALT 377U/L ( $<45$ ), AST 245U/L ( $<35$ ). Subsequent investigations including a computed tomogram and liver biopsy confirmed metastatic cholangiocarcinoma. Comorbidities included hypertension, obesity, dyslipidaemia and type 2 diabetes mellitus. The patient was taking rosuvastatin, aspirin, atenolol, dapagliflozin, amlodipine, valsartan, hydrochlorothiazide and metformin.

Throughout the admission the patient was hyponatraemic. Paired testing confirmed a serum sodium 118mmol/L (135–145), calculated serum osmolality 260mmol/L (275–295), urine osmolality 243mmol/kg and urine sodium 23mmol/L. Renal function, cortisol, thyroid function, protein and glucose were unremarkable. He was clinically euvolaemic and displayed no neurological symptoms.

The thiazide was ceased without improvement of sodium levels. Measured osmolality 5 days later was 268mmol/kg with an osmolar gap of 14mmol/kg, serum sodium was 119mmol/L. A lipid profile to exclude pseudohyponatraemia showed an elevated cholesterol and LDL at 14.9 and 14mmol/L respectively, triglycerides 1.6mmol/L, HDL 0.2mmol/L, VLDL 0.7mmol/L, apolipoprotein A1 was low at 0.27g/L and apolipoprotein B normal at 1.11g/L. Lipid electrophoresis revealed lipoprotein X (figure 1). This was confirmed by the characteristic pattern on ALP isoenzyme electrophoresis (figure 2). Sodium was re-measured by direct ISE to correct for pseudohyponatraemia secondary to lipoprotein X and showed a sodium of 131mmol/L. Subsequent measurements by direct ISE showed normal sodium results. After stent placement and resolution of cholestasis, a repeated lipid profile 1 month later showed resolution of lipoprotein X.

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## Correlation of pre-operative sestamibi scintigraphy with operative findings in primary hyperparathyroidism

**Lauren Burrage<sup>1</sup>, Shyam Sunder<sup>1</sup>, Michael Donovan<sup>2</sup>**

1. *Department of Endocrinology and Diabetes, Sunshine Coast Health Service, Sunshine Coast, Queensland, Australia*

2. *Department of Surgery, Sunshine Coast Health Service, Sunshine Coast, Queensland, Australia*

**Background:** Minimally invasive parathyroidectomy is an appealing option for treatment of primary hyperparathyroidism (1). Good quality pre-operative localisation imaging is essential to facilitate the surgical approach. Several imaging modalities are available, however there are advantages and disadvantages to each. The literature reports a wide range of accuracies for each modality (2, 3, 4). The purpose of this study was to define the accuracy and positive predictive value (PPV) of Sestamibi scintigraphy for pre-operative localisation in primary hyperparathyroidism in a cohort of patients from a large regional hospital that includes parathyroid adenoma and hyperplasia. Furthermore, we aimed to evaluate the role of inter-observer variation in interpretation of Sestamibi scintigraphy.

**Method:** A retrospective chart review was conducted on all parathyroid surgeries completed at Nambour General Hospital from January 2010 to February 2017. There was a total of 140 patients. 117 patients met inclusion criteria. Sestamibi reports were reviewed and recorded as correlating with the correct side or quadrant as compared with operative and histopathologic findings.

**Results:** 103 patients (88%) had adenomas and 14 patients (12%) had parathyroid hyperplasia. Sestamibi scans had an accuracy of 60% and PPV of 71% for localising the correct quadrant and accuracy of 73% and PPV of 87% for localising the correct side. However, 76% of negative Sestamibi scans had an adenoma subsequently confirmed at operation. There were 12 imaging providers in total. Accuracy and PPV were calculated for the 4 major providers. Using the Marascuillo method, there was no significant difference in accuracies or PPV between imaging providers ( $P < 0.05$ ).

**Conclusion:** The accuracy of Sestamibi scintigraphy for pre-operative localisation in our cohort of patients is similar to literature reports. The low accuracy of negative scans suggests that additional imaging (including 4D-computed tomography or high resolution ultrasonography) should be performed to reduce the need for bilateral (classical) neck exploration.

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### Glucocorticoids promote mitochondrial fatty acid oxidation in fetal cardiomyocytes

Jessica R Ivy<sup>1</sup>, Roderick N Carter<sup>1</sup>, Jin-Feng Zhao<sup>2</sup>, Emma J Agnew<sup>1</sup>, Charlotte B Buckley<sup>1</sup>, Ian G Ganley<sup>2</sup>, Nicholas M Morton<sup>1</sup>, Karen E Chapman<sup>1</sup>

1. *University/BHF Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, UK*
2. *School of Life Sciences, University of Dundee, Dundee, UK*

Perinatal heart maturation is associated with a switch in energy substrate preference from glucose metabolism to fatty acid (FA) oxidation. The cause of this metabolic switch is unknown. The late gestational surge in glucocorticoids (GC) is critical for the structural and functional maturation of the fetal heart and may contribute to metabolic maturation. Here, we hypothesized that GC promote a switch to fatty acid oxidation in late gestation fetal cardiomyocytes. Primary mouse fetal cardiomyocytes were cultured following collagenase and pancreatin digestion of embryonic day (E)14.5-15.5 hearts. Two days later, cells were treated with RU486 (GR antagonist) or vehicle for 30 minutes prior to 24h treatment with 1mM dexamethasone. Mitochondrial respiration and glycolysis were measured using a Seahorse XF24 Analyzer. Respiration was measured in the presence of the FA, palmitate (100mM) and the mitochondrial FA uptake blocker etomoxir (6mM) or vehicle. Mitophagy was assessed following dexamethasone treatment of cultures of fetal cardiomyocytes from MitoQC mice in which an increase in red puncta is indicative of mitophagy. Mitochondrial volume was measured using MitoGraph software following staining with Mito-tracker deep red FM or using MitoQC cardiomyocytes. Dexamethasone did not alter glycolysis. In the presence of palmitate, dexamethasone increased basal respiration and ATP production. This was attenuated by etomoxir or RU486. Neither mitochondrial volume or mitophagy were affected by dexamethasone. Consistent with an increase in FA oxidation, dexamethasone increased the expression of genes involved in FA uptake (*Cd36, Cpt1a, Cpt1b*) and utilization (*Lcad, Mcad, Lipin1, Ppargc1a*) but not *Sirt1* (involved in autophagy and metabolism) and *Scad* (short chain FA utilization). These data support a glucocorticoid-induced switch in substrate preference towards FA oxidation in fetal cardiomyocytes through changes in gene expression rather than gross changes in mitochondrial volume or inducing mitochondrial turnover.

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### The effect of medium chain triglycerides on type 2 diabetes mellitus associated disorders

Yang Chen<sup>1</sup>, Chen Chen<sup>1</sup>, Lili Huang<sup>1</sup>

1. *School of biomedical science, The University of Queensland, Brisbane, QLD, AUSTRALIA*

Medium-chain triglycerides (MCTs) are triglycerides containing medium-chain fatty acids, which have chain lengths of 6–12 carbon atoms. MCTs have been widely used in different diets as a healthy replacement of long chain fatty acids and combined with medications to treat digestive diseases. However, limited study has focused on the effect of MCTs on obese type 2 diabetes mellitus (T2DM) and its complications, including non-alcoholic fatty liver disease (NAFLD) and cardiovascular disorders. It has been reported that MCT based diets can effectively attenuate the progression of NAFLD and improve cardiovascular functions. It remains unclear whether MCT diet can demonstrate similar positive effects on liver and heart under diabetic conditions. To determine the side effects and therapeutic effects, both wild type and diabetic mice were treated with normal chow, MCT based diet and high fat diet (HFD). 8-week-old male WT mice under normal diet and MCT diet for 12 weeks showed similar body weight, glucose tolerance, electrocardiogram, liver triglyceride content and hormone profiles, including insulin and growth hormone, while mice under HFD showed deterioration in those parameters. While showing increased energy intake under both HFD and MCT based diet group, only MCT group showed increased energy expenditure and maintained total energy balance. Following the WT experiments, 10 week-old melanocortin 4 receptor knockout (MC4RKO) mice, an obese mice model due to hyperphagia, were under HFD for 6 weeks to induce T2DM and switched to pair feeding with the same energy intake from normal, MCT diet and HFD for 6 weeks. MCT diet group showed improved glucose tolerance, insulin sensitivity, and reduced liver triglyceride content among all groups. Our study demonstrated that MCT diet has no significant side effect for normal mice and sustained healthy metabolic profiles. MCT diet may have potential positive effects on liver and cardiac function under T2DM.

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### Treating Obesity by Activating Beige and Brown Fat; Human Transplant Studies.

Sarah Davis<sup>1</sup>, Jenny Gunton<sup>1</sup>

1. *Westmead Institute for Medical Research, Westmead, NSW, Australia*

The aim of this project is to “brown” and activate human adipose tissue to increase energy expenditure for the treatment of obesity and diabetes. To determine the whole-body effects of browning agents on energy balance and glucose and fat handling (metabolism) in mice which have received human fat transplants. Obesity occurs when energy intake exceeds energy expenditure and two-thirds of Australian adults are now overweight or obese. Rodents are known to possess significant quantities of brown adipose tissue (BAT) a thermogenic organ with a unique protein; uncoupling protein 1 (UCP1). When activated, UCP1 allows brown fat cells to generate heat and can burn more energy than any other organ in the body (per gram of weight). Recent metabolic imaging have shown that most human adults possess small amounts of BAT (<50g). It has been

estimated that activation of BAT could increase resting daily energy expenditure by up to 20% and improve blood glucose (diabetes) and lipids in the circulation of overweight people. Thus, BAT represents an important therapeutic target for drugs that could increase resting energy expenditure to treat obesity and related diseases. These experiments will involve pharmacological treatment of mice (which have been transplanted with human Fat) and energy expenditure, glucose and lipid metabolism measured. The mice are given an intervention to investigate agents which may increase browning or activation of human fat. Target agents may include exercise, cold exposure, diet manipulation,  $\beta$ 3-Adrenergic Receptor Agonists, anti-inflammatory pharmacology and alteration of gut microflora.

## Androgen receptor signalling in the female adrenal cortex is dispensable for X-zone regression but protects against early onset spindle cell development

**Anne-Louise Gannon**<sup>1,2</sup>, **Laura O'Hara**<sup>2,3</sup>, **J. Ian Mason**<sup>2</sup>, **Laura Milne**<sup>2</sup>, **Sarah E Smith**<sup>2</sup>, **Rod T Mitchell**<sup>2</sup>, **Lee B Smith**<sup>1,2</sup>

1. The University of Newcastle, Callaghan, NSW, Australia

2. CRH, The University of Edinburgh, Edinburgh, Scotland

3. Centre for Discovery Brain Sciences, The University of Edinburgh, Edinburgh, Scotland

Androgens have been shown to be integral during ovarian folliculogenesis, embryonic implantation and uterine and breast development<sup>1</sup>. Adrenal androgens are essential for these processes; however, the focus of adrenal androgen research is centred on disruption to production of androgens from the adrenal with little research investigating the role of androgen receptor (AR) signalling in the adrenal. This could be in part due to the perceived lack of suitable rodent model, as the mouse adrenal does not produce androgens due to methylation of *Cyp17a1* during development<sup>2</sup>. Despite this, studies in female mice show that androgens are able to influence the adrenal cortex. The transient X-zone of the mouse adrenal cortex, thought to be a homologue for the human adrenal foetal zone, is essential for providing stem cells for the adult adrenal cortex. The X-zone regresses following pregnancy, however, studies have shown that the X-zone regresses following treatment with testosterone in virgin mice<sup>3</sup>. Furthermore, loss of circulating androgens through gonadectomy results in a resurgence of the X-zone and development of adrenocortical tumours<sup>4</sup>. The mechanism by which androgens regulate these processes and the adrenal cortex remains largely unknown.

To dissect the role of AR-signalling in the female mouse adrenal, we utilised a *Cyp11a1-Cre* that permits tissue-specific ablation of AR from the adrenal cortex.

Results demonstrate AR is dispensable for the postnatal developing female adrenal and surprisingly during X-zone regression following pregnancy. However, following disruption to adrenal AR, elevated serum corticosterone is observed in postpartum females and development of spindle cell hyperplasia in young adult females that progress with age.

These results point to potentially undefined roles for adrenal AR in postpartum-stress regulation. Furthermore, dysfunctional adrenal androgen signalling could be a possible mechanism in the development of adrenal spindle cell hyperplasia and could act as a potential therapeutic target.

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## NCKX3 loss in mice lead to abnormal motor function and social behavior

**Dinh Nam Tran**<sup>1</sup>, **Jae-Hwan Lee**<sup>1</sup>, **Bo Hui Jeon**<sup>1</sup>, **Eui-Man Jung**<sup>1</sup>, **Eui-Bae Jeung**<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

NCKX3 (sodium/potassium/calcium exchanger 3) is an important component of intracellular  $Ca^{2+}$  homeostasis.  $Ca^{2+}$  homeostasis has been extensively studied in various cell systems. Dysregulation of  $Ca^{2+}$  homeostasis can induce the excitotoxic and neurodegeneration in central nervous system. NCKX3 gene is highly expressed in thalamic nuclei, in hippocampal CA1 neurons, and in layer IV of the cerebral cortex in the mouse brain. Here, we examined the effects of inactivation of NCKX3 in mice. Mice lacking NCKX3 at 6 week-age were used for behavior assays. NCKX3<sup>-/-</sup> mice show increased moving distances in the open field test. In the sociability test, NCKX3<sup>-/-</sup> mice have reduced time spent on general sniffing, anogenital sniffing, and following behavior but increased in fighting. In the rotarod test, there were abnormal in motor learnings in NCKX3<sup>-/-</sup> mice. There was no change in recognition memory in the novel object recognition test. During acquisition phase in the Morris water maze test, there was no different in escape latency time between wild-type and NCKX3<sup>-/-</sup> mice. This indicated NCKX3 mutation did not impair to spatial learning in mice. The data show an essential in vivo role for NCKX3 in motor functions and social behaviors in mice.

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### Calbindin-D<sub>9k</sub> prevent ER stress induced pancreatic beta cell death

Dinh Nam Tran<sup>1</sup>, Changhwan Ahn<sup>1</sup>, Jae-Hwan Lee<sup>1</sup>, Yeong-Min Yoo<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

Intracellular calcium ion is tightly regulated to maintain cellular function and cell survival. signals have been proposed to activate signal for hormone secretion. Calbindin-D<sub>9k</sub> (CaBP-9k) is responsible for regulation of the distribution of cytosolic free calcium ion. The previous study demonstrated that calcium binding protein CaBP-9k contribute to control signal-dependent NAD(P)H formation, respiration, and ATP changes in intact cells. Those regulation determine cell survival and secretory function. Furthermore, in the latest article demonstrated that CaBP-9k expression in insulin secreting and CaBP-9k depletion cause hypoinsulinemia. CaBP-9k KO mice accumulate only a few amounts of abdominal fat compare to wild-type mice result from hypoinsulinemia. Decreased insulin levels impede fat storage into the adipose tissues and other metabolic organs like liver and skeletal muscles. On the other hand, insulin resistance leads to an increase in the amount of fatty acids in the blood circulation due to the loss of insulin's ability to suppress lipolysis. Therefore, the phenotypes in aged CaBP-9k KO mice are related with decrease insulin secretion or production. 6 months old CaBP-9k KO mice showed decreased islet volume, increased cell death marker such as capase-3 and TUNEL staining resulting from endoplasmic reticulum stress which can lead pancreatic  $\beta$  cell death. Collectively, our findings indicate that CaBP-9k play a critical role for protection of pancreatic  $\beta$  cell survival from ER stress which contribute to glucose homeostasis accompanying lipid metabolism.

### The effects of dexamethasone for calcium channel and mucin-related gene expression in A549 cell line

Bo Hui Jeon<sup>1</sup>, Dinh Nam Tran<sup>1</sup>, Yeong-Min Yoo<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

Calcium is important for physiological functioning in many tissues and is essential in mucus secretion. Previously reported, Mucin secretion is regulated predominantly by cytosolic calcium-dependent pathways. Cytosolic of calcium are regulated by calcium channels such as TRPV6, NCX1, and PMCA1. A549 cell line was treated with 10<sup>-8</sup>M dexamethasone (DEX) and 10<sup>-6</sup>M RU486. Subsequently, the expression of TRPV6, NCX1, and PMCA1 in A549 cell line were examined. There was no significant differences in PMCA1 expressions in DEX-treated groups, but TRPV6 was increased in DEX-treated groups and was recovered by DEX+RU486 treatment. NCX1 was decreased in DEX-treated groups and was recovered by RU486 treatment. In addition, mucin secretion, related genes MUC4 and MUC5AC, was also decreased by DEX treatment. Control of calcium channel gene expression may affect the control of mucus secretion in the lung cancer. These results could be used for understanding the basis of treatment mucin secretion related disease such as cancer.

### Regulatory effect of systemic corticosteroid dexamethasone on tracheal calcium processing proteins and mucin secretion

Bo Hui Jeon<sup>1</sup>, Bonn Lee<sup>1</sup>, Eui-Man Jung<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

Systemic glucocorticoid dexamethasone has known for exerting an inhibitory effect on tracheal mucin secretion and therefore considered the primary option for treating acute asthma exacerbation. However, the mechanism underlying glucocorticoid-induced decreased in mucosubstances is unclear. Recent studies have reported that dexamethasone exerts an inhibition on mucin and mucosubstances in the lung by modulating the expression of calcium-processing genes. However, the expression of the calcium-processing genes in trachea are not examined yet. Thus, the present study is the first to report glucocorticoid-induced regulation of tracheal calcium processing genes such as transient receptor potential vanilloid-4 (*Trpv4*), transient receptor potential vanilloid-6 (*Trpv6*), calbindin-D<sub>9k</sub> (*CaBP-9k*), and plasma membrane Ca<sup>2+</sup>-ATPase (*Pmca1*) in the mice. In the study, mice were subcutaneously injected with systemic dexamethasone for 5days, or injected with estradiol or progesterone for 3 days. The tracheae were collected by dividing them into cervical and thoracic sections based on its anatomical structure. Quantitative PCR was performed to investigate mRNA expression of calcium-processing genes. Immunohistochemistry and immunofluorescence were performed to localize the calcium-processing proteins. Tracheal mucins were detected by performing Alcian blue-periodic acid-Schiff staining. The expression of TRPV4, TRPV6, CaBP-9k, and PMCA1 proteins was localized in the tracheal epithelium, submucosal glands, cartilages and muscles. Dexamethasone treatment decreased the mRNA expression of the four calcium-processing genes and mucin 1, mucin 4, mucin 5ac, and mucin 5b genes. Dexamethasone inhibited in the secretion of mucosubstances in the trachea. Our findings suggest that glucocorticoids regulate the tracheal expression of calcium-processing genes and tracheal mucin secretion.

### The effect of steroid hormone on the calcium-processing proteins in the immature rat brain

Seon Myeong Go<sup>1</sup>, Seon Young Park<sup>1</sup>, Jae-Hwan Lee<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>



1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

The cytosolic calcium concentration is regulated by calcium-processing proteins such as TRPV5, TRPV6, NCX1, and PMCA1. Those calcium-processing proteins are important for physiological functions in the brain. The effects of steroid hormones on calcium-processing protein expressions in the brains are undescribed. Thus, the effects of steroid hormones on the distribution, localization, and expressions of calcium-processing proteins in the brain were analyzed. Immature female rats were injected for 5 days with estrogen (E2), progesterone (P4), dexamethasone (DEX), and their antagonists (ICI 182,780 and RU486). The localization and expression of calcium-processing proteins in rat brain were observed by immunofluorescence and western blot analyses, respectively. We found that TRPV5 and TRPV6 proteins were highly expressed in the cerebral cortex (CT), hypothalamus (HY), and brain stem (BS) compared to that in the olfactory bulb (OB) and cerebellum (CB). Also, the NCX1 protein was highly expressed in CT and BS compared to that in OB, HY, and CB, and PMCA1 protein was highly expressed in CT compared to that in other brain regions. Furthermore, expression levels of calcium-processing proteins were regulated by E2, P4, and/or DEX in the CT and HY. In summary, calcium-processing proteins are widely expressed in the immature rat brain, and expressions of calcium-processing proteins in CT and HY are regulated by E2, P4, and/or DEX and can be recovered by antagonist treatment. These results indicate that steroid hormone regulation of calcium-processing proteins may serve as a critical regulator of cytosolic calcium absorption and release in the brain.

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### Distribution of and steroid hormone effects on calbindin-D9k (CaBP-9k) in the immature rat

Seon Mi Park<sup>1</sup>, Seon Young Park<sup>1</sup>, Dinh Nam Tran<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

Calbindin-D<sub>9k</sub> (CaBP-9k), one of the major calcium-binding and calcium-buffering proteins, is important in the physiological functioning of organs. The neuroanatomical localization of CaBP-9k in the rodent brain has not been reported; thus, this study investigated the neuroanatomical distribution of CaBP-9k and the regulation of CaBP-9k expression on steroid hormones in the immature rat brain. To confirm the influence of steroid hormones on CaBP-9k expression, immature female rats were injected for 5 days with estrogen (E2), progesterone (P4), dexamethasone (DEX), and their antagonists (ICI 182, 780 and RU486). The localization and expression of the CaBP-9k protein in brain regions were identified by immunofluorescence and western blot assays, respectively. We observed that CaBP-9k expression was especially strong in hypothalamus, cerebellum, and brain stem. In addition, CaBP-9k was colocalized with mature-, GABAergic, dopaminergic, and oxytocinergic neurons. We also observed that the CaBP-9k protein level was significantly increased by P4 and reversed by antagonist RU486 treatment in immature rat brain. In summary, CaBP-9k positive cells have a wide distribution in the immature rat brain, and CaBP-9k expression is regulated by P4. We suggest that CaBP-9k expression regulated by steroid hormone may serve as an important regulator of cytosolic calcium concentration in the brain.

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### Effects of endocrine disrupting chemical on calcium signaling in differentiated cardiomyocyte from mouse embryonic stem cell

Jae-Hwan Lee<sup>1</sup>, Seon Myeong Go<sup>1</sup>, Yeong-Min Yoo<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

Endocrine-disrupting chemicals (EDCs) have similar structures with steroids hormones, which can interfere with hormone synthesis and normal physiological functions of male and female reproductive organs. Sex steroid hormones influence calcium signaling of the cardiac muscle in early embryo development. Progesterone (P4) has been reported to reduce blood pressure. To confirm the effect of P4, octyl-phenol (OP) and bisphenol A (BPA) on early differentiation of mouse embryonic stem cells (mESCs) into cardiomyocytes, P4, OP and BPA were treated at two days after attachment and media were replaced every two days. In addition, mifepristone (RU486) is a synthetic steroid that has an affinity for progesterone receptor (Pgr) and was treated for one day starting on day 11. To investigate the calcium signaling, the expression of calcium channel gene and contraction-related genes was analyzed. Beating ratio was decreased in P4, OP and BPA treatment. The *Pgr* mRNA level was significantly increased in P4, OP and BPA-treated group. However, the mRNA level of calcium channel gene, *Trpv2*, was significantly decreased in the P4, OP and BPA-treated group. In addition, expressions of contraction-related genes such as *Ryr2*, *Cam2* and *Mlck3* were significantly decreased in the P4, OP and BPA-treated group. Interestingly, treatment of RU486 rescues altered calcium channel gene and contraction-related genes. P4, OP and BPA treatments resulted in the reduction of intracellular calcium level. Taken together, these results suggest that OP and BPA may impact on the inhibition of cardiomyocytes differentiation of mESCs, results in disruption of cardiomyocytes differentiation of mESCs.

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### Second-phase validation study of developmental toxicity test using mouse embryonic stem cells derived embryoid bodies

Jae-Hwan Lee<sup>1</sup>, Seon Mi Park<sup>1</sup>, Eui-Man Jung<sup>1</sup>, Eui-Bae Jeung<sup>1</sup>

1. Chungbuk National University, Seowon-gu, Chungbuk, South Korea

The embryoid body test (EBT) is a developmental toxicity test method that assesses the half inhibitory concentrations of substances in the area of embryoid bodies (EBs), and in the viability of mouse embryonic stem cells (ESCs) and fibroblasts (3T3 cells). In the previous pre-validation study evaluated the predictive accuracy of the EBT using 26 coded test substances and highly accurate (above 80%) when substances were classified using the predictive model. EBT used two same endpoints

as EST, the half inhibition concentrations for cell viability of mouse ESCs (IC50 E14) and 3T3 fibroblasts (IC50 3T3), but replaced the half inhibition concentration for cardiac differentiation (ID50 CM) with the half inhibition concentration for EB area (ID50 EB). We used the hanging drop method to form an embryoid bodies. In order to verify the proposed EBT method in this study, inter-laboratory reproducibility (5 substances in common) and predictive capacity (10 substances in each laboratory) tests were performed. To ensure reliability of the study results, the tests were conducted using identity-coded test substances. The results of statistical analysis of the inter-laboratory reproducibility test indicated that reproducibility accuracy 87%, sensitivity 78%, and specificity 100%. The results of statistical analysis of the predictive capacity test indicated that the lead laboratory had reproducibility accuracy 80%, sensitivity 86%, and specificity 67%. Participatory laboratory 1 had reproducibility accuracy 80%, sensitivity 71%, and specificity 100% and participatory laboratory 2 had reproducibility accuracy 80%, sensitivity 86%, and specificity 67%. The results of the intra- and inter-laboratory 2tests were highly accuracy 83%, sensitivity 80%, and specificity 89% when substances were classified using the predictive model. EBT can accurately classify various embryotoxicants in a short time with less effort and greater validation.

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## Evidence based education for primary care physicians for diabetes management in India

**Pushkar Kumar<sup>1</sup>, Sandeep Bhalla<sup>1</sup>, V Mohan<sup>2</sup>, Ranjit Unnikrishnan<sup>2</sup>, Deepak Monga<sup>1</sup>**

1. Public Health Foundation of India, Gurugram, Haryana, India

2. Dr Mohan Diabetes Education Academy, Chennai, Tamil Nadu, India

**Background:** India with the second highest number of individuals with diabetes in world with primary care physicians being the main workforce to deal with any health related problem at the grass root level. Every year more than 50,000 medical graduates join the health workforce from different medical institutions. Despite this, there is a huge disproportion between patients to doctor ratio. In addition, these PCPs are unable to manage the 72.9 million people suffering from diabetes all across the country due to paucity of diabetes related education and training programs.

**Intervention:** An integrated education program for primary care physicians (PCPs) was launched by Public Health Foundation of India and Dr Mohan's Diabetes Education Academy. This program was supported by an education fund from a funding partner with the ultimate goal of providing evidence based care to individuals with diabetes and reducing future risk of complications. This educational program aims to build the skills and core competencies of primary care physicians, and create a network among primary care physicians, endocrinologists and diabetologists to establish robust linkages for better patient outcomes and since its inception (2010), it has trained more than 10000 PCPs. The course has been recognized by IDF and SAFES.

**Results:** Significant knowledge improvement ( $P$  value <0.005) was noticed among PCPs after attending the contact sessions. The course facilitated a systematic approach for treatment of diabetes by majority of the participants in their daily clinical practice. PCPs found themselves more confident (91%) in managing not only diabetes but also related complications after completing the program. After completion of the program, PCPs managed the diabetes cases routinely and with confidence; and referring complicated case to appropriate healthcare institute (89.4%). Some of the PCPs started displaying IEC aids in their clinics and upgraded their in-clinic facilities for better diagnosis and counselling.

**Conclusion:** This effective program in building capacity and skills of PCPs in the management of diabetes needs potential pathways for scale up to reach out to a wider audience of healthcare providers.

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## The incidence of adrenal insufficiency in hospitalised patients with primary and secondary adrenal malignancies: An analysis of hospital admission data, NSW, 2006-2017

**Anna J Lubomski<sup>1</sup>, Henrik Falhammar<sup>2</sup>, David J Torpy<sup>3,4</sup>, Louise Rushworth<sup>1</sup>**

1. School of Medicine, University of Notre Dame Australia, Sydney, NSW, Australia

2. Endocrine and Metabolic Unit, Royal Adelaide Hospital and University of Adelaide, Adelaide, SA, Australia

3. Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden

4. Menzies School of Health Research and Royal Darwin Hospital, Tiwi, NT, Australia

### Background:

Adrenal metastases (AM) are common in patients with metastatic malignant disease. Bilateral AMs may cause adrenal insufficiency (AI) and affected patients are at risk of an adrenal crisis (AC). Symptoms of AI are similar to those of advanced malignancy, and the incidence of AI/AC metastatic malignancy is uncertain.

### Methods:

This retrospective study evaluated data on all admissions to NSW hospitals between 2006 and 2017 from the NSW Ministry of Health Admitted Patient Data Collection (APDC). Patient demographics, their primary malignancies, a principal or comorbid diagnosis of AI, and the incidence of a diagnosed AC were assessed.

### Results:

There was a total of 14,665 hospital admissions with a principal or comorbid diagnosis of AM in NSW over the study period, corresponding to 1222.1 admissions/year. The majority (62.0%, n=9094) of patients were male. The mean patient age was 67.2 (+/-12.1) years. The most common primary malignancies were: lung 50.5% (n=7403), melanoma 8.7% (n=1276), kidney 6.7% (n=987), and breast 4.7% (n=689). A principal or comorbid diagnosis of AI was recorded in 162 patients (1.1% of all admissions) and 19 (11.7% of AI diagnoses, 0.1% of all admissions) of these were classified as an AC. An AC was more common in men (89.5%, n=17, p<0.05), while there was no difference between the sexes in the incidence of AI. Four patients (21%) with an AC died during the admission.

### Conclusion:

AI, due to AM can be managed by glucocorticoid replacement therapy, arises in only in a small proportion of patients with AM and can be associated with an AC. AI due to other causes may also occur. The results of this study support an approach to management of AM patients that aims to detect and manage AI based on early evaluation of indicative AI symptoms rather than systematic screening of asymptomatic patients.

### Rescue of the HSD17B3 knock-out model using lentiviral delivery system.

**Diane Rebourcet<sup>1</sup>, Rosa Mackay<sup>2</sup>, Annalucia Darbey<sup>1</sup>, Peter O'Shaughnessy<sup>3</sup>, Ana Monteiro<sup>3</sup>, Mike Curley<sup>2</sup>, Nathan Jeffery<sup>2</sup>, Laura Milne<sup>2</sup>, Sarah Smith<sup>2</sup>, Lee B Smith<sup>1,2</sup>**

1. *The University of Newcastle, Callaghan, NSW, Australia*

2. *MRC-Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK*

3. *Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK*

Male health and wellbeing is androgen dependent. Low circulating testosterone concentration is associated with many increasingly prevalent chronic and age-related clinical conditions (metabolic disorders such as obesity, cardiovascular disease) (1-2). The cause/consequence relationship to perturbed androgen action is unclear and androgen therapy is able to alleviate symptoms in many cases. However, the use of androgen therapy remains controversial due to a lack of solid scientific evidence regarding the cost/benefits and the potential adverse risks (3). An approach to improve endogenous androgen production, could be via modulating gene expression in testis somatic cells using a lentiviral delivery system.

Androgens are synthesized by Leydig cells within the interstitial compartment. The final step in testosterone production, controlled by hydroxysteroid-dehydrogenase-17-beta-3 (HSD17B3), has been shown to occur in different compartments depending on the stage of development of the testis (3). In fetal life, the expression of HSD17B3 is restricted to the seminiferous tubules, in Sertoli cells and in adulthood, to the Leydig cells. With a view to support a healthy androgen profile throughout life and alleviate the secondary effects of testosterone replacement therapy, we exploited the HSD17B3 knock-out model, which displays similar ageing related disorders such as altered hormonal profile (testosterone and gonadotrophins) and obesity as the males aged. To determine whether we could rescue the phenotype observed in mutant males, we used an in vivo lentiviral delivery of HSD17B3 cDNA or control constructs, specifically targeting adult Sertoli cells in knock-out and wild type males.

Our preliminary data shows that the lentiviral delivery of HSD17B3 cDNA system is able to rescue the phenotype observed in the knock-out, as their bodyweight curve decreases significantly following the treatment. This opens doors to new approaches as a refinement over current androgen therapy strategies to compensate for male reproductive disorders and to support a healthy androgen profile throughout life.

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### Transthyretin uptake by hepatocytes is regulated by high density lipoprotein and is independent of scavenger receptor class B member 1

**Kerry Richard<sup>1,2,3</sup>, Kelly A Landers<sup>1</sup>, Michael C d'Emden<sup>4,3</sup>**

1. *Pathology Queensland, Queensland Health, Herston, Queensland, Australia*

2. *Queensland University of Technology, Brisbane, Qld, Australia*

3. *University of Queensland, Herston, Queensland, Australia*

4. *Endocrinology and Diabetes, Royal Brisbane and Women's Hospital, Herston, Queensland, Australia*

Thyroid hormone is essential for the normal function of cells and is transported in serum bound to several proteins including transthyretin. For many years, it was thought that only free hormone could be transported into cells however, there is now evidence that hormone binding protein dependent uptake mechanisms also exist. Our recent study showed that transthyretin and transthyretin bound to thyroxine (T4) are endocytosed by placental trophoblasts through the high-density lipoprotein receptor, Scavenger Receptor Class B Type 1 (SR-B1) [1].

The liver plays an important role in the transport, metabolism and excretion of thyroid hormones and is an important site of hormone activity. SR-B1 is also expressed in hepatocytes and we sought to determine if hepatocyte SR-B1 was involved in transthyretin or transthyretin-T4 uptake and whether uptake was affected by high density lipoprotein.

Transthyretin and transthyretin-T4 uptake by hepatocytes is not dependent on SR-B1. Knockdown of SR-B1 expression using siRNA resulted in increased transthyretin-T4 uptake and had no significant effect on transthyretin uptake. High density lipoprotein treatment of hepatocytes decreased SR-B1 expression in hepatocytes but increased uptake of transthyretin-T4. Addition of HDL to uptake experiments blocked both transthyretin and transthyretin-T4 uptake.

Hepatocyte uptake of transthyretin-T4 is not dependent on SR-B1 expression but is affected by SR-B1 levels. HDL also decreases both transthyretin and transthyretin-T4 uptake. This suggests that multiple lipoprotein receptors may be involved in the regulation of uptake of transthyretin-T4 in different cell types. Further study is required to identify the specific transporters involved.

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## Structural properties of a prehistoric human skeleton found in Malaysia

**Ima Nirwana Soelaiman<sup>1</sup>, Kok-Yong Chin<sup>1</sup>, Elvy S Mohd. Ramli<sup>2</sup>, Faridah Mohd. Nor<sup>3</sup>, Zuliskandar Ramli<sup>4</sup>**

1. Dept. of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, FT, Malaysia

2. Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Wilayah Persekutuan, Malaysia

3. Dept. of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, FT, Malaysia

4. Institute of the Malay World and Civilisation, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

**Background:** The limestone caves and rock shelters of the Nenggiri Valley in the state of Kelantan are sites of archaeological excavation since early 20<sup>th</sup> century. Recently, in November 2018, an almost complete female skeleton was uncovered in Gua Chawan, one of the caves in the Nenggiri Valley. Her age was estimated to be in the mid-thirties based on dental examination. The skeleton was carbon-dated by Beta Analytics, USA to be around 8000 years old, i.e. corresponding to the early Mesolithic period. No study on the structural properties of prehistoric Malaysian skeletal remains have ever been done. **Objective:** Our objective was to determine the mineral content, density, and microarchitecture of this prehistoric skeleton and compare it with modern human skeletons. **Methodology:** The right humerus from the prehistoric skeleton was compared with 6 modern humeri obtained from the Anatomy Department. The humeri were weighed and measured. Bone mineral content and density were measured using Dual-Energy X-Ray Absorptiometer (DXA). Bone microstructure was determined using micro Computed Tomography (microCT). **Results:** Amongst the 7 humeri studied, the prehistoric humerus was the second longest, but second lightest in weight. The bone mineral density (BMD) was the second highest. MicroCT scan was done on the prehistoric humerus and two other humeri with almost similar BMD. MicroCT data showed that the prehistoric humerus had higher bone volume and trabecular number, while the trabecular separation was the smallest. **Conclusion:** The prehistoric skeleton was more dense than the modern bones, maybe due to the higher levels of physical activity and healthier, more organic nutritional intake of that time period. **Limitations:** Only one prehistoric skeleton was available for study, while the age and gender of the modern humeri were not known. Other factors related to the environment in the cave and length of time the skeleton was there were not considered.

## Effects of Chronic Moderate Intensity Exercise on Blood Glucose, Fasting Insulin, FFA and Insulin Resistance in Male Wistar Rats Diabetes Mellitus

**Aywar Zamri<sup>1</sup>, Achmad Rudijanto<sup>2</sup>, Putu M Arsana<sup>2</sup>**

1. Internal Medicine, Internal Medicine Consultant Specialist Education Program (PPDS PDK) in the field of Endocrine Metabolism and Diabetes in the Faculty of Medicine, University of Brawijaya / RSUD Dr. Saiful Anwar, Malang, Jawa Timur, Indonesia

2. Internal Medicine, Staff of the Division of Metabolic and Diabetes Endocrinology, Department of Internal Medicine, University of Brawijaya / Regional General Hospital, Dr. Saiful Anwar, Malang, Jawa Timur, Indonesia

**Objective:** To assess whether chronic moderate intensity exercise had effects on blood glucose, fasting insulin, FFA and insulin resistance in male diabetes mellitus (DM) Wistar rats.

**Method:** A 10 weeks experimental laboratorial study with Wistar Diabetes *Rattus Novergicus Strain* rats using Pretest-Posttest Comparison Group Design. 20 rats were divided into four groups of rats, 5 rats per group. The four groups were: normal rats with sedentary lifestyle, normal rats with exercise, DM rats with sedentary lifestyle and DM rats with exercise. In the exercise groups (normal and DM), rats performed a treadmill for 10 weeks whereas speed and duration were gradually increased every two weeks, starting at 10 m/minute for 10 minutes and increased until 26 m/minute for 1 hour at week 9 and 10. Blood plasma examination was performed in each group for fasting blood glucose, fasting insulin, FFA and HOMA-IR was calculated at baseline and the end of week 10

**Results:** There were no significant differences between before and after exercise in the group of normal exercise rats and DM exercise rats but there was a tendency of decreasing plasma glucose (pre 143.59; post 121.65,  $p0.39$ ), insulin (pre 16.56; post 15.38,  $p0.32$ ), FFA (pre 19.93; post 14.05,  $p0.36$ ) and HOMA-IR (pre 5.87; posts 4.62,  $p0.27$ ) in normal rats before and after exercise. Likewise, there was a downward trend in DM rats exercise blood glucose levels (pre 242.26; Post 222.03,  $p0.63$ ), insulin (pre 26.5; post 24.45,  $p0.12$ ), HOMA-IR (pre 15.58; post 13.5,  $p0.37$ ) and FFA (pre 26.5; post 24.45,  $p0.12$ ) before and after exercise.

**Conclusion:** There was a decrease in insulin resistance as indicated by a tendency to decrease HOMA-IR, fasting insulin, plasma glucose, plasma FFA. It might require longer than 10 weeks of exercise to see a significant decrease on these parameters.

## Visual loss in pregnancy

**Caroline Bachmeier<sup>1</sup>, Cameron Snell<sup>2</sup>, Adam Morton<sup>1</sup>**

1. Endocrinology department, Queensland Diabetes and Endocrine Centre, Mater Hospital, Brisbane, QLD, Australia

Visual loss in pregnancy may be caused by a variety of reasons including pituitary adenomas. Prolactinomas (PRLs) are the commonest hormone-secreting tumours in pregnant women. As most PRLs present with menstrual abnormalities, infertility or galactorrhoea, they are most commonly diagnosed before pregnancy. We present the case of a 30 year-old primigravida who presented at 36<sup>+</sup> weeks gestation with headaches and left sided visual loss. Magnetic resonance imaging (MRI) of the pituitary gland confirmed a 10mm x 11mm left suprasellar mass. Results of her anterior pituitary function were unremarkable for her gestational age. Post-partum she underwent an endoscopic endonasal resection of the pituitary tumour. The histology was consistent with a prolactinoma (PRL). Literature review reveals only one possible case of a new diagnosis of a prolactinoma during pregnancy. It highlights the importance to consider a wide range of differential diagnoses when assessing visual loss in pregnancy.

## Altered gut microbiota composition in early pregnancy in women treated with thyroxine

**Helen L Barrett**<sup>1,2</sup>, **Leonie K Callaway**<sup>3</sup>, **H.David McIntyre**<sup>4,2</sup>, **Marloes Dekker Nitert**<sup>5</sup>

1. Mater Research Institute - The University of QLD, Brisbane

2. Endocrinology, Mater Misericordiae Ltd, Brisbane

3. Royal Brisbane and Women's Hospital, Herston, QLD, Australia

4. School of Medicine, University of Queensland, Herston, QLD, Australia

5. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD

### Background:

Gut bacteria are known to take up thyroid hormone and could play a role in the recycling of iodothyronines by deconjugating bile-conjugated iodothyronines. Outside pregnancy, hyperthyroidism is associated with altered gut microbiota composition and hypothyroidism with small intestinal bacterial overgrowth. The absorption of orally administered thyroxine therapy is not complete in the small intestine and some thyroxine will reach the gut microbiota in the colon. Thyroxine need increases in pregnancy, likely due to physiological changes, but a significant portion reaches the colon where it could alter the composition of the gut microbiota and alter intraluminal metabolism. Here, we investigated if gut microbiota composition is different in women on thyroxine treatment in early pregnancy.

**Methods:** Overweight and obese women who participated in the SPRING study and reported using thyroxine were matched with controls not on thyroxine in a 1:2 ratio. Gut microbiota composition at 16 weeks gestation was determined by sequencing the V6-V8 region of the 16S rRNA gene using the QIIME pipeline for analysis. The PiCRuSt was used for predicting the functional capacity of the gut microbiota.

**Results:** There was no difference in the alpha and beta diversity of the gut microbiota between 8 women on thyroxine and their 16 matched controls at 16 weeks gestation. The gut microbiota of the women on thyroxine showed increased abundance of the genera *Bifidobacterium* (P=0.045) and *Bilophila* (P=0.039) but decreased abundance of the *Archaea* member *Methanobrevibacter* (P=0.047). Functional prediction analysis indicated increased abundance of bacterial pathways involving DNA replication; DNA repair and recombination; pyrimidine metabolism and amino acid metabolism in women taking thyroxine but lower abundance of the methane metabolism pathway.

**Conclusion:** These results in a small cohort of overweight and obese pregnant women suggest that thyroxine treatment is associated with altered composition of the gut microbiota in early pregnancy.

## The effect of patient-managed stress dosing on electrolytes and blood pressure in acute illness in children with congenital adrenal hyperplasia

**Georgina L Christ**<sup>1</sup>, **David J Torpy**<sup>2</sup>, **Ann M Maguire**<sup>3,4</sup>, **Maria Quartararo**<sup>1</sup>, **Henrik Falhammar**<sup>5,6,7</sup>, **Bruce R King**<sup>8,9</sup>, **Craig F Munns**<sup>3,4</sup>, **Shihab Hameed**<sup>4,10,11</sup>, **Louise Rushworth**<sup>1</sup>

1. School of Medicine, Sydney, University of Notre Dame Australia, Darlinghurst, NSW, Australia

2. Endocrine and Metabolic Unit, Royal Adelaide Hospital and University of Adelaide, Adelaide, NSW, Australia

3. The Children's Hospital, Westmead, NSW, Australia

4. Sydney Medical School, The University of Sydney, Camperdown, NSW, Australia

5. Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, , Sweden

6. Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, , Sweden

7. Menzies School of Health Research and Royal Darwin Hospital, Tiwi, NT, Australia

8. John Hunter Children's Hospital, Newcastle, NSW, Australia

9. University of Newcastle, Newcastle, NSW, Australia

10. Sydney Children's Hospital, Randwick, NSW, Australia

11. School of Women's and Children's Health, University of New South Wales, Kensington, NSW, Australia

**Background:** Adrenal crises (AC) are acute episodes of adrenal insufficiency (AI). Manifestations include hypotension and electrolyte disturbances. Glucocorticoid stress dosing (SD) can prevent AC progression but its effect on physiological parameters has not been assessed in a "real world setting".

**Aim:** To assess the effect of prior self-managed glucocorticoid dose escalation on physiological markers in children with AI presenting to hospital for an acute illness.

**Methods:** An audit of records of all children with congenital adrenal hyperplasia (CAH) and an acute medical illness attending a paediatric referral hospital between 2000 and 2016. Potassium, sodium and glucose levels, and hypotension (classified using age-related normal levels or delayed capillary return), were compared between children who had and had not used SD.

**Results:** There were 321 attendances by patients with CAH and an acute illness during the study period. Any form of SD was used by 64.2% (n=206); IM was used by 22.1% (n=71) and oral only by 41.7% (n=134). Use of SD (oral and/or IM hydrocortisone) was associated with a significantly lower mean potassium level (SD= 4.02+/- 0.71 and No SD=4.27+/- 0.79 mmol/l, p<0.05). More patients (71.6%, n=151) had used SD in the normokalaemic group than among those with hyperkalaemia (28.6%, n=4), p<0.01. Linear regression analysis showed that age (in years): beta =-0.04 (-0.06, -0.02), diarrhoea: beta= 0.41 (0.21,0.61); and SD: beta= 0.21 (0.02,0.41) each exerted an independent significant lowering effect on potassium levels. SD was not significantly associated with sodium or glucose concentrations or with estimates of hypotension.

**Conclusion:** Glucocorticoid dose escalation, as it is implemented in the community, results in a significant reduction in hyperkalaemia and lowers mean potassium levels in patients with AI and an acute illness but does not alter significantly sodium and glucose concentrations. The incidence of hypotension was not associated with SD in this population.

## Efficacy and safety of fast-acting insulin aspart compared with insulin aspart, both in combination with insulin degludec, in children and adolescents with type 1 diabetes: the onset 7 trial

**Maria Craig**<sup>1</sup>, **Bruce Bode**<sup>2</sup>, **Violeta Iotova**<sup>3</sup>, **Margarita Kovarenko**<sup>4</sup>, **Lori Laffel**<sup>5</sup>, **Paturi V Rao**<sup>6</sup>, **Henning Andersen**<sup>7</sup>, **Srikanth Deenadayalan**<sup>7</sup>, **Steffen Falgreen Larsen**<sup>7</sup>, **Thomas Danne**<sup>8</sup>

1. *The Children's Hospital at Westmead and St George Hospital, Sydney, NSW, Australia*

2. *Atlanta Diabetes Associates, Atlanta, Georgia, USA*

3. *University Hospital St. Marina, Medical University Varna, Varna, Bulgaria*

4. *Pediatric Department, Novosibirsk State Medical University of The Ministry of Healthcare of the Russian Federation, Novosibirsk, Russia*

5. *Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts, USA*

6. *Diabetes Research Society, Hyderabad, India*

7. *Novo Nordisk A/S, Søborg, Denmark*

8. *Children's Hospital Auf der Bult, Hannover, Germany*

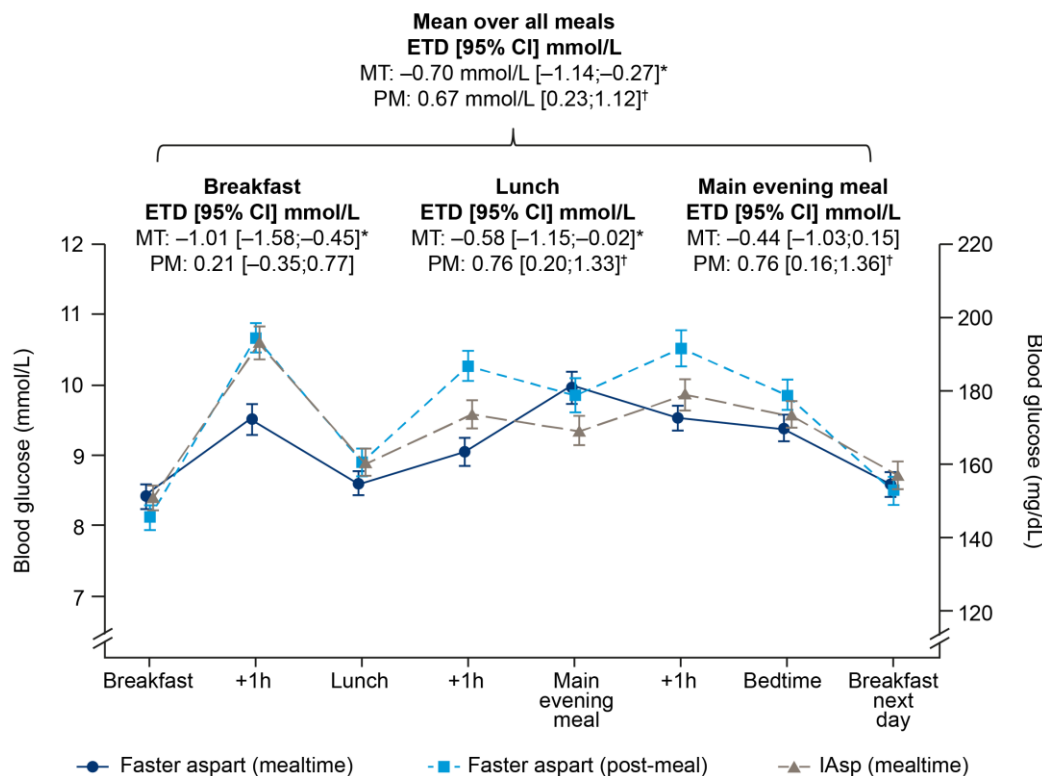
**Objectives:** To confirm the efficacy and safety of fast-acting insulin aspart (faster aspart) versus insulin aspart (IAsp) in a paediatric sample with type 1 diabetes (T1D).

**Methods:** A treat-to-target, 26-week, multicentre trial randomised participants (1 to <18 years old) following a 12-week run-in period to double-blind mealtime faster aspart (n=260) or IAsp (n=258), or open-label post-meal faster aspart (n=259), each with daily insulin degludec treatment (NCT02670915). All available information regardless of treatment discontinuation was used for evaluation.

**Results:** At week 26, non-inferiority (0.4% margin) for the primary endpoint, change from baseline in HbA<sub>1c</sub>, was confirmed for mealtime and post-meal faster aspart versus IAsp, with a statistically significant difference in favour of mealtime faster aspart (estimated treatment difference [95% CI]: -0.17% [-0.30;-0.03]; -1.82 mmol/mol [-3.28;-0.36]). Change from baseline in 1-hour postprandial glucose significantly favoured mealtime faster aspart versus IAsp at breakfast, lunch and mean over all meals (Figure). No significant differences in overall rate of treatment-emergent severe or blood glucose (BG)-confirmed hypoglycaemic episodes (BG <3.1 mmol/L [56 mg/dL]) were observed. Mean total daily insulin dose on treatment was 0.92 U/kg (mealtime faster aspart), 0.92 U/kg (post-meal faster aspart) and 0.88 U/kg (IAsp).

**Conclusion:** Mealtime and post-meal faster aspart with insulin degludec provided effective glycaemic control (superior for mealtime faster aspart) versus IAsp, with no additional safety risks in children and adolescents with T1D.

Figure: 8-point SMBG profiles 26 weeks after randomisation



Mealtime: defined as injecting 0–2 min before the meal. Post-meal: defined as injecting 20 min after the start of the meal. Change from baseline in postprandial glucose (SMBG) was analysed using an analysis of variance model after multiple imputation. Error bars: +/- standard error (mean).

\*In favour of mealtime faster aspart versus IAsp; †in favour of IAsp versus post-meal faster aspart.

CI, confidence interval; ETD, estimated treatment difference; faster aspart, fast-acting insulin aspart; IAsp, insulin aspart; MT, mealtime comparison; PM, post-meal comparison; SMBG, self-measured blood glucose.

## Cardiac paragangliomas in two siblings with *SDHB* mutations: challenges of management.

Simon Ryder<sup>1,2</sup>, Jade Eccles-Smith<sup>1</sup>, John Younger<sup>3</sup>, Amanda Love<sup>1</sup>, Emma Duncan<sup>1,2,4</sup>

1. Department of Endocrinology, The Royal Brisbane and Women's Hospital, Herston, QLD, Australia

2. The University of Queensland, Brisbane, QLD, Australia

3. Department of Cardiology, The Royal Brisbane and Women's Hospital, Herston, QLD, Australia

4. Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia

Paragangliomas (PG) and pheochromocytomas (PCC) are amongst the most heritable tumours. Germline mutations are identified in 35-40% of cases and to date, 21 PCC/PG susceptibility genes have been described. *SDHB* is a known PCC/PG susceptibility gene, and codes for a succinate dehydrogenase subunit. *SDHB* mutations have a penetrance of 25-40% however 25-30% of *SDHB*-associated tumours are malignant.

Cardiac paragangliomas (PGs) are rare (<0.3%) mediastinal tumours, comprising 1-3% of primary cardiac tumours and 2% of all PGs. Prior to functional imaging, most tumours were diagnosed only after the onset of symptoms or signs, and thus were often large and unresectable. Cardiac PGs have been more commonly reported with *SDHD* mutations.

We present two siblings from a large kindred with an *SDHB* mutation (c.286+2T>G (IVS3+2T>G)). Of 28 carriers, six have evidenced PGs to date, including four siblings and one of their offspring. Two of these siblings developed cardiac PGs, identified through FDG-PET screening. Gated cardiac MRI quantified both lesions (<2cm diameter) and both siblings underwent successful resection of their tumours. No variants in *SDHD* were identified (one affected sibling screened). The asymmetrical penetrance of the *SDHB* mutation in this kindred suggests the potential role of a modifier, however further work is required to explore this hypothesis. Furthermore, due to the rarity of condition, the approach to ongoing surveillance remains to be defined.

1. Benn, D. E., et al. (2015). "15 YEARS OF PARAGANGLIOMA: Clinical manifestations of paraganglioma syndromes types 1-5." *Endocr Relat Cancer* 22(4): T91-103. 2. Ghayee, H. K., et al. (2009). "Mediastinal paragangliomas: association with mutations in the succinate dehydrogenase genes and aggressive behavior." *Endocr Relat Cancer* 16(1): 291-299. 3. Martucci, V. L., et al. (2015). "Succinate dehydrogenase gene mutations in cardiac paragangliomas." *Am J Cardiol* 115(12): 1753-1759. 4. Michalowska, I., et al. (2016). "Mediastinal paragangliomas related to *SDHB* gene

## An audit of sodium-glucose co-transporter 2 inhibitors in a specialist outpatient service: are the pharmaceutical benefits scheme approval guidelines too restrictive?

**Judith R Fleming<sup>1</sup>**

1. *Launceston General Hospital, Launceston, Tasmania, Australia*

Background: Launceston is an isolated city with a relatively high prevalence of diabetes mellitus at around 5.3%<sup>1</sup>. SGLT2 inhibitors were enlisted on the Australian PBS between 2013 and 2015 to be prescribed under strict criteria<sup>2</sup> – similar to that of NICE (UK) and the NZF (New Zealand). Aims: To explore the affect of SGLT2 inhibitors on BMI, blood pressure, renal function and HbA1c when prescribed both within and outwith PBS guidelines. Methods: Records of the presenting outpatient patient cohort (n=60) currently prescribed/newly commenced on SGLT2 inhibitors between 1st January 2018 and 31st July 2018 inclusive were scrutinised for demographics and changes in aforementioned variables. Results: 64% of patients were prescribed SGLT2 inhibitors within PBS guidelines. Statistically significant reductions were seen in HbA1c, weight and BMI (p=0.0008, p=0.0010, p=0.0171 respectively) with no significant effect on renal function (p=0.6043). Similarly in the group prescribed SGLT2 inhibitors outwith PBS guidelines, statistically significant reductions were seen in HbA1c, weight and BMI (p values of p=0.0285, p=0.0143, p=0.0087 respectively) with no significant effect on renal function (p=0.7280). Mean HbA1c reduction was greater in this group. Co-prescription of GLP-1 agonists outwith PBS guidelines was common but afforded no statistically significant difference in HbA1c or weight. Conclusions: The addition of SGLT2 inhibitors as anti-diabetic agents in a specialist outpatient service have shown significant improvements in HbA1c, weight and BMI with no significant deterioration in renal function. It should be considered that SGLT2 inhibitors may show more net benefit if the PBS guidelines were relaxed.

- 1) Australian Bureau of Statistics. National Health Survey 2014-2015. 2018 Aug [cited 2018 Nov]. <http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4364.0.55.001~2014-15~Main%20Features~Tasmania~10007>
- 2) PBS: The Pharmaceutical Benefits Scheme. Department of Health. 2018 Nov [cited 2018 Nov]. <http://www.pbs.gov.au/pbs/home>

## Clinical outcomes in a hospital bariatric medicine clinic

**George Roubos<sup>1</sup>, Peter Nguyen<sup>1</sup>, Spiros Fourlanos<sup>2,3</sup>, John M Wentworth<sup>2</sup>**

1. *University of Melbourne, Parkville, Victoria, Australia*

2. *Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia*

3. *Department of Medicine, Royal Melbourne Hospital, Parkville, Victoria, Australia*

### Aim

To investigate the clinical outcomes following personalised medical management in an Endocrinologist-led outpatient clinic.

### Background

Obesity has been identified in over one-third of hospital inpatients at the Royal Melbourne Hospital. This audit was performed to benchmark the Royal Melbourne Hospital Bariatric Medicine Clinic (RMH BMC) which provides personalised medical management plans for weight loss.

### Methods

This retrospective cohort study collected data from patient records, pathology results and patient surveys. The study included all patients who attended the clinic from February 2012 to November 2018 for at least one visit. BMC data were captured at baseline, 3, 6 and 12-month time points. The primary outcome measure was weight loss at 12 months. Secondary endpoints were waist circumference, biochemical markers and quality-of-life.

### Results

The RMH BMC cohort comprised 313 patients (213 female). The cohort mean ( $\pm$ sd) age, weight and BMI at baseline were 46.1( $\pm$ 14.0)years, 129.0( $\pm$ 31.7)kg, and 46.5( $\pm$ 9.8)kgm<sup>2</sup>, respectively. Patient attrition was high, with only 113 (36%) patients attending for 12 months. After 12 months the mean weight loss was 4.1( $\pm$ 8.7)kg (3.3% of baseline) and their waist circumference decreased by 6.2( $\pm$ 11.2)cm. Sixty-eight patients (60%) lost 1kg or more, and 42 (37%) lost greater than 5% body weight. Mean quality-of-life physical domain score increased from 34 at baseline to 44 after 12 months, a significant improvement relative to the Australian population mean of 50. In contrast, biochemical markers, including HbA1c and fasting glucose, did not change significantly.

### Conclusion

The RMH BMC population had high rates of loss to follow up by 12 months. Of those who attended for 12 months, approximately one third attained more than 5% weight loss, comparable to other reported medical interventions. Clinic attendance delivered substantial improvement in physical quality of life but resulted in only modest weight loss with no significant impact on biochemical markers.



## Predictors of weight loss with personalised management in a hospital bariatric medicine clinic

Peter PN Nguyen<sup>1</sup>, George GR Roubos<sup>1</sup>, John JW Wentworth<sup>2</sup>, Spiros SF Fournalanos<sup>2,1</sup>

1. University of Melbourne, Melbourne, Victoria, Australia

2. Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

**Introduction:** The increasing prevalence of obesity in conjunction with the lack of effective non-surgical treatments highlights the need for better treatment strategies. To improve patient selection for lifestyle intervention, we sought to determine the patient characteristics associated with favourable weight outcomes following 12 months of personalised management in the Endocrinologist-led Royal Melbourne Hospital Bariatric Medicine Clinic (RMH BMC).

**Methods:** A retrospective assessment of medical records, patient surveys and pathology results was performed in 313 patients who attended the RMH BMC between 2012 and 2018. The final cohort comprised 113 adults (age, 48.4(±14.2)y; BMI, 44.9(±7.7)kg/m<sup>2</sup>) who completed 12 months follow-up. Thirty-six clinical, psychosocial and biological variables were investigated in relation to the primary outcome measure of weight loss (% bodyweight).

**Results:** Of all starting participants, there was a high loss to follow up rate of 64% (n = 200) whom did not complete 12 months of clinical care. Those remaining had a mean of 4.1 ± 8.7 kg or 3.3(± 6.8)% weight loss. In a multivariate correlation/regression analysis significant predictors of higher percentage weight loss were; clinic attendance (p=0.004), consultant at initial visit (p=0.009), clinician adherence (p=0.018), dietitian adherence (p=0.049), excess alcohol intake (p=0.005), older age at clinic entry (p=0.026), higher age of obesity onset (p=0.009), osteoarthritis of weight-bearing joints (p=0.002), higher Edmonton Obesity Stage (p=0.019), higher baseline HbA1c (p=0.001), higher baseline and fasting blood glucose (p=0.035). Significant negative predictors were a diagnosis of depression (p=0.045). All predictors were independently associated with weight loss.

**Conclusion:** Predictors of weight loss span different domains, reflecting the multifactorial nature of obesity. These findings suggest the medical management for weight loss is more likely to be effective in patients who are older, became obese at an older age, have osteoarthritis or excessive alcohol intake.

## Pre-hospital management of acute illness in Addison's Disease: an audit of patients attending a referral hospital in a large regional area

Thomas Goubar<sup>1</sup>, Louise Rushworth<sup>1</sup>, David J Torpy<sup>2</sup>, Shaun McGrath<sup>3</sup>

1. The University of Notre Dame Australia, School of Medicine, Sydney, Darlinghurst, NSW, Australia

2. Endocrine and Metabolic Unit, Royal Adelaide Hospital and University of Adelaide, Adelaide, South Australia, Australia

3. Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

**Background:** Adrenal crises (AC) cause morbidity and mortality in patients with Addison's disease (primary adrenal insufficiency (PAI)). Patient-initiated oral stress dosing, with parenteral hydrocortisone for those unable to take tablets, is recommended to avert ACs. While these should be effective, the continued incidence of ACs remains largely unexplained.

**Methods:** An audit of all attendances between 2000 and 2017 by adult patients with treated PAI to one regional referral centre in New South Wales, Australia. Measurements were those taken on arrival at hospital.

**Results:** There were 252 attendances by 56 patients with treated PAI during the study period. Women comprised 60.7% (n=34) of the patients. Mean age of attendees was 53.7 (19.6) years. The majority (83.7%, n= 211) of patients were admitted. Nearly half (45.2%, n=114) the patients had an infection. Among the 252 attendances, there were 61 (24.2%) ACs diagnosed by the treating clinician. Only 17.9% (n=45) of the hospital presentations followed any form of stress dosing. Intramuscular (IM) hydrocortisone was used before 7 (2.8%) attendances only and no subcutaneous (SC) administration of hydrocortisone was recorded. Among patients with a clinician diagnosed AC, only 32.8% (n=20) had used stress dosing before presentation. Vomiting was reported by 47.6% (n=120) of the patients on arrival but only 33 (27.5%) of these attempted stress dosing and only 5 patients with vomiting used IM hydrocortisone. In 11 attendances, a diagnosis of diabetic ketoacidosis was recorded. There were 5 (2.0%) in-hospital deaths. Younger age, vomiting and the number of prior presentations all significantly predicted stress dose use (p<0.05).

**Conclusion:** Dose escalation strategies are not used universally or correctly by all acutely unwell patients with PAI, and many patients do not use IM or SC hydrocortisone injections. Previous treatment in hospital increases the likelihood of stress dosing and offers the opportunity for reinforcement of prevention strategies.

## Skeletal benefit/risk of long-term denosumab therapy: A virtual twin analysis of fractures prevented to skeletal safety events observed

Serge Ferrari<sup>1</sup>, E Michelle Lewiecki<sup>2</sup>, P W Butler<sup>3</sup>, David L Kendler<sup>4</sup>, Nicola Napoli<sup>5</sup>, S Huang<sup>3</sup>, Daria B Crittenden<sup>3</sup>, Nico Pannacciulli<sup>3</sup>, Ethel Siris<sup>6</sup>, Neil C Binkley<sup>7</sup>, Jeffrey Hassall<sup>8</sup>

1. Geneva University Hospital, Geneva, Switzerland

2. New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA

3. Amgen Inc., Thousand Oaks, CA, USA

4. University of British Columbia, Vancouver, BC, Canada

5. Università Campus Bio-Medico di Roma, , Rome, Italy

6. Columbia University Medical Center, New York, NY, USA

7. University of Wisconsin-Madison, Madison, WI, USA

8. Amgen Australia, North Ryde, NSW, Australia

**Purpose:** Osteoporosis is a chronic disease, but atypical femoral fracture (AFF) and osteonecrosis of the jaw (ONJ) remain a concern with long-term treatment. Ten years of denosumab (DMAb) therapy in postmenopausal women with osteoporosis has previously demonstrated sustained and low fracture rates, with low adverse event rates. Here, we generated a DMAb skeletal benefit/risk ratio derived from observed data and model-based estimates.

**Methods:** Exposure-adjusted subject incidence per 100,000 subject-years of clinical, major osteoporotic, vertebral, nonvertebral, and hip fractures was calculated for long-term (LT) subjects randomized to DMAb in the 3-year FREEDOM trial and enrolled in the 7-year Extension (follow-up time 3–10 years). Due to the lack of a long-term placebo group, fracture rates in a hypothetical cohort of placebo controls (virtual twins [VT]) were estimated using a regression model. The number of fractures prevented per 100,000 subject-years was calculated as (VT rate – LT rate). AFF and ONJ incidences on DMAb were based on observed cases in the LT group during the Extension; the VT group was assumed to have no AFF or ONJ in the absence of treatment. A skeletal benefit/risk ratio was calculated from fractures prevented per AFF or ONJ observed.

**Results:** This analysis included 2343 subjects. An estimated 1403 clinical fractures were prevented per 100,000 subject-years (Table). There was 1 case of AFF and 7 ONJ (mild and moderate), corresponding to rates of 5 (AFF) and 35 (ONJ) per 100,000 subject-years. Hence, there were 281 and 40 clinical fractures prevented per AFF and ONJ observed, respectively. The benefit/risk ratio for other fracture endpoints is shown below (Table).

**Conclusions:** As long-term placebo-controlled fracture outcome studies in postmenopausal OP are not ethical, the virtual twin model provides a reasonable estimate of untreated fracture rates. Using this model, long-term DMAb therapy has a highly favorable benefit/risk profile.

**Table. Ratio of Fractures Prevented per Bone Safety Event Observed in Long-term FREEDOM Extension Subjects Using the Virtual Twin Method**

	Exposure-adjusted subject incidence per 100,000 subject-years				
	Clinical fractures N = 2343 Exp = 18295	Major osteoporotic fractures <sup>a</sup> N = 2343 Exp = 18772	Vertebral fractures N = 2116 <sup>b</sup> Exp = 18385	Non-vertebral fractures N = 2343 Exp = 18451	Hip fractures N = 2343 Exp = 19742
Long-term	1777	1525	901	1528	149
Twin	3180	2699	1879	2924	297
Ratio <sup>c</sup>	0.56	0.57	0.48	0.53	0.54
Fractures prevented	1403	1174	978	1396	148
Ratio of fractures prevented per AE observed <sup>d</sup>					
AFF (5) <sup>e</sup>	280.6	234.8	195.6	279.2	29.6
ONJ (35) <sup>e, f</sup>	40.1	33.5	27.9	39.9	4.2

Based on LT denosumab subjects. All results are based on 5000 bootstrap samples.  
N = number of LT subjects who had fracture outcome data; Exp = exposure time in subject years indicating the time from FREEDOM baseline to the onset of first event (for subjects who had a fracture event) or end of study or last assessment date, whichever was greater (for subjects who did not have a fracture event)

<sup>a</sup> Major osteoporotic fractures were defined as clinical spine, forearm, hip, or shoulder fracture

<sup>b</sup> 227 LT subjects had missing vertebral fracture outcome data during FREEDOM and/or its Extension

<sup>c</sup> Calculated as (LT rate) / (VT rate)

<sup>d</sup> Calculated as (VT rate – LT rate) / AE rate

<sup>e</sup> The number in parentheses indicates the AE subject incidence rate per 100,000 subject-years in LT subjects

<sup>f</sup> There were 2 mild, 5 moderate, and no severe cases of ONJ in the LT group

AE: Adverse event; AFF: atypical femoral fracture; ONJ: osteonecrosis of the jaw

### Subgroup analysis of the effect of denosumab compared with risedronate on percentage change in lumbar spine bone mineral density at 24 months in glucocorticoid-treated individuals

Kenneth Saag<sup>1</sup>, Nico Pannaciuoli<sup>2</sup>, P Geusens<sup>3</sup>, J Adachi<sup>4</sup>, E Lespessailles<sup>5</sup>, J Malouf-Sierra<sup>6</sup>, B Langdah<sup>7</sup>, P W Butler<sup>2</sup>, X Yin<sup>2</sup>, W F Lems<sup>8</sup>, Jeffrey Hassall<sup>9</sup>

1. University of Alabama, Birmingham, AL, USA

2. Amgen Inc., Thousand Oaks, CA, USA

3. Maastricht University, Maastricht, The Netherlands

4. McMaster University, Hamilton, ON, Canada

5. University Hospital Orleans, Orleans, France

6. Hospital San Pablo, Barcelona, Spain

7. Aarhus University Hospital, Aarhus, Denmark

8. VU University Medical Centre, Amsterdam, The Netherlands

9. Amgen Australia, North Ryde, NSW, Australia

**Purpose:** We previously demonstrated denosumab (DMAb) increased lumbar spine (LS) and total hip (TH) BMD significantly more than risedronate (RIS) at 12 and 24 months (mos) in glucocorticoid (GC)-treated individuals. Prespecified subgroup analyses of LS BMD at 12 mos indicated that DMAb was superior to RIS across 7 subgroups of GC-treated individuals. This analysis explored the effects of DMAb and RIS on LS BMD in the same subgroups of GC-treated individuals at 24 mos.

**Methods:** The phase 3, randomized, double-blind, double-dummy, active-controlled study enrolled women and men age ≥18 years receiving ≥7.5 mg prednisone or equivalent daily for <3 mos (GC-initiating [GC-I]) or ≥3 mos (GC-continuing [GC-C]) before screening. Subjects were randomized 1:1 to DMAb 60 mg SC every 6 mos or RIS 5 mg PO daily for 24 mos. All subjects were to receive daily calcium (≥1000 mg) and vitamin D (≥800 IU). The treatment difference (DMAb – RIS) for percentage change from baseline in LS BMD at 24 mos was estimated in the GC-I and GC-C subpopulations, both overall and in 7 prespecified subgroups.

**Results:** The study enrolled 795 subjects. Baseline characteristics were balanced between treatment groups within each subpopulation. DMAB was superior to RIS for gains in LS BMD at 24 mos in both the GC-I and GC-C subpopulations. Within each subgroup (Table), DMAB was consistently associated with a greater increase in LS BMD at 24 mos compared with RIS.

**Conclusion:** DMAB consistently increased LS BMD more than RIS at 24 mos in both GC-I and GC-C subpopulations, with no evidence of directional heterogeneity in treatment effect across 7 prespecified subgroups of GC-treated individuals. DMAB may be a useful addition to the osteoporosis armamentarium in the common clinical setting of GC use.

**Table. Treatment Difference (DMAB – RIS) in Lumbar Spine BMD Percentage Change From Baseline at Month 24**

	Glucocorticoid-initiating Subpopulation (N = 133 RIS / 128 DMAB)				Glucocorticoid-continuing Subpopulation (N = 230 RIS / 228 DMAB)			
	DMAB (n)	RIS (n)	Least Square Mean Estimate of Difference (95% CI)	Interaction P value Quantitative (Qualitative)	DMAB (n)	RIS (n)	Least Square Mean Estimate of Difference (95% CI)	Interaction P value Quantitative
<b>Overall subpopulation</b>	107	113	4.5 (3.2, 5.8)*	–	183	174	3.2 (2.0, 4.3)*	–
<b>Sex</b>								
Female	69	72	5.7 (4.0, 7.5)*	0.013	141	131	3.7 (2.4, 5.0)*	
Male	38	41	2.1 (0.1, 4.1) <sup>†</sup>	(0.50 <sup>†</sup> )	42	43	1.4 (-1.1, 4.0)	0.13
<b>Race</b>								
Caucasian	92	97	3.9 (2.4, 5.3)*	0.024	166	158	3.3 (2.1, 4.5)*	
Not Caucasian	15	16	7.6 (3.8, 11.4)*	(0.50 <sup>†</sup> )	17	16	1.9 (-1.6, 5.4)	0.63
<b>Age</b>								
< 60 years	28	41	2.9 (0.1, 5.8) <sup>†</sup>		79	86	2.9 (1.2, 4.6) <sup>†</sup>	
≥ 60 years	79	72	5.1 (3.6, 6.6)*	0.089	104	88	3.4 (1.9, 5.0)*	0.72
<b>Baseline LS T-score</b>								
≤ -2.5	17	21	4.6 (1.0, 8.1) <sup>†</sup>		66	68	3.4 (1.1, 5.6) <sup>†</sup>	
> -2.5	90	92	3.9 (2.5, 5.2)*	0.066	117	106	2.9 (1.6, 4.2)*	0.44
<b>Baseline LS T-score</b>								
≤ -1.0	55	60	5.4 (3.6, 7.3)*		142	141	3.2 (1.8, 4.5)*	
> -1.0	52	53	2.8 (1.0, 4.6) <sup>†</sup>	0.13	41	33	2.9 (1.0, 4.8) <sup>†</sup>	0.89
<b>Geographic region</b>								
Europe	65	73	4.0 (2.4, 5.6)*		135	122	3.2 (1.8, 4.6)*	
Non-Europe	42	40	5.3 (2.9, 7.8)*	0.24	48	52	2.6 (0.8, 4.5) <sup>†</sup>	0.90
<b>Menopausal status</b>								
Premenopausal	9	3	11.4 (1.2, 21.7) <sup>†</sup>		20	20	3.9 (0.2, 7.6) <sup>†</sup>	
Postmenopausal	59	67	5.7 (3.9, 7.6)*	0.77	119	109	3.7 (2.3, 5.1)*	0.98
<b>Baseline GC dose<sup>‡</sup></b>								
≥ 7.5 to < 10 mg	20	28	6.3 (2.8, 9.7)*		61	67	3.4 (1.6, 5.2)*	
≥ 10 mg	85	85	4.3 (2.8, 5.8)*	0.57	121	107	3.2 (1.7, 4.7)*	0.88

CI = confidence interval; N = Number of randomized subjects with a baseline measurement and ≥ 1 postbaseline measurement for the lumbar spine BMD; n=number of subjects with observed value. \*p<0.001, DMAB vs RIS. <sup>†</sup>p<0.05, DMAB vs RIS. <sup>‡</sup>Qualitative p-values >0.05 support consistent directionality of treatment effect within the subgroups. <sup>§</sup>In prednisone equivalents.

## Systemic inflammation and The Effect of a GLP-1 Agonist in Adults with Prader-Willi Syndrome

**Amanda Hor<sup>1,2,3</sup>, Jarron Dodds<sup>1,4</sup>, Louise Purtell<sup>1</sup>, Lesley Campbell<sup>1,2,3</sup>, Alexander Viardot<sup>1,2,3</sup>**

1. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

2. St Vincent's Hospital, Sydney, NSW, Australia

3. University of New South Wales, Sydney, NSW, Australia

4. School of Medicine, University of Notre Dame, Sydney, NSW, Australia

Introduction:

Prader – Willi syndrome (PWS) is one of the most common known genetic obesity disorders. Individuals with PWS have reduced life expectancy due to cardiovascular disease. Increased systemic low-grade inflammation is postulated to contribute. Whilst increased systemic inflammation is associated with excess adiposity, increased visceral fat mass and decreased insulin sensitivity in obesity, an overactivation of the innate immune system has been reported in PWS independent of central adiposity and insulin resistance. This chronic inflammatory process could present a novel treatment target to reduce cardiovascular morbidity and mortality in PWS.

Aims:

1. To assess circulating cytokine profile, fasting and postprandially, to estimate systemic immune activation in PWS, compared to lean and adiposity-matched obese subjects.
2. To determine the acute effect of a GLP-1 receptor agonist on systemic inflammation in PWS.

Methods:

Baseline and postprandial inflammatory cytokine levels were measured in 9 PWS adults and compared with 11 adiposity-matched obese, and 10 healthy lean subjects. In a single-blinded, crossover design, 6 PWS and 11 obese subjects, received either a single dose of 10 mcg exenatide (Byetta) or normal saline subcutaneously 15 minutes before a 600kCAL test meal. IL-6, the only cytokine high in PWS compared to control subjects, was measured at baseline and for 240 min after the test meal.

**Results:**

E-selectin, MIC-1 and PAI-1 were elevated in PWS compared to lean but not different to obese. sICAM-1 levels were not different between the groups. IL-6 was higher in PWS than in Obese and Lean. Single dose GLP-1 agonist did not significantly lower IL-6 response post-prandially.

**Conclusion:**

There was no generally increased immune activation observed in PWS. However, the increased IL-6 levels fasting and post-prandially appears to be specific to PWS and merits further investigation regarding its possible contribution to the cardiovascular risk.

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## **Is depression a risk factor for inadequate glycemic control in young adults with type 2 diabetes? Sri Lankan perspective**

**Anuradha Jayasuriya<sup>1</sup>, Uditha Bulugahapitiya<sup>1</sup>, Udai Wijetunga<sup>1</sup>, Gowri M Rathnayake<sup>1</sup>, Athula Sumathipala<sup>2</sup>**

1. *Diabetes and Endocrinology clinic, Colombo South Teaching Hospital, Kalubowila, Western province, Sri Lanka*

2. *Research Institute for Primary Care & Health Sciences, Faculty of Medicine & Health Sciences, Keele University, New Castle, Staffordshire, ST5 5BG, United Kingdom*

**Abstract**

**Background**

Presence of depression in young adults with type 2 diabetes has a negative impact on diabetes self care and adherence to medications leading to impaired glycemic control. Hence timely detection and effective treatment of depression among young adults is increasingly recognized as an essential component of high quality clinical care.

**Objectives**

This research aims to determine the prevalence of depression in young adults with type 2 diabetes in a cohort of patients attending a tertiary care setting and to describe the effects of depression on their glycemic control and other parameters.

**Research design and Methods**

A descriptive cross sectional study was conducted at diabetes clinic of Colombo South Teaching Hospital in Sri Lanka where 140 patients with type 2 diabetes, aged between 18 to 35 years were enrolled as study subjects. Self administered, validated questionnaires, Patient health questionnaire- 9 (PHQ-9) and Short Form 36 Health survey questionnaire (SF-36) were used to assess the depression and quality of life respectively.

**Results**

Estimated prevalence of depression was 55.2% in study population ; majority 37.7% had mild depression whereas 14.2% were diagnosed with moderate depression. However only 4.7% had severe depression. Glycemic control among depressed young adults was significantly impaired ( P value 0.03, mean fasting blood sugar/FBS of 155mg/dl in the depressed compared to mean FBS of 131mg/dl in non-depressed. Despite higher mean post prandial blood sugar/PPBS ( 183 mg/dl Vs.164mg/dl) and Glycated hemoglobin/HbA1c (8.5% Vs. 7.4% ) values observed in depressed young adults, these were not statistically significant. There were no significant between-group difference in mean values of systolic blood pressure(122mmHg Vs. 124 mmHg) and body mass index(26.2 vs. 25.1).

**Conclusion**

Depression in young adults with diabetes is an important comorbidity which requires early diagnosis and careful management in order to achieve optimum glycemic control.

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## **Does depression in young Sri Lankan patients with type 2 diabetes contribute to impaired quality of life? Real world data in a tertiary care setting**

**Anuradha Jayasuriya<sup>1</sup>, Uditha Bulugahapitiya<sup>1</sup>, Udai Wijetunga<sup>1</sup>, Gowri M Rathnayake<sup>1</sup>**

1. *Diabetes and Endocrinology clinic, Colombo South Teaching Hospital, Kalubowila, Western province, Sri Lanka*

**Abstract**

**Background**

Improvement in quality of life is the ultimate goal of all health interventions and presence of diabetes itself impairs the quality of life of patients leading to poor physical and mental well being. Furthermore individuals diagnosed with depression and Diabetes have an additional worse outcome.

### **Objectives**

The aim of the study was to estimate the prevalence of depression and impairment of quality of life in young patients with type 2 diabetes.

### **Research design and Methods**

This analytical cross sectional study consisted of 140 subjects with type 2 diabetes aged between 18 and 35 years attending Diabetes clinic at Colombo South Teaching Hospital, Sri Lanka. Prevalence of depression and dimensions of quality of life were assessed separately using self administered validated questionnaires.

### **Results**

Out of total study subjects, females constituted 70.8% and mean age was 30 years. Substantial proportion of young adults (55.1%) were diagnosed to have depression. Physical function as well as physical health was significantly impaired in the depressed group compared to those of non depressed group with p value <0.0001 and < 0.001 respectively. Moreover both energy levels and social functions were significantly decreased in those with depression with p values < 0.0001 for both dimensions. However there were no significant group differences in total pain or emotional problems experienced in 2 groups. Furthermore impaired physical functioning contributed to inadequate glycemic control with significantly higher FBS levels. ( P value 0.04 )

### **Conclusions**

Depression in young patients with type2 diabetes has a significant negative impact on most components of quality of life. Hence this highlights the importance of identification and proper treatment of depression to prevent deterioration of quality of life.

## Real world data on prevalence of oral candidiasis and its associations among patients with diabetes in a tertiary care centre in Sri Lanka

Rukshini Chethana Wijesuriya<sup>1</sup>, Tharundi Erandika Wijesinghe<sup>1</sup>, Shalini Devanthika Dias<sup>1</sup>, Charmini Anuradha Jayasuriya<sup>2</sup>, Uditha Bulugahapitiya<sup>2</sup>

1. Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Rathmalana, Western province, Sri Lanka

2. Diabetes and Endocrinology clinic, Colombo South Teaching Hospital, Kalubowila, Western province, Sri Lanka

### Background

Oral existence of *Candida* species both as a commensal and opportunistic pathogen in immunocompromised individuals is well recognized. Diabetes is associated with plethora of oral complications including oral candidiasis.

### Objectives

This research aims to determine the prevalence of candidiasis and its associations with diabetes mellitus (DM). Additionally it aims to estimate the isolation and identification of *Candida* species and its correlations with biochemical and microbiological parameters and other associated factors.

### Research design and Methods

An analytical cross sectional study was conducted at the Diabetes and Endocrinology clinic, Colombo South Teaching Hospital, Sri Lanka where 100 patients with diabetes and 100 subjects without diabetes were recruited as study group and control group respectively. Blood, saliva and an oral rinse were obtained from each subject for the estimation of fasting blood sugar (FBS), glycated hemoglobin (HbA1c), fasting salivary glucose (FSG) and for the detection of *Candida* species.

### Results

Mean age of the study and control groups was 45 years and more than 90% of study subjects and 88% in control group were females. Prevalence of oral candidiasis among patients with type 2 diabetes was 66% whereas that in control group was 46%. Significant correlations ( $P < 0.05$ ) were observed between *Candida* growth and fasting blood sugar levels, the logarithmic values of the total Colony forming units of *Candida* and FSG levels ( $P < 0.05$ ). Furthermore significant associations were detected between *Candida* growth and wearing of dentures ( $P < 0.001$ ), their duration ( $P < 0.01$ ) and FSG levels ( $P < 0.05$ ).

### Conclusions

Patients with diabetes are at high risk of development of oral candidiasis compared with those without and *Candida* growth in the oral cavity is significantly associated with glycemic control and wearing of dentures. This study emphasizes the necessity of routine dental work up and appropriate oral hygiene in patients with diabetes.

## Progressive increase in bone turnover despite improvement in markers of calcium homeostasis over twelve months post sleeve gastrectomy in obese subjects

Chris Kwan<sup>1,2</sup>, Anthony Russell<sup>3,2</sup>, Viral Chikani<sup>3,2</sup>, Anthony Cheng<sup>4,2</sup>, Chung Won<sup>4,2</sup>, Moe Thuzar<sup>3,2</sup>

1. Department of Internal Medicine, Princess Alexandra Hospital, Brisbane, Queensland, Australia

2. Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia

3. Department of Endocrinology & Diabetes, Princess Alexandra Hospital, Brisbane, Queensland, Australia

4. Department of Surgery, Princess Alexandra Hospital and Queen Elizabeth II Hospitals, Brisbane, Queensland, Australia

### Introduction

Bariatric surgery, including sleeve gastrectomy, is associated with unfavourable effects upon bone health; the mechanism of which is unclear.<sup>1</sup> The study aims to examine changes in calcium homeostasis and bone turnover over time after sleeve gastrectomy.

### Methods

This is a single-centre retrospective cohort study involving 102 people (84 women) with mean  $\pm$  SEM age  $42 \pm 2$  years and baseline BMI  $49.6 \pm 0.7$  kg/m<sup>2</sup> who underwent sleeve gastrectomy between January 2015 and December 2016 and were followed for up to 12 months post-surgery under a multidisciplinary team. Data on changes in weight, serum markers of calcium

homeostasis and bone turnover (P1NP) and hormone profiles were collected from electronic medical records, and analysed using repeated measures ANOVA.

## Results

Body weight reduced significantly post-surgery (140.0±4.6 preop, 114.8±4.1 at 3 months, 106.2±4.0 at 6 months, 100.0±4.2kg at 12 months; P<0.001). P1NP significantly and progressively increased (48.7±8.4 pre-op, 59.5±8.2 at 3 months, 67.0±8.9 at 6 months, 68.1±8.9mcg/L at 12 months; P=0.001). Vitamin D levels significantly increased with supplementation of vitamin D (62±7 pre-op, 81±6 at 3 months, 79±8 at 6 months, 79±7nmol/L at 12 months; P<0.001), and parathyroid hormone levels significantly decreased (6.8±1.1 pre-op, 5.9±1.1 at 3 months, 5.8±0.9 at 6 months, 5.8±1.2pmol/L at 12 months; P=0.01) over 12 months post-surgery. Testosterone in men tended to increase (10.43±3.2 pre-op, 16.5±7.3 at 3 months, 18.3±5.1 at 6 months, 18.6±7.5nmol/L, P=0.12). There were no significant changes in serum calcium, phosphate, thyroid function or renal function over time. Gender did not significantly influence P1NP changes over time.

## Conclusions

Bone turnover progressively increased over 12 months post sleeve gastrectomy, which likely contributes to the well-known adverse effect of bariatric surgery on bone health. This occurred despite improvement in calcium homeostasis and sex hormone profiles. Future studies need to focus on other mechanisms of increased bone turnover.

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## Incidence and natural history of hypophysitis secondary to immune checkpoint inhibitor therapy at a tertiary centre

**Nicole Lafontaine Bedecarratz<sup>1,2</sup>, Sylvia Ye<sup>2</sup>, Phillip Parente<sup>2</sup>, Christopher Gilfillan<sup>2</sup>**

1. Dept Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, VIC

2. Eastern Health, Melbourne, VIC, Australia

**Background-** Check-point inhibitor therapy has revolutionised treatment in oncology. Because of their mechanism of action, they can lead to a number of immune related adverse effects including hypophysitis. There have been a few studies looking at the long term outcomes of patients with ipilimumab-induced hypophysitis however, there are limited studies including patients receiving PD1 inhibitors or combination therapy.

**Aim-** To determine the incidence of hypophysitis associated with ipilimumab, nivolumab and pembrolizumab at our center and describe its natural history.

**Methods-** We performed a retrospective study of patients treated with pembrolizumab, nivolumab, ipilimumab or combination of these drugs between Aug 2013 and Jun 2018. Files were reviewed for cancer history and immune related adverse effects. Patients with hypophysitis had their clinical status, biochemistry and treatment documented at 0,1.5, 3, 6, 9 months and at the most recent follow up.

**Results-** Of 115 patients identified, 14 developed hypophysitis. Of these, 8 of 17 patients had received combination ipilimumab and nivolumab, 1 of 6 had received ipilimumab, 4 of 61 had received nivolumab and 1 of 23 had received pembrolizumab. The median time to hypophysitis from initial dose was 98 days.

At diagnosis, 78% had thyroid axis involvement, 3/11 had resolved at 6 weeks. Only 50% had a paired ACTH/cortisol requested at diagnosis. 100% had evidence of adrenal insufficiency with no patient recovering their adrenal axis. Gonadal axis was involved in 5/8 at diagnosis and 3/5 had normalized at 6 weeks. All patients received high dose steroids at diagnosis with a large range of time to physiological dosing (4-311 days), median 56.

**Conclusion-** Hypophysitis is a common adverse effect, especially with combination ipilimumab and nivolumab. Most hormone deficiencies present at 6 weeks appear to be permanent. It is important to have early endocrinology involvement to ensure appropriate investigation and management.

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## Watch What Happens Neck: A case report of hyperparathyroidism-jaw tumour syndrome in a 35 year old lady

**Andrew Lin<sup>1</sup>, Katherine Benson<sup>1,2</sup>**

1. Concord Hospital, Sydney, NSW, Australia

2. Sydney University Medical School, Sydney, NSW, Australia

### Introduction:

Hyperparathyroidism-Jaw Tumour Syndrome (HPT-JT) is a rare, autosomal-dominant condition caused by inactivating mutations of the *CDC73* gene which encodes the protein parafibromin<sup>1</sup>. It is characterised by hyperparathyroidism, ossifying fibromas of the maxilla and mandible; and lesions of the kidneys and uterus<sup>2</sup>. Penetrance is high with most developing hyperparathyroidism before age 50<sup>3</sup>.



### Case

A 35 year old lady was referred for assessment of primary hyperparathyroidism (Figure 1). Her main symptoms were lethargy and thirst. There was a past history of wrist fracture in her 20's and previous fibroid removal. She had no regular medications. She had 3 children with un-complicated pregnancies. Examination revealed no palpable neck masses or signs of hormonal deficiency, excess or a specific endocrine syndrome. Interestingly, she had a strong family history of parathyroid disorders. Her paternal grandfather had hyperparathyroidism; paternal uncle had parathyroid carcinoma and a paternal cousin had parathyroidectomy for unknown reasons.

### report:

She was referred for parathyroidectomy and a large parathyroid adenoma was excised. Immunohistochemistry staining for parafibromin was negative. Genetic testing confirmed our patient carried a germline mutation in *CDC73* diagnostic of HPT-JT.

OPG and renal ultrasound were normal. Pelvic ultrasound revealed a lower uterine mass 40x30x30mm. She underwent hysterectomy and bilateral salpingectomy which revealed a cervical adenosarcoma. Histological margins were intact and there was no lymphovascular invasion. The adenosarcoma stained negative for parafibromin confirming its relation to HPT-JT.

One year post-parathyroidectomy our patient's calcium and PTH levels remain normal. One of her children also tested positive for *CDC73* mutation. Both our patient and her child undergo regular monitoring with blood tests, OPG and ultrasounds of the kidneys and pelvis.

### Conclusion:

HPT-JT is a rare disorder characterised by hyperparathyroidism and lesions of the jaw, kidneys and uterus. Regular biochemical and radiological monitoring is crucial for the ongoing management of patients with this condition.

	Result	Reference
Sodium	140	135 – 145mmol/L
Potassium	4.7	3.5 – 5.5mmol/L
Chloride	108	95 – 110mmol/L
Bicarbonate	23	20 – 32mmol/L
Urea	5.1	2.5 – 7.0mmol/L
Creatinine	75	45 – 85µmol/L
Corrected Calcium	2.92	2.15 – 2.55mmol/L
Magnesium	0.91	0.65 – 1.0mmol/L
Phosphate	0.85	0.8 – 1.5mmol/L
Albumin	43	37 – 48g/L
PTH	25.1	1.6 – 6.9pmol/L
Vitamin D	79	50 – 140nmol/L

**Ultrasound and Sestamibi:**  
Suggestive of parathyroid adenoma on the **right side** inferior to the thyroid

**DEXA:**  
Hip: Normal  
Distal radius: Osteoporosis range

**Urinary calcium excretion**  
10.4mmol/day

**Normal chromogranin A, gastrin, prolactin, IGF-1, glucose, insulin**

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3. Van der Tuin et al. (2017). *CDC73*-Related Disorders: Clinical Manifestations and Case Detection in Primary Hyperparathyroidism. *Journal of Clinical Endocrinology and Metabolism*. 102 (12), 4534-4540.

### Treatment induced neuropathy of diabetes an under-recognised cause of peripheral neuropathy

**Andrew G Lin<sup>1</sup>, Kay Hau Aaron Choy<sup>1</sup>, Minoli Abeysekera<sup>1</sup>, Kirtan Ganda<sup>1,2</sup>**

1. Concord Hospital, Sydney, NSW, Australia

2. Sydney University Medical School, Sydney, NSW, Australia

**Case:** A 27-year-old female with a seven-year history of type 1 diabetes mellitus (T1DM) was hospitalised and successfully managed for diabetic ketoacidosis (DKA) secondary to persistent nausea, vomiting and poor sick day management. On specific questioning, her vomiting was induced by oxycodone and pregabalin which were being trialled as treatment for a three-month history of worsening, lower back pain and severe distal lower limb, nocturnal neuropathic pain, associated with paraesthesia. Trials of oxycodone, meloxicam and pregabalin were ineffective.

Since her diagnosis, her glycaemic control was poor resulting in multiple episodes of DKA. However, recent lifestyle modifications and better family support resulted in rapid improvement in glycaemic control, evidenced by a reduction in HbA1c from 13.3% to 6.4% within four months.

Examination was consistent with a lower limb sensory neuropathy with reduced sensation to monofilament testing up to the ankles bilaterally. There was no lower limb weakness. There was no focal spinal tenderness and her lumbar flexion was normal.

Investigations for autoimmune, thyroid and nutritional causes of neuropathy were unremarkable. A MRI of the spine did not reveal a cause for the symptoms.

Her symptoms were strongly suggestive of treatment-induced neuropathy of diabetes (TIND). Given pain refractory to pre-existing therapy, duloxetine was commenced and an assessment for consideration of local nerve decompression was arranged.

**Discussion:** TIND is an under-recognised iatrogenic complication of diabetes management with unclear incidence, however may be up to 90% amongst those with a > 5% change in HbA1c over 3 months.<sup>1,2,3</sup> The pain is often severe and difficult to manage with analgesics but usually improves gradually in the long-term.<sup>1</sup> It is also associated with faster progression of retinopathy and microalbuminuria.<sup>3</sup> Awareness of this iatrogenic complication of rapid improvement in glycaemic control is important to reduce the risk of morbidity amongst those with long term poor glycaemic control.

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## Cushing's syndrome caused by ectopic ACTH/CRH production from neuroendocrine transformation of prostate cancer: two case reports

**Andrew G Lin<sup>1</sup>, Veronica Wong<sup>1</sup>, Kirtan Ganda<sup>1</sup>**

*1. Concord Hospital, Sydney, NSW, Australia*

### Introduction:

Ectopic ACTH/CRH production accounts for <5% of cases of Cushing's syndrome. We describe two patients with neuroendocrine small cell transformation of prostate adenocarcinoma leading to ectopic Cushing's syndrome. This is a rare cause of ectopic Cushing's syndrome with only 30 cases reported in the literature.

### Case

An 81-year-old gentleman presented with dyspnoea, lower limb oedema and weight loss over four weeks. He had a history of prostate cancer, which was treated with androgen deprivation therapy. His investigations were consistent with ACTH dependent Cushing's syndrome due to an ectopic source (Table 1).

FDG-PET showed a lesion in the prostatic bed. Biopsy revealed small cell neuroendocrine tumour. He was commenced on metyrapone and ketoconazole therapy for hypercortisolism. He developed brachiocephalic vein thrombosis and hospital acquired pneumonia. He declined palliative treatment, after which he survived a further 26 days before succumbing to his illness 64 days after presentation.

### Case

A 70-year-old gentleman presented with facial and peripheral oedema, and cognitive decline. He had a history of prostate cancer treated with prostatectomy in 2010 followed by salvage radiotherapy and androgen deprivation therapy. He then had bicalutamide therapy but required docetaxel chemotherapy six months before presentation. Investigations were consistent with ACTH dependent Cushing's syndrome due to an ectopic source (Table 2).

CT scan revealed subtle hypodense lesions within the liver; when biopsied they showed high grade neuroendocrine metastases. Metyrapone was trialled unsuccessfully. Chemotherapy normalised cortisol levels but was complicated by pancytopenia and urosepsis. He was then commenced on goserelin. ACTH-dependent hypercortisolism recurred six months later with abiraterone trialled unsuccessfully. A palliative approach was adopted and he died twelve months after presentation.

### Conclusions:

Prostate cancers have the potential for neuroendocrine transformation. These two cases illustrate the difficulties controlling both the underlying malignancy and hypercortisolism.

Table 1. Investigations for Case 1. Cortisol failed to suppress >50% after 8mg dexamethasone suppression testing.

	Value	Reference Range
ACTH	85.7 pmol/L	≤ 10 pmol/L
Cortisol	2,072 nmol/L	170 – 500 nmol/L
Potassium	3.3 mmol/L	3.5 – 5.2 mmol/L
Bicarbonate	38 mmol/L	22 – 30 mmol/L
24 hour urinary free cortisol	5,885 nmol/L	50 – 250 nmol/L
PSA	0.33µg/L	0.25 – 9.00 µg/L

Table 2. Investigations for Case 2. Cortisol failed to suppress after 8mg dexamethasone suppression test.

	Value	Reference Range
ACTH	67.7 pmol/L	≤ 10 pmol/L
Cortisol	2,248 nmol/L	170 – 500 nmol/L
Potassium	3.1 mmol/L	3.5 – 5.2 mmol/L
Bicarbonate	31 mmol/L	22 – 30 mmol/L
PSA	10.4 µg/L	0.25 – 9.00 µg/L

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## Feasibility Comparison of Human Chorionic Gonadotropin (hCG) Combined with Menotropin (hMG) Therapy and Gonadotropin-Releasing Hormone (GnRH) Pump Treatment for Male Adolescents with Congenital Hypogonadotropic Hypogonadism (CHH)

Ying L Liu<sup>1</sup>, Chunxiu G Ggong<sup>1</sup>

1. Beijing Children's Hospital, Beijing, China

**Objective:** The efficacy and safety of human chorionic gonadotropin (hCG)/Menotropin (hMG) and gonadotropin-releasing hormone (GnRH) pump in male adolescents with congenital hypogonadotropic hypogonadism were compared. As a possible clinical treatment program, it was proved being feasibility.

**Methods:** A prospective and non-randomized cohort control trial was conducted in male adolescents congenital hypogonadotropic hypogonadism (CHH) patients. The treatment was divided into study (0-3 months) and extension (3-12 months) periods. The testicular volume (TV), penis length (PL) and diameter (PD), and sex hormone levels were compared between the two groups during the treatment.

**Results:** After 3 months of treatment, the TV of hCG/hMG and GnRH groups increased to 5.1±2.7 mL (from 2.5±1.3 mL) vs 4.6±2.7 mL (from 2.7±1.5 mL), respectively ( $p > 0.05$ ). PL reached to 6.9±1.7cm (from 4.6±1.4cm) vs 5.1±1.6cm (from 4.3±1.4cm) ( $p < 0.05$ ). PD were 2.5±0.5cm (from 1.6±0.4cm) vs 1.9±0.6cm (from 1.5±0.5cm) ( $p < 0.05$ ). Testosterone was 459±163 (from 21.5±8.3) VS 99±95 ng/dL (from 20.7±6.0 ng/dL). Their PL, PD, and testosterone of the hCG/hMG group were still significantly higher than those of GnRH group (18 cases), yet, TV was not different ( $p > 0.05$ ) in the expansion period (after 6-month treatment) in 8 cases. However, except testosterone was still higher in hCG/hMG group ( $p < 0.05$ ), other indices did not show and statistical difference ( $p > 0.05$ ) after 9-month treatment.

**Conclusion:** The hCG/hMG treatment is feasible. The initial 3 months could be used as a window to observe the treatment effectiveness. The long-term effectiveness, strengths, and weaknesses of it require further research.

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## Use of liraglutide and dulaglutide for weight loss: real-world observations on efficacy and tolerability from a community clinic

Stephen Ludgate<sup>1</sup>, Kenneth Ho<sup>1</sup>

1. Department of Endocrinology, Ryde Hospital, Sydney, NSW, Australia

GLP-1 agonists are commonly prescribed for the treatment of T2DM with obesity, but have also shown efficacy in obesity alone. We conducted a retrospective study to examine the efficacy of liraglutide (Saxenda, Victoza) and dulaglutide (Trulicity). We examined weight change, duration of use, prescribed dose, tolerability and whether lifestyle changes were implemented. Results were analysed for combined GLP-1 agonist use, and liraglutide and dulaglutide alone using ANOVA and shown as mean + SEM.

143 patients were prescribed GLP-1 agonists (75% liraglutide, 25% dulaglutide). 64.3% were female, 54.6% had a diagnosis of diabetes and 77.6% were co-prescribed metformin. Liraglutide users were asked to stay on the maximum tolerated dose for as long as possible. Doses were 0.6mg (22%), 1.2mg (37%), 1.8mg (17%), 2.4mg (7%) and 3.0mg (17%). Dulaglutide users received fixed dose 1.5mg weekly. 83.8% of all GLP-1 agonists users experienced reduced appetite while 30.3% reported adverse side effects. Dulaglutide users had less side effects than liraglutide users (8.3%vs37.4%). 63.1% modified their diet, while 17.7% increased exercise. The mean weight loss of patients using dulaglutide was greater than those using liraglutide (6.13kg+0.45 vs. 4.87kg+0.56) over a mean of 5.03+0.29 months compared to 4.89+0.49 months respectively. Patients who used GLP-1 agonists for longer had greater reduction in weight ( $p=0.003$ ). In such patients, the addition of metformin( $p=0.009$ ), appetite reduction ( $p=0.004$ ) and absence of adverse side effects ( $p=0.002$ ) were associated with greater weight loss. Diet modification was also associated with increased weight loss however increased exercise was not.

We recommend liraglutide be used at lower doses to improve patient compliance, treatment duration and efficacy.

## Congenital isolated hypogonadotropic hypogonadism in a cohort of male refugees from the Middle East

**Brendan J Nolan<sup>1,2</sup>, Ada S Cheung<sup>1,2</sup>, Mathis Grossmann<sup>1,2</sup>**

1. Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia

2. Department of Medicine (Austin Health), University of Melbourne, HEIDELBERG, VIC, Australia

**Background:** Congenital isolated hypogonadotropic hypogonadism (ciHH) is a rare condition with an estimated prevalence of 1 in 10,000-100,000. No study has reported clinical features from a cohort from the Middle East. We present the clinical phenotype, laboratory and imaging results of a cohort of male refugees from the Middle East who presented to our Andrology Clinic in Melbourne, Australia.

**Clinical phenotype:** Four men (median age 36 years (range 25-47)) were included (Table 1). Testicular volume measured median 2.5 mL (range 2-10) and all had lack of virilization. One patient had congenital deafness and reported a family history of consanguinity. No patient reported anosmia or had clinical evidence of synkinesia. Minimal trauma fractures were reported in one patient. One patient had a history of coeliac disease but no others had risk factors for osteoporosis. Two patients were treatment naïve, while one had previously received six months of therapy with human chorionic gonadotropin, and another three years of testosterone.

**Laboratory and imaging results:** Median testosterone concentration at initial consultation measured 0.8 (range 0.3-1.3) nmol/L, with undetectable oestradiol concentration (measured by immunoassay), and normal luteinizing hormone 0.5 (range 0-3.6) U/L. Remainder of pituitary hormone testing was unremarkable. Karyotype was 46,XY in three patients tested. Whole exome sequencing was performed in one patient but did not reveal pathogenic mutation. MRI pituitary was normal in three patients with normal CT head in the other. All had osteoporosis on DEXA. Two patients had normal renal tract ultrasound excluding renal agenesis.

**Treatment:** All patients are currently treated with intramuscular testosterone undecanoate. Concurrent intravenous zoledronic acid was prescribed for two patients.

**Conclusion:** In a cohort of men with ciHH, there was evidence of resultant complications from hypogonadism but limited other clinical features. Further longitudinal data will establish the influence of testosterone replacement on outcomes including bone density.

**Table 1:** Baseline patient characteristics

Patient	Country of birth	Age at review	Testosterone (nmol/L)	Oestradiol (pmol/L)	LH (U/L)	Lumbar spine BMD (g/cm <sup>2</sup> )	Femoral neck BMD (g/cm <sup>2</sup> )
1	Syria	47	0.8	<18	<0.1	0.706 (T-score -4.2)	0.712 (T-score -2.8)
2	Iran	25	0.3	<18	0.4	0.811 (T-score -3.4)	0.700 (T-score -2.5)
3	Iraq	44	0.8	<18	0.6	0.667 (T-score -3.5)	0.544 (T-score -2.7)
4	Iraq	29	1.3	<19	3.6	0.873 (T-score -2.9)	0.768 (T-score -2.3)

## Hypoglycaemia with mealtime fast-acting insulin aspart versus insulin aspart across two large type 1 diabetes trials

**David O'Neal<sup>1</sup>, Christophe De Block<sup>2</sup>, Anders Carlson<sup>3</sup>, Ludger Rose<sup>4</sup>, Theis Gondolf<sup>5</sup>, Anders Gorst-Rasmussen<sup>5</sup>, Wendy Lane<sup>6</sup>**

1. University of Melbourne Department of Medicine, St Vincent's Hospital, Melbourne, Victoria, Australia

2. Department of Endocrinology-Diabetology-Metabolism, Antwerp University Hospital, Antwerp, Belgium

3. International Diabetes Center, Minneapolis, Minnesota, USA

4. Institute for Diabetes Research, Münster, Germany

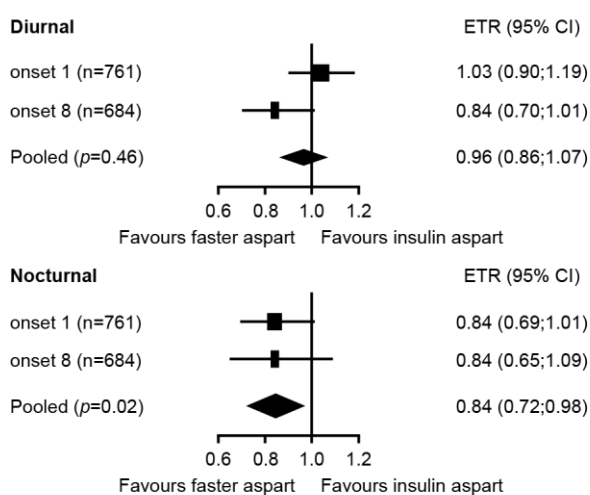
5. Novo Nordisk A/S, Søborg, Denmark

6. Mountain Diabetes and Endocrine Center, Asheville, North Carolina, USA

Hypoglycaemia is a ubiquitous challenge with insulin treatment in type 1 diabetes (T1D), with nocturnal episodes of particular concern. Severe (as defined by the American Diabetes Association) or blood glucose-confirmed (<56 mg/dL [3.1 mmol/L]) hypoglycaemia was investigated across two double-blind, treat-to-target, randomised trials assessing the efficacy and safety of mealtime fast-acting insulin aspart (faster aspart) versus insulin aspart (IAsp) by multiple daily injections in adults with T1D: a 52-week trial in combination with insulin detemir (onset 1; n=761), and a 26-week trial in combination with insulin degludec (onset 8; n=684). Faster aspart was confirmed to be non-inferior to IAsp regarding change from baseline in HbA<sub>1c</sub> in both trials, with a statistically significantly greater HbA<sub>1c</sub> reduction with faster aspart in onset 1. Importantly, nocturnal hypoglycaemia rates were consistently lower with faster aspart versus IAsp in both trials (pooled estimated treatment rate ratio [ETR] 0.84 [95% CI 0.72;0.98];  $p=0.02$ ) (Figure), while no significant difference was observed for overall (pooled ETR 0.94 [95% CI: 0.85;1.05]) and diurnal hypoglycaemia (pooled ETR 0.96 [95% CI 0.86;1.07]) (Figure) with some heterogeneity across trials. In summary, analysis across two large trials supports the safety of mealtime faster aspart, with lower rates of nocturnal hypoglycaemia with faster aspart versus IAsp.

ClinicalTrials.gov: NCT01831765; NCT02500706

**Figure: Diurnal and nocturnal severe or blood glucose-confirmed hypoglycaemic events\***



\*An episode that is severe (requiring assistance of another person to actively administer carbohydrate or glucagon, or take other corrective actions) or blood glucose-confirmed by a plasma glucose value <56 mg/dL (3.1 mmol/L) with or without symptoms consistent with hypoglycaemia.

Pooled ETR and CI is obtained from a fixed-effects meta-analysis. Study duration of onset 1 was 52 weeks and onset 8 was 26 weeks. For onset 1, weighted contribution is 63.5% for diurnal hypoglycaemia and 65.2% for nocturnal hypoglycaemia. For onset 8, weighted contribution is 36.5% for diurnal hypoglycaemia and 34.9% for nocturnal hypoglycaemia.

CI, confidence interval; ETR, estimated treatment rate ratio; faster aspart, fast-acting insulin aspart; N, total number of subjects in faster aspart/insulin aspart arm.

## Hypoparathyroidism after total thyroidectomy with or without lymph node dissection: A retrospective audit of rates, determinants and recovery.

**Michael Papanikolas<sup>1</sup>, Tamara Preda<sup>1</sup>, Peter Campbell<sup>1</sup>**

1. Department of Breast, Endocrine, Head and Neck Surgery, SWSLHD, Liverpool, NSW, Australia

Introduction:

Hypoparathyroidism is characterised by symptoms of hypocalcaemia secondary to inadequate parathyroid hormone (PTH). Acquired hypoparathyroidism is most commonly seen after total thyroidectomy with or without dissection of the central neck nodes (CLND) (1). CLND is thought to confer higher rates of post operative hypoparathyroidism as the inferior parathyroids are at risk of devascularisation. Our aim was to determine institutional rates of temporary and permanent hypoparathyroidism in total thyroidectomy patients.

Methods:

Audit data for 275 consecutive patients undergoing total or completion thyroidectomy between November 2016 - May 2019 in the Department of Endocrine Surgery at Liverpool Hospital NSW, was retrospectively reviewed. Patients with parathyroid pathology to be treated concurrently were excluded. Serum PTH & corrected calcium levels were checked in recovery and on day 1 and 2 post operatively. Results were subdivided into 3 groups:

Normal (PTH in recovery >2.0 pmol/L) [RR 2.0-6.0pmol/L]

Transient hypoparathyroidism (PTH Day 0 <2.0 pmol/L ; PTH Day 2 normal)

Hypoparathyroid (PTH Day 0 & 2 below normal).

The hypoparathyroid cohort had serial PTH and corrected calcium measured.

Clinicopathologic characteristics including extent of nodal surgery were compared between groups.

Results:

110 patients (40%) were hypoparathyroid at Day 0; one third (37 patients) had recovered by Day 2 and were considered transiently affected. 73/275 (26.5%) were discharged on treatment for hypoparathyroidism.

Follow-up data was available for 62/73 patients; 15 (5.7%) had persistent hypoparathyroidism over the following 3 months. 53% of these patients had undergone lymph nodes dissection compared to 19% in the normal group.

5/14 patients (35.7%) who had unilateral CLND and 2/3 who had bilateral CLND were hypoparathyroid at 3 months.

Conclusion:

Despite variable techniques employed for parathyroid preservation an overall low rate of chronic hypoparathyroidism is displayed. CLND is a strong risk factor for hypoparathyroidism.

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## Oma, oedema, cerebrovascular trauma- an enigma to fathom: Protean presentations of hypercatecholaminism

**Smitha S Rao<sup>1</sup>, Dhalapathy Sadacharan<sup>1</sup>, Ferdinant Jabamalai<sup>1</sup>**

1. Madras medical college, Chennai, Tamilnadu, India

**Background:** Non secretory adrenal adenomas constitute majority of adrenal incidentalomas. 5% of these are pheochromocytomas (PCC) with the classical triad of headache, palpitations and diaphoresis. Atypical presentations are seen in 9-10% of these patients.

**Aim:** To highlight unusual presentations with the difficulties associated with diagnosis and management.

**Materials and methods:** Out of a total of 18 cases of PCC/ paragangliomas (PGL), over a period of 2 years from June 2017 to 2019, we report a series of 6 unusual cases. The diagnostic pitfalls and difficult management strategies have been described.

**Results:** Our series had 2 males and 4 females. The varied presentations in our cases were: acute intestinal pseudoobstruction, right sided neck mass, colossal adrenal incidentaloma, right hemiparesis with acute coronary events, congestive cardiac failure and a rare abdominal infrarenal tumour. Amongst the 6 cases, 3 of them had normal blood pressure. Normotensive PCC are rare entities, described in a few studies. All of our cases had a biochemical and histopathological confirmation of PCC/PGL with surgical excision and cure. PCC presenting with intestinal pseudoobstruction and a tumour of 20x16 cm were the rarest of them all, which have been only reported anecdotally.

**Conclusion:** This series reconfirms the masquerading nature of pheochromocytomas. Two extremely rare presentations were the highlights of our study. A keen suspicion and adequate preoperative evaluation dictates management in such cases. Diagnostic and management protocols may vary based on the presentation contributing for a learning experience.

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## Patterns of illness and pre-hospital management in patients with Addison's Disease with and without Type 1 Diabetes Mellitus attending a Sydney Hospital

**Brienna Mortimer<sup>1</sup>, Vaidehi Naganur<sup>1</sup>, Paul Satouris<sup>1</sup>, Jerry Greenfield<sup>2</sup>, David J Torpy<sup>3</sup>, Louise Rushworth<sup>1</sup>**

1. The University of Notre Dame, Sydney, Sydney

2. St Vincent's Health Australia, Sydney

3. Royal Adelaide Hospital, Adelaide

### Background

Patients with Addison's disease (AD) and comorbid Type1 diabetes mellitus (T1DM) are at increased risk of acute metabolic disorders relative to patients with a single condition. The reasons for this are unknown.

### Methods

All attendances for a medical illness by AD patients at the emergency department of a Sydney hospital between 2000 and 2017 were reviewed. Physiological parameters and illness management strategies were compared between AD patients, those with T1DM and AD combined, and a control group of patients with T1DM who were matched to the diabetic AD patients by age and sex.

### Results

Of the 46 AD hospital attendances for acute medical problems, six (13.0%) were due to missed medication/non-compliance/acute alcohol intoxication and one to excessive exercise. The remaining 39 presentations represented 20 AD (28 attendances) and 5 AD/T1DM patients (11 presentations). There were 17 (43.6%) diagnosed adrenal crises (63.6% (n=7) in AD/T1DM and 35.7% (n=10) in AD only) and stress doses preceded 61.5% (n=24) attendances. Four patients used intramuscular hydrocortisone. Patients who used stress doses had a history of more presentations than those who did not (2.0+/-1.3 vs 1.2+/-0.5, p=0.01). AD/T1DM patients had more hypoglycaemia (27.3% vs 0%, p<0.05); fever 54.5% vs 14.3%, p<0.05; and infection (63.6% vs 46.4%, p=ns). There were 40 presentations for 11 T1DM control patients, none with diabetic ketoacidosis. More control patients had hyperglycaemia (62.5% vs 18.2%, p<0.01) and fewer had hypoglycaemia (10.0% vs 27.3%, p=NS) than diabetic AD patients.

### Conclusion

Patients with AD develop acute illness due to a range of issues, including medication non-adherence and psychosocial factors. More prior hospital presentations increased stress dose use. Patients with combined disease had 7/11 presentations with an

AC diagnosis. They also had a higher incidence of hypoglycaemia than AD only patients and a lower incidence of hyperglycaemia than T1DM control patients.

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## Targeting inpatient rehabilitation units to improve antiresorptive therapy administration rates after hip fracture

**Matthew Sawyer<sup>1</sup>, Ie-Wen Sim<sup>1,2,3</sup>**

1. Endocrinology, Eastern Health, Melbourne, VIC, Australia

2. Endocrinology, Monash Health, Melbourne, VIC, Australia

3. Endocrinology, Western Health, Melbourne, VIC, Australia

**Background:** Acute osteoporotic hip fractures are a major and increasing burden in Australia and confer a high risk for further fragility fractures. Despite recommendations from the Australian Hip Fracture Care Clinical Care Standard, the rate of administration of antiresorptive therapy (ART) prior to discharge from hospital remains poor, leaving ongoing opportunity for strategies to improve care.

**Objectives:** To examine whether targeted education would result in higher rates of administration of ART in patients admitted to rehabilitation units following an osteoporotic hip fracture.

**Methods:** An audit of patients with an osteoporotic hip fracture discharged from rehabilitation units at Eastern Health between October 2018 and May 2019 inclusive was conducted. An educational session encouraging administration of ART prior to discharge as per the Australian Hip Fracture Care Clinical Care Standard was provided to the rehabilitation team in March 2019. Rates of consideration and administration of ART were compared before and after the intervention.

**Results:** 133 cases of osteoporotic hip fracture were identified, including 99 in the pre-intervention period and 34 thereafter. In the pre-intervention period, ART was considered in 55 patients (55.6%) and 35 patients (35.3%) continued or received treatment prior to discharge. In the first two months after the educational intervention, there was no demonstrable improvement in the number of patients considered for or being the recipient of ART, with 18 patients considered for treatment (OR 0.9,  $p=0.79$ ) and 7 patients receiving or continuing treatment (OR 0.47,  $p=0.11$ ). Documented reasons for not receiving ART included vitamin D deficiency, deferral of treatment decisions until outpatient review and patients declining treatment.

**Conclusion:** Interim analysis of this ongoing project did not demonstrate improvement in rates of administration of ART post-hip fracture after implementation of a brief educational intervention. Understanding the factors that contribute to patients not receiving treatment will identify targets for future interventions.

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## Rate and Extent of Testicular Function Recovery After Ceasing Androgen Abuse

**Nandini Shankara Narayana<sup>1,2,3</sup>, Sasha Savkovic<sup>2</sup>, Reena Desai<sup>1</sup>, Carolyn Fennell<sup>2</sup>, Leo Turner<sup>2</sup>, Veena Jayadev<sup>2</sup>, Ann J Conway<sup>2,4</sup>, Christopher Yu<sup>5</sup>, Leonard Kritharides<sup>5,4</sup>, David Handelsman<sup>1,2,4</sup>**

1. Andrology, ANZAC Research Institute, Concord, NSW, Australia

2. Andrology, Concord Repatriation General Hospital, Concord, NSW

3. Concord and Central Clinical School, University of Sydney, Sydney, NSW, Australia

4. Concord Clinical School, University of Sydney, Sydney, NSW, Australia

5. Cardiology, Concord Repatriation General Hospital, Concord, NSW, Australia

Supraphysiological androgen administration suppresses testicular functions causing reduced sperm output and testosterone secretion; however, the rate and extent of testicular function recovery after cessation is not reported. We undertook a cross-sectional, observational study of current and past androgen abusers together with healthy non-user controls to determine the rate and extent of recovery of sperm output and reproductive hormones after cessation of androgen intake.

We recruited (via social media) age-matched (mean 34 years), regularly exercising volunteers comprising 41 current and 31 past users ( $\geq 3$  months (median 300 days) since last use) with 21 healthy non-user controls. Each underwent physical examination and provided serum (steroids by LC-MS; LH, FSH, SHBG, hematology and biochemistry) and semen sample (WHO). Current users, compared with past and non-users, had significant suppression of mean (orchidometry) testicular volume (TV, 14.3, 18.6, 23.2 ml), sperm output (excluding 6 vasectomized men, median 4, 215, 203 million/ejaculate) and mean serum LH (0.5, 5.5, 5.2 IU/L), FSH (0.5, 4.7, 4.9 IU/L), SHBG (17.2, 33.9, 42.0 nmol/L) and had significant increases in serum T (133.6, 21.5, 29.8 nmol/L), DHT (1.5, 0.5, 0.7 ng/ml), E2 (146, 41, 48 pg/ml), E1 (65, 32, 38 pg/ml), 3 $\alpha$ -androstenediol (2.2, 0.4, 0.6 ng/ml), hemoglobin (164, 154, 151 g/l). All but TV and SHBG were not significantly different between past and non-users consistent with full recovery. Rate of recovery for androgen-suppressed variables (estimated as the time to reach the mean for non-users), was 9 months for serum LH, 14.2 months for sperm output and 18.7 months for serum FSH.

We conclude that suppressed testicular function due to androgen abuse is mostly reversible with recovery taking 9 to 18 months after ceasing androgen intake. Suppressed serum LH and FSH represent convenient, useful and underutilized markers of androgen abuse and recovery.



## Severe hypertriglyceridemia-associated acute pancreatitis: a prospective study of incidence and management.

**Hong Lin Evelyn Tan**<sup>2,1</sup>, **Georgina McDonald**<sup>1,3</sup>, **Alexander Payne**<sup>3</sup>, **William Yu**<sup>3</sup>, **Zahrul Ismadi**<sup>4</sup>, **Amanda Caswell**<sup>1,4</sup>, **Huy Tran**<sup>1,4</sup>, **Jon Gani**<sup>1,5</sup>, **Katie Wynne**<sup>2,1</sup>

1. School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

2. Department of Diabetes & Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

3. Department of Medicine, John Hunter Hospital, New Lambton Heights, NSW, Australia

4. Department of Clinical Chemistry, John Hunter Hospital, New Lambton Heights, NSW, Australia

5. Department of Surgery, John Hunter Hospital, New Lambton Heights, NSW, Australia

**Introduction** Hypertriglyceridemia-associated acute pancreatitis (HTGAP) is thought to account for 2-6% of acute pancreatitis (AP) and has been associated with increased severity and morbidity.<sup>1</sup> Despite being the third most common cause of AP, triglyceride levels are not routinely measured. This study prospectively measured triglyceride levels to establish incidence in a tertiary Australian centre.

**Aims and Methods** A prospective cohort was collated over 18 months in a tertiary referral hospital. Adults with AP had admission triglyceride levels measured if lipase was 3x the upper reference limit. HTGAP was defined as AP with triglycerides >11.1mmol/L.<sup>1,2</sup> Incidence, severity and management strategies were recorded.

**Results** Of 301 episodes of AP, 253(84%) had triglycerides measured and were included. HTGAP was diagnosed in 10 of 253(4%) AP cases. Type 2 diabetes (n=6) and overweight status (BMI>25kg/m<sup>2</sup>, n=6) were common. Alcohol misuse (n=4) and gallstones (n=3) may have contributed to AP. Severe hypertriglyceridemia was present in the HTGAP group (49.0±10.2 vs. 1.8±0.1mmol/L). Despite their age (49±2 vs. 54±1years, p=0.36), the HTGAP group had longer hospital admissions (8.0±1.9 vs. 5.4±0.7days, p=0.03). ICU admissions were significantly increased (OR 15, 95% CI 4-58) in the HTGAP group (5/10 vs. 15/243 admissions, p<0.001) and constituted 25%(5/20) of total ICU admissions for AP. All patients were managed with a low-fat (<30g) diet. Four patients received intravenous insulin with resolution of hypertriglyceridemia over 3-5 days; one patient with diabetes had mild hypoglycaemia requiring 10% dextrose infusion. Oral therapy was commenced prior to discharge (statin n=7, fibrate n=5).

**Conclusion** HTGAP occurred in 4% of AP cases and was associated with high risk of ICU admission and longer length of stay. Intravenous insulin results in a rapid reduction of triglyceride levels.<sup>2</sup> Plasmapheresis, albeit efficacious in reducing triglyceride levels, has complications and is not recommended.<sup>2</sup> A novel management protocol is proposed for testing in future studies.

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## Perinatal mental health in women with polycystic ovary syndrome

**Chau Thien Tay**<sup>1,2</sup>, **Helena Teede**<sup>1,2</sup>, **Jacqueline Boyle**<sup>1</sup>, **Jayashri Kulkarni**<sup>3</sup>, **Deborah Loxton**<sup>4</sup>, **Anju Joham**<sup>1,2</sup>

1. Monash Centre for Health Research and Implementation, Clayton, VIC, Australia

2. Department of Diabetes and Vascular Medicine, Monash Health, Clayton, VIC, Australia

3. The Monash Alfred Psychiatry Research Centre, Monash University and The Alfred Hospital, Melbourne, Victoria, Australia

4. Research Centre for Generational Health and Ageing, University of Newcastle, Callaghan, New South Wales, Australia

**Background:** Women with polycystic ovary syndrome (PCOS) have many risk factors associated with perinatal mental disorders such as pre-existing mental health disorders, infertility and obstetric complications. However, little is known about perinatal mental health in women with PCOS. We aimed to examine the prevalence of common perinatal mental disorders in women with PCOS and study the relationship between PCOS and common perinatal disorders.

**Methods:** A cross-sectional study was performed on 5239 women (PCOS: n=436; non-PCOS: n=4803) born between 1973-78 from the Australian Longitudinal Study on Women's Health. Main outcomes measured were self-reported antenatal depression, antenatal anxiety, postnatal depression and postnatal anxiety. Main exposure was self-reported PCOS status. Other important factors examined included body mass index, reproductive history (miscarriage, infertility, assisted reproductive treatment etc.) and obstetric complications (gestational diabetes, hypertension in pregnancy, pre-term labour, small or large for gestational age etc.).  $\chi^2$  tests were used to examine the differences in prevalence between groups whereas logistic regression analyses were performed to examine the relationship between PCOS and perinatal mental disorders.

**Results:** Compared to women not reporting PCOS, women reporting PCOS had higher prevalence of any perinatal mental disorders (33.5% vs 23.8%), antenatal depression (8.9% vs 4.4%), antenatal anxiety (11.7% vs 5.6%), postnatal depression (26.8% vs 18.6%) and postnatal anxiety (18.4% vs 12.0%). PCOS was significantly associated with increased odds for perinatal mental disorders in crude analysis (OR 1.6, 95% CI 1.3-2.0) and after controlling for sociodemographic and lifestyle factors, body mass index, reproductive history and obstetric complications (adjusted OR 1.5, 95% CI 1.2-2.0).

**Conclusion and relevance:** There is a high prevalence of common perinatal mental disorders in PCOS. Aligned with recent international guidelines, increased awareness and screening for mental health disorders is important in PCOS and here we emphasize the importance of this during the perinatal period.

## Tumour localisation of pheochromocytoma or paraganglioma based on plasma metanephrines

**Danielle Tu<sup>1</sup>, Hamish Russell<sup>1</sup>**

*1. Diabetes and Endocrinology, Liverpool Hospital, Liverpool, NSW, Australia*

### Background

Pheochromocytomas (PHE) and paragangliomas (PPL) are rare catecholamine-producing neuroendocrine tumours that arise from chromaffin cells within or outside of the adrenals respectively.<sup>1</sup> Based on previous observations, PPL predominantly secrete noradrenaline (noradrenergic phenotype) while adrenaline-secreting (adrenergic phenotype) tumours are usually confined to the adrenals.<sup>1-3</sup> Further studies indicate that half of PHE have an adrenergic phenotype while the other half produce a variable mixture of adrenaline and noradrenaline.<sup>1-4</sup> Patterns of increases in their metabolites can help predict tumour location.<sup>1,2</sup> Significant elevations in metanephrines are invariably due to an adrenal tumour.<sup>1,2</sup> Although isolated increases in normetanephrine cannot predict tumour location, when accompanied with a considerable rise in methoxy-tyramine, the tumour is almost always extra-adrenal.<sup>1</sup>

### Methods

We analysed plasma metanephrines of 20 patients with PHE or PPL and determined their phenotype by calculating the percentage increase of plasma metanephrines. An increase in plasma free metanephrines >10% of the combined increases in normetanephrines and metanephrines were consistent with an adrenergic phenotype while negligible increases <10% were consistent with a noradrenergic phenotype.<sup>2</sup>

### Results

13 out of 20 patients were diagnosed with PHE. 6 out of 13 (46%) patients with PHE have an adrenergic phenotype and the other 7 (54%) patients presented with a noradrenergic phenotype. 6 of 7 (86%) patients with PPL have a noradrenergic phenotype.

### Conclusion

Measurement of plasma metanephrines not only assist in diagnosing PHE and PPL, but can help predict its location. Our results are consistent with the current literature.

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## Body fat loss after bariatric surgery in Sri Lankan adults

**Udai Wijetunga<sup>1</sup>, Uditha Bulugahapitiya<sup>1</sup>, Thejana Wijeratne<sup>2</sup>, Gowri Ratnayake<sup>1</sup>, Milanka Wategama<sup>1</sup>, Anuradha Jayasuriya<sup>1</sup>, Somasundaram Praveenan<sup>1</sup>, Asela Gunawardena<sup>1</sup>**

*1. Endocrinology and Diabetes Unit, Colombo South Teaching Hospital, Colombo, Sri Lanka*

*2. Department of Surgery, Colombo South Teaching Hospital, Colombo, Sri Lanka*

Increased body fat percentage is related to increased cardiovascular mortality and this effect is more pronounced in South Asians. Bariatric surgery is one of the most effective interventions to lose excess body fat in obese patients. In this study we aimed to assess the reduction of body fat after bariatric surgery among obese Sri Lankans. We did a retrospective analysis of medical records of 192 obese patients who underwent bariatric surgery at Colombo South Teaching Hospital, Sri Lanka. Body fat percentage was recorded by bioelectrical impedance analysis. Percentage body fat loss (PBFL) was calculated to assess the body fat content in the body weight lost. The PBFL was calculated at each follow up visit by the following formula, (loss of body fat weight / loss total body weight) x100. Overall 75.5% were females. The mean age was 38.2 (±10.2) years. Mean body weight and body mass index were 114.6 (± 22.4) kg, and 45.0 (± 6.7) kg/m<sup>2</sup> respectively. At 18 months after surgery, the mean body weight loss was 30.6 (± 9.1) kg. At 1 month, 3 months, 6 months, 12 months and 18 months after bariatric surgery, the mean body fat weight loss was 6.0 (±6.0) kg, 11.5 (±6.8) kg, 17.0 (±6.7) kg, 20.1 (±7.2) kg and 21.3 (±7.4) kg respectively. The PBFL at these time intervals were 62.3%, 65.9%, 68.5%, 66.2% and 67.3% respectively. In conclusion bariatric surgery resulted in a progressive loss of body fat with a mean body fat weight loss of 21.3 kg at 18 months. There was a consistent PBFL of almost 70% meaning that the patients lost almost 700 g of body fat per every 1 kg of body weight lost. Thus bariatric surgery seems to be a very effective intervention in reducing body fat in obese Sri Lankans.

### Sri Lankan men lose more weight in the short term than women after bariatric surgery

**Udai Wijetunga<sup>1</sup>, Uditha Bulugahapitiya<sup>1</sup>, Thejana Wijeratne<sup>2</sup>, Gowri Ratnayake<sup>1</sup>, Milanka Wattedgama<sup>1</sup>, Anuradha Jayasuriya<sup>1</sup>, Somasundaram Praveenan<sup>1</sup>, Asela Gunawardena<sup>1</sup>**

1. *Endocrinology and Diabetes Unit, Colombo South Teaching Hospital, Colombo, Sri Lanka*

2. *Department of Surgery, Colombo South Teaching Hospital, Colombo, Sri Lanka*

Bariatric surgery is the most effective established intervention for weight loss in higher grades of obesity. Bariatric surgery is associated with approximately 30% body weight loss. Weight loss is associated with cardiovascular risk reduction especially in patients with higher grades of obesity. Thus early weight loss after an intervention may result in an early reduction of cardiovascular risk especially in patients at a higher risk. The effect of gender on weight loss after bariatric surgery is not clear and there is conflicting evidence. In this study we aimed to assess effect of gender on short term weight loss among Sri Lankan obese patients undergoing bariatric surgery. We did a retrospective analysis of medical records of 192 obese patients who underwent bariatric surgery at Colombo South Teaching Hospital, Sri Lanka. Overall 75.5% were females. The mean age was 38.2 ( $\pm 10.2$ ) years. Mean body weight and body mass index were 114.6 ( $\pm 22.4$ ) kg, and 45.0 ( $\pm 6.7$ ) kg/m<sup>2</sup> respectively. Males lost more body weight than females at 1 week (14.0 vs 6.8 kg,  $p < 0.001$ ), 1 month (15.4 vs 9.8 kg,  $p < 0.001$ ), 3 months (27.3 vs 17.3 kg,  $p < 0.001$ ) and 6 months (34.2 vs 24.2 kg,  $p < 0.005$ ). The trend was similar for loss of body weight percentage at 1 week (9.5% vs 6.4%,  $p < 0.001$ ), 1 month (11.5% vs 9.2%,  $p = 0.001$ ), 3 months (19.6% vs 16.2%,  $p < 0.001$ ) and 6 months (25.4% vs 22.7%,  $p = 0.05$ ). In conclusion, Sri Lankan males lose significantly more weight than females during the first 6 months after bariatric surgery. At 6 months men lose an extra 10 kg when compared to their female counterparts. Thus Sri Lankan males may gain more cardiovascular and other health benefits in the short term than females due to bariatric surgery.

### Vitamin D deficiency and insufficiency among obese Sri Lankans undergoing bariatric surgery

**Udai Wijetunga<sup>1</sup>, Uditha Bulugahapitiya<sup>1</sup>, Thejana Wijeratne<sup>2</sup>, Gowri Ratnayake<sup>1</sup>, Milanka Wattedgama<sup>1</sup>, Anuradha Jayasuriya<sup>1</sup>, Somasundaram Praveenan<sup>1</sup>, Asela Gunawardena<sup>1</sup>**

1. *Endocrinology and Diabetes Unit, Colombo South Teaching Hospital, Colombo, Sri Lanka*

2. *Department of Surgery, Colombo South Teaching Hospital, Colombo, Sri Lanka*

Vitamin D deficiency is common among South Asians with prevalence rates ranging from 30% to 90%. It is especially prevalent among obese individuals. Vitamin D deficiency causes osteoporosis leading to fragility fractures. It is also implicated in development of cardiovascular disease and cancer. In this study we aimed to assess the prevalence of vitamin D deficiency and insufficiency among Sri Lankan obese patients undergoing bariatric surgery. We did a retrospective analysis of medical records of 80 obese patients who underwent bariatric surgery at Colombo South Teaching Hospital, Sri Lanka. Overall 85.0% were females. Vitamin D deficiency was defined as 25-hydroxyvitamin D level less than 20 ng/ml. Vitamin D insufficiency was defined as 25-hydroxyvitamin D level between 20 ng/ml and 30 ng/ml. Vitamin D sufficiency was defined as 25-hydroxyvitamin D level more than 30 ng/ml. The mean age was 39.1 ( $\pm 10.6$ ) years. Mean body weight and body mass index were 114.6 ( $\pm 23.4$ ) kg, and 45.9 ( $\pm 7.3$ ) kg/m<sup>2</sup> respectively. Mean 25 hydroxyvitamin D level was 17.0 ( $\pm 7.5$ ) ng/ml. Overall 72.5% of patients were vitamin D deficient ( $< 20$ ng/ml) while 23.8% were vitamin D insufficient (20-30 ng/ml). Only 3.7% of patients were vitamin D sufficient. There was no significant difference in vitamin D levels among males and females (18.0 vs 16.8 ng/ml,  $p = 0.62$ ). Age of patient, body weight or BMI were not associated with vitamin D levels. In conclusion, vitamin D deficiency is extremely common among obese Sri Lankan patients undergoing bariatric surgery with 96.3% of individuals being either vitamin D deficient or insufficient. Thus it is essential to check vitamin D levels in patients undergoing bariatric surgery and to correct the deficiency preoperatively. In addition, obese Sri Lankans should be considered for routine screening for vitamin D deficiency due to the very high prevalence rate in this population.

### Spaced education; a novel tool to teach junior doctors insulin management.

**Ayanthi Wijewardene<sup>1</sup>, Christopher White<sup>2</sup>**

1. *Department of Endocrinology, Concord Hospital, Concord, NSW, Australia*

2. *Department of Endocrinology, Prince of Wales Hospital, Randwick, NSW, Australia*

Diabetes is the fastest growing chronic condition in Australia. In 2015-2016 the Australian Institute of Health and Welfare found 1,053,700 patients were hospitalised with diabetes as an additional diagnosis and 50,000 hospitalisations as the principal diagnosis. Traditionally, insulin education of junior doctors occurs in a didactic form often limited to intern orientation. Junior doctors are not confident in managing insulin with George et al finding only 18% of junior doctors were comfortable adjusting diabetic medications pre-operatively.

Spaced education is an online education form that uses repetition of core principles often in multiple-choice questions. Forty-three doctors from a tertiary hospital were enrolled to participate in a spaced education program which involved 15 questions covering common inpatient insulin management issues. Two questions were provided in each session and two sessions were given each week. Questions were repeated after 7 days if answered incorrectly and after 13 days if answered correctly. Two attempts of the question had to be answered correctly before a question was retired and a maximum of three attempts were given to each question.

The program had an 83.7% participation rate. In all categories participants improved their knowledge with repeat questioning except on insulin management in TPN which had initial correct response of 100%. The greatest improvement occurred in learning types of insulin and insulin management in steroid therapy. Engagement levels varied depending on the level of training of the participant. A survey of participants found the program easy to use.

Our project shows the utility of online education tools to assist in education of junior doctors. As technology improves strategies to improve doctors knowledge needs to be adapted. We have found that a spaced education online tool is effective and easy to use in the education of insulin for junior doctors.

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## Hyponatraemia as a surrogate marker for frailty and geriatric syndrome: a study in an Australian regional hospital

**Ru H New<sup>1</sup>, Daryl H Wong<sup>2</sup>, Jim H Wong<sup>3</sup>, Shirantha Adikari<sup>3</sup>**

1. *University of Sydney, Sydney, NSW, Australia*

2. *Medicine, University of Queensland, Brisbane, QLD, Australia*

3. *Cairns Hospital, Redlynch, QLD, Australia*

**Background:** Hyponatremia is the most common electrolyte imbalance in Australian hospitals today<sup>1-3</sup>, particularly with respect to the geriatric population.<sup>1,2,4</sup> Although mild hyponatremia is usually asymptomatic,<sup>1</sup> studies have inferred that hyponatremia is associated with increased falls,<sup>1,5</sup> frailty,<sup>6</sup> delirium,<sup>2</sup> dementia,<sup>3</sup> and mortality.<sup>1,6</sup> Currently, an objective measurable marker of frailty has not been found.

**Aim:** To investigate the prevalence of hyponatremia in the geriatric population as well as its impact on frailty and geriatric syndrome.

**Methods:** Retrospective study of 235 patients aged 70 and above who were admitted in the Geriatric Unit in Cairns Hospital from 1<sup>st</sup> January 2018 to 30<sup>th</sup> June 2018. Comparison were made between patients with normal serum sodium, with those with mild, moderate and severe hyponatremia in several aspects of geriatric syndrome. Data collated was analysed using Microsoft Excel and presented in mean values, frequencies and percentages. Student t-test and N-1 Pearson's Chi-squared test<sup>7,8</sup> were used to determine statistical significance.

**Results:** Patients with hyponatremia were associated with higher CSHA (Canadian Study of Health and Aging) Clinical Frailty Scale scores<sup>9</sup> (mean 3.79+/-1.79 in patients with normal sodium versus 4.29+/-1.75, p=0.04 in patients with hyponatraemia), history of falls (61.04% versus 74.68%, p=0.03), and prolonged hospital admission (average 21.48 days +/-18.51 versus 31.43 days +/-18.31, p<0.001). There also appeared to be a mild increase risk of delirium in patients with hyponatremia (31.17% versus 41.77%, p=0.12).

**Conclusion:** Hyponatremia is associated with several aspects of geriatric syndrome. These findings support the use of hyponatremia as a potential surrogate marker of frailty and geriatric syndrome with further implications in prognostic capabilities.

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## Efficacy and Cost Effectiveness of Thyroid Function Tests – An Analysis of Outcomes in the Geriatric wards of a Secondary Hospital in Regional Australia

**Jim Wong<sup>1</sup>, Shirantha Adikari<sup>1</sup>, Ru H New<sup>2</sup>**

1. Geriatrics, Cairns Hospital, Cairns, QLD, Australia

2. University of Sydney, Sydney, NSW, Australia

**Introduction:** Thyroid function tests (TFT's) incorporating Thyroid Stimulating Hormone (TSH) and Free Thyroxine (fT4) are some of the most commonly ordered investigations in Geriatric wards. The cost of such clinical practice is currently costing Medicare \$203 million per year with an average growth of 6.2% per annum. (1)

**Aim:** To analyze current practice and determine if TFT's (TSH and fT4 testing) are related to any trends in clinical efficiency, health outcomes and cost effectiveness for both patients and the health service.

**Method:** Retrospective study of 329 admissions between 1<sup>st</sup> January 2018 to 30<sup>th</sup> June 2018 were studied. 10 variables were analysed and 4 outcomes were measured. Partition modelling was used to identify variables which could significantly affect outcomes and chi square analysis was utilized to determine statistical significance.

**Results:** Total of 329 admissions met inclusion criteria (mean age 80.2, SD = 9.6, male = 45%, n = 148). 219 had TFT's tested at some stage during their admission, of which, 0.45% (n = 1) were positive for true hypothyroidism (TSH > 4.5 mU/L and fT4 <7 pmol/L) while 10% (n = 22) resulted in subclinical hypothyroidism (TSH > 4.5 mU/L but fT4 within normal range). Of the positive tests, 42% already had a diagnosis of hypothyroidism, and only 13.6% stimulated a change in management. Overall, only 0.30% of the cohort was deemed to have benefited from TFT screening within the 6-month study period.

**Conclusion:**

Our recommendation is that TFT's as a routine screening test in an inpatient setting are not a cost effective measure. Number needed to diagnose was 330 tests, resulting in \$11,484 per patient with hypothyroidism. Emphasis should be placed on history and clinical examination, prior to ordering further investigations to confirm or rebuke a clinical diagnosis, as opposed to routine screening.

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## **Trabecular bone score is reduced in thalassaemia major**

**Nisal Punchihewa<sup>1</sup>, Phillip Wong<sup>2,3,4</sup>, Anne Trinh<sup>2,3,4</sup>, Frances Milat<sup>2,3,4</sup>**

1. Clinical School, Monash University, Clayton, Vic, Australia

2. Hudson Institute, Clayton, VIC, Australia

3. Department of Endocrinology, Monash Health, Clayton, VIC, Australia

4. Department of Medicine, Monash University, Clayton, VIC, Australia

Thalassaemia major (TM) is a condition of ineffective erythropoiesis requiring chronic transfusion therapy. This leads to severe iron overload and requires concomitant iron chelation therapy to reduce end-organ complications. Osteoporosis, kidney stones and hypercalciuria are common and major cause of morbidity. Trabecular bone score (TBS) is a measure of bone texture and bone microarchitecture at the lumbar spine.

We conducted a retrospective cross-sectional study of patients with thalassaemia major at Monash Health, the centre for haemoglobinopathy in Australia. We investigated bone parameters using Dual energy X-ray absorptiometry (DXA) and TBS and documented fracture prevalence. Clinical and biochemical risk factors for bone disease were confirmed through the medical records. Non-parametric data was analysed using Mann U Whitney and logistic and linear regression models.

We analysed 71 subjects with TM receiving deferasirox (n=59) and deferoxamine (n=12). The mean age was 44.4±10 (years) and 43% were male. TBS scores (L2-4) were reduced compared to aged matched controls<sup>(1)</sup>, male: 1.24 Z-scores at all sites were reduced: lumbar spine: -1.75 (-4.6 to 1.7), femoral neck: -1.10 (-4.8 to 1.0) and total body: -1.0 (-4.4 to 1.5). Fractures were confirmed in 28% of subjects: non-vertebral (27.7%) vs spinal fractures (13.9%). Lumbar spine and femoral neck Z scores were significantly associated with fractures (P<0.05) but TBS was not.

Kidney stones were highly prevalent (40.6%) and spot urine calcium/creatinine ratio was elevated 0.95 (0.05 to 9.1) (Normal <0.5). Kidney stones were associated with non-vertebral fractures (P=0.03) and lowTBS (P=0.006) after adjusting for urine calcium/creatinine ratio. Conventional DXA parameters were not significantly associated with kidney stones.

Abnormal bone microarchitecture, as measured by TBS is present in thalassaemia major. The significant association between kidney stones and TBS deserves further study given the established links between hypercalciuria, kidney stones and osteoporosis in thalassaemia major.

## Harnessing electronic health record data to optimise screening and management of diabetes mellitus in patients with acute myocardial infarction (AMI)

Linda Wu<sup>1</sup>, Charmaine S Tam<sup>2,3</sup>, Richard W Morris<sup>2,3</sup>, Aldo Saavedra<sup>3</sup>, Jonathan Morris<sup>2,4,5</sup>, Gregory Fulcher<sup>6,2</sup>

1. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Northern Clinical School, University of Sydney, Sydney, NSW, Australia

3. Centre for Translational Data Science, University of Sydney, Sydney, NSW, Australia

4. Clinical and Population Perinatal Health Research, Kolling Institute of Medical Research, St Leonards, NSW, Australia

5. Department of Obstetrics and Gynaecology, Royal North Shore Hospital, St Leonards, New South Wales, Australia

6. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

### Introduction

Studies have demonstrated that patients with diabetes mellitus (DM) presenting with acute myocardial infarction (AMI) have higher risk of mortality, cardiac failure and readmission in comparison to non-diabetic patients<sup>1,2</sup>. 'Big data' from electronic health records (eMR) has been used extensively in overseas registries but data in the Australian setting is limited.

### Aim

To characterise the current screening, management and outcomes of patients with DM presenting with chest pain in a local health district (LHD) using data extracted from eMR.

### Methods

Patients presenting with chest pain to emergency departments within the LHD from April to June 2017 were included in preliminary analysis. AMI was defined as ICD10 code STEMI/NSTEMI (I21.0-4). DM was defined as HbA1c>6.5% or DM medication on discharge or the presence of "diabetes" and related terms in any clinical documentation.

### Results

From 25,984 presentations of with chest pain, 365 patients had AMI (226 males; mean age= 75y). Prevalence of diabetes was 26% (101/365). 67% (68/101) had pre-existing diabetes, of which 49% (33/68) had a HbA1c test performed and 39% (13/33) had a HbA1c>8%. Of patients without known diabetes (n=297), 23% (68/297) had HbA1c performed and 12% (8/68) had HbA1c>6.5%. 2/8 were discharged with medications for treatment of diabetes. Length of stay (LOS) was longer in those with DM (mean LOS 9.0 days) in comparison to those without (mean LOS 7.1 days).

### Conclusion

Data extraction via eMR can clarify areas of practice in which management could be optimised. Screening for diabetes is practice changing yet screening rates are low even in this high risk cohort. Endpoints such as mortality and readmission are being evaluated, together with the impact of Endocrine involvement on risk-factor modification. These data will define a high risk cohort for intensive management strategies.

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## Body weight and fat mass may negatively influence responses to high-dose vitamin D supplementation in overweight and obese older adults

Cecilia L H Xu<sup>1,2</sup>, Alexander J Rodriguez<sup>1</sup>, Mavil M Cervo<sup>1</sup>, Jakub Mesinovic<sup>1</sup>, Lachlan McMillan<sup>1</sup>, Catherine Shore-Lorenti<sup>1</sup>, Peter R Ebeling<sup>1</sup>, David Scott<sup>1,3</sup>

1. Department of Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia

2. Austin Health, Heidelberg, VIC, Australia

3. Department of Medicine and Australian Institute for Musculoskeletal Science, The University of Melbourne, St Albans, Victoria, Australia

**BACKGROUND:** Overweight and obese older adults have increased risk of vitamin D deficiency. High-dose vitamin D<sub>3</sub> supplementation is an effective treatment for vitamin D deficiency. However, high body fat mass and unfavourable body fat distribution may reduce responsiveness to supplementation.

**AIM:** To evaluate body weight and body composition as predictors of increases in serum 25-hydroxyvitamin D (25(OH)D) concentrations following 12 weeks of vitamin D<sub>3</sub> supplementation in overweight and obese older adults with low 25(OH)D.

**METHOD:** Nineteen overweight and obese adults aged ≥50 years with baseline serum 25(OH)D concentrations <50 nmol/L were given 4000 IU/d of oral vitamin D<sub>3</sub> for 12 weeks. Body weight, body mass index (BMI), waist circumference, waist-hip ratio, and body fat parameters estimated by dual-energy x-ray absorptiometry were measured at baseline and 12 weeks.

**RESULTS:** Mean±SD 25(OH)D was 41±10 nmol/L at baseline and 84±15 nmol/L at 12 weeks (p<0.05) with 100% of participants becoming vitamin D replete at 12 weeks. Serum 25(OH)D did not correlate with anthropometric or body composition measures at baseline. Body weight at baseline (r=-0.44, p=0.06) and android-to-gynoid fat ratio at baseline (r=-0.40, p=0.09) were both negatively correlated with changes in serum 25(OH)D.

**CONCLUSIONS:** Higher body weight and android relative to gynoid fat mass may predict a poorer response to vitamin D<sub>3</sub> supplementation in overweight and obese older adults in line with theories around fat sequestration of vitamin D. Larger clinical trials are required to confirm these negative effects of body weight and fat mass on responses to vitamin D supplementation.

## Prevalence of primary aldosteronism in Australian primary care

Renata Libianto<sup>1</sup>, Morag J Young<sup>1</sup>, Jimmy Shen<sup>2,1</sup>, Grant Russell<sup>3</sup>, Michael Stowasser<sup>4</sup>, Stella Gwini<sup>5,6</sup>, Peta Nuttall<sup>1</sup>, Peter J Fuller<sup>2,1</sup>, Jun Yang<sup>7,2,1</sup>

1. Hudson Institute of Medical Research, Clayton, VIC, Australia
2. Endocrinology, Monash Health, Clayton, Victoria, Australia
3. Department of General Practice, Monash University, Clayton, Victoria, Australia
4. Faculty of Medicine, University of Queensland, St Lucia, QLD, Australia
5. Department of Epidemiology and Preventive Medicine, Monash University, Clayton, Victoria, Australia
6. University Hospital Geelong, Barwon Health, Geelong, Victoria, Australia
7. Department of Medicine, Monash University, Clayton, VIC, Australia

**Background:** Primary aldosteronism (PA) is the most common endocrine cause of hypertension with a reported prevalence of 3 – 13% in primary care. Timely diagnosis is important, since PA carries a worse prognosis compared to blood pressure-matched essential hypertension. However, even though effective targeted treatments are available, evidence suggests that PA is substantially under-diagnosed. A recent survey of Victorian primary care clinics revealed that < 0.1% of 7000+ hypertensive patients had a diagnosis of PA. This study is the first in Australia to establish the prevalence of PA in primary care populations.

**Methods:** Twenty primary care clinics across rural and urban Victoria were invited to screen patients with newly diagnosed, untreated hypertension, for PA by measuring their aldosterone-to-renin ratio (ARR). Those with ARR > 70 pmol/mU underwent confirmatory testing with a recumbent saline infusion test. Plasma aldosterone concentration (PAC) >140 pmol/L after the infusion of 2L normal saline confirmed the diagnosis.

**Results:** Of 156 patients screened, 46 had an ARR > 70. Of the 27 undertaking the saline infusion test, 23 had PAC > 140 pmol/L post saline infusion, leading to a prevalence estimate of 25%. If the most stringent cut-off for post-saline PAC is used (> 280 pmol/L), 6 patients would have PA, leading to a prevalence estimate of 7%. Baseline characteristics, in particular, age, blood pressure, and potassium levels did not discriminate between those with and without PA.

**Conclusions:** PA is under-diagnosed in Australian primary care, and screening has identified a prevalence of up to 25% amongst patients with newly diagnosed, treatment-naïve hypertension. Whilst the prevalence is highly dependent on the diagnostic criteria, it is much higher than currently observed. Increased awareness and access to a streamlined care pathway are needed to improve the diagnostic rate of this potentially curable cause of hypertension.

## A prodigious presentation in a previously well indigenous woman

Matthew Balcerek<sup>1</sup>, Thomas Dover<sup>2</sup>

1. Sunshine Coast University Hospital, Birtinya, QLD, Australia
2. Ipswich General Hospital, Ipswich, Queensland, Australia

### Case

A 54-year-old indigenous female presented with polydipsia, peripheral oedema and generalised weakness of two-weeks duration. She was hypertensive (155/80mmHg), hyperglycaemic (19mmol/L), and hypokalaemic (2.4mmol/L). She was known to Endocrinology who were managing her T2DM (HbA1c 6.9%) and toxic multinodular goiter (euthyroid post radioactive iodine ablation).

She appeared overtly Cushingoid and biochemistry was consistent with ACTH-dependent Cushing's syndrome, likely ectopic source (Fig-1). Peripheral CRH stimulation was not consistent with Cushing's disease (Fig-2), and CT Pituitary did not visualise an adenoma. Metyrapone was commenced with daily cortisol monitoring.

Figure 1. Screening and confirmatory tests for hypercortisolism

	Sample 1	Sample 2	Sample 3	Reference Range
24-hour urinary free cortisol	Cortisol 7570 nmol/24h Volume 3.28L	Cortisol 12300 nmol/24h Volume 1.08L	Cortisol 4440 nmol/24h Volume 0.80L	Cortisol 10-120 nmol/24h (0.8-2.0L)
Late night salivary cortisol	398 nmol/L (23:30)	103 nmol/L (23:15)	563 nmol/L (23:28)	0-5 nmol/L (23:00)
1mg DST	Morning cortisol (pre 1mg Dex) 2040 nmol/L (08:57)	Morning cortisol (post 1mg Dex) 1960 nmol/L (08:14) ACTH 220 ng/L	-	Morning cortisol <50 nmol/L indicates normal suppression
8mg DST	Morning cortisol (pre 8mg Dex) 1960 nmol/L (08:24)*	Morning cortisol (post 8mg Dex) 2270 nmol/L (08:26) ACTH 290 ng/L	-	Decrease >50% suggests Cushing's disease (though 10% of ECS suppress)

Figure 2. Peripheral CRH Stimulation without significant augmentation

Time (min)*	ACTH (ng/L)	Cortisol (nmol/L)
-5	330	2780
0	260	2660
15	390	2580
30	220	2670
45	400	2900
60	380	2760
90	250	2640

\*Time since intravenous CRH administration (100 µg)

CT chest revealed a locally invasive right upper lobe lesion. Endobronchial biopsy confirmed malignant small cell neuroendocrine carcinoma and cytotoxic chemotherapy was commenced.

She was admitted post cycle-1 chemotherapy with hypotension (BP 60/40mmHg) and received 100mg IV hydrocortisone, achieving rapid haemodynamic stability. We hypothesise her presentation was related to acute adrenal insufficiency from chemotherapy induced tumour-lysis, resulting in marked reduction in plasma ACTH (66% decrease), and concomitant cortisol blockade (metyrapone).

Re-staging CT three months later showed disease progression which correlated clinically with a worsening of her Cushing's syndrome and multiple infective complications. At the time of writing, plasma cortisol remains >2000 nmol/L despite metyrapone, ketoconazole and mitotane triple therapy. Chemotherapy has been withdrawn with provision of best supportive cares.

## Discussion

Ectopic Cushing's syndrome (ECS) accounts for 10–15% of Cushing's syndrome cases<sup>1</sup>. It is caused by a variety of extrapituitary tumours, usually malignant. Onset of clinical features is usually abrupt and prodigious hypercortisolism, hypokalaemia and markedly elevated plasma ACTH concentrations should raise clinical suspicion.

SCLC and ECS carry a poor prognosis due to poor chemotherapy responsiveness, severe infectious complications and high VTE risk. Care should be taken post chemotherapy if patients are adequately blocked with steroidogenesis inhibitors, as tumour-lysis may lead to rapid reductions in ACTH secretion and clinical manifestations of secondary adrenal insufficiency.

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## An unusual diagnosis to swallow

**Matthew Balcerek<sup>1</sup>, Brett Sillars<sup>1</sup>, Sophie Poulter<sup>1</sup>**

1. *Sunshine Coast University Hospital, Birtinya, QLD, Australia*

### Background

Paragangliomas (PGLs) are uncommon neuroendocrine tumours arising from extra-adrenal autonomic ganglia<sup>1</sup>. Head and neck PGLs comprise the majority (up to 69%)<sup>2</sup> and are typically parasympathetic. Fewer than 5% are secretory<sup>2</sup>.

Approximately 30% of PGLs are caused by hereditary mutations, with up to 56% of parasympathetic PGLs harbouring SDHD germline mutations<sup>3</sup>. Male to female ratio is equal among hereditary PGLs whereas sporadic tumours predominate in women (71%)<sup>4</sup>.



The carotid body, jugulotympanic paraganglia and ganglion nodosum are the most common parasympathetic sites of origin<sup>5</sup>. Occurrence in oral structures is extremely rare.

### Case

A 46-year-old woman presented with a rapidly progressive left lateral tongue mass. Contrast CT revealed a 3.2x4.2cm lesion with patchy uptake and significant mass effect. MRI showed a well-defined solidly enhancing lesion in the genioglossus which encroached upon, but did not cross the midline.

She proceeded to core needle biopsy and histology confirmed PGL with classic zellballen arrangement and positive staining for chromogranin, synaptophysin and S100 on immunohistochemistry.

There were no convincing clinical symptoms or signs of catecholamine excess and biochemical assessment is outlined below (Fig. 1). There was no family history of pheochromocytoma, PGL or hereditary syndromes.

Figure 1. Plasma metanephrines and urinary biogenic amines

		Sample 1	Sample 2	Sample 3	Reference Range
<b>Plasma metanephrines</b>	Adrenaline	0.3	-	-	< 1.0 nmol/L
	Noradrenaline	1.9	-	-	< 3.5 nmol/L
	Normetadrenaline	425	414	-	< 900 pmol/L
	Metadrenaline	47	70	-	< 500 pmol/L
	3 Methoxy Tyramine	1	7	-	< 110 pmol/L
<b>Urinary biogenic amines</b>	Adrenaline	-	11	16	< 80 nmol/24h
	Noradrenaline	-	299	449	< 750 nmol/24h
	Dopamine	-	2010	1780	200-3500 nmol/24h
	Metadrenaline	0.32	0.32	0.8	< 1.7 umol/24h
	Normetadrenaline	2.7	2.6	2.6	< 2.3 umol/24h
	Normetadrenaline/Cr	-	-	0.16	<0.25
	3 Methoxy Tyramine	2.0	1.4	0.9	< 1.3 umol/24h

DOTATATE-PET revealed intense avidity at the site of the tongue lesion without synchronous disease. Genetic panel was negative. Given the PGL location and absence of secretory features, alpha blockade was not recommended. She underwent surgical resection of the lesion without complication (histology pending).

### Discussion

Our case highlights an unusually located sporadic paraganglioma of the tongue, which was successfully surgically resected without perioperative blockade. Only five cases have previously been described. Whilst sporadic PGLs are more common, especially in females, it is of paramount importance to screen for hereditary syndromes. This is especially true in head and neck PGLs where more than half arise in the setting of a known genetic syndrome.

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## When should back pain be of grave concern?

**Matthew Balcerek<sup>1</sup>, Thomas Dover<sup>2</sup>**

1. *Sunshine Coast University Hospital, Birtinya, QLD, Australia*

2. *Ipswich General Hospital, Ipswich, Queensland, Australia*

### Case

A 37-year-old Caucasian male was referred with 20kg unintentional weight loss, multiple vertebral fractures and osteoporosis on DEXA. Thoraco-lumbar x-ray revealed morphometric fractures of T10/T11/T12/L1 and CT lumbar spine showed additional crush fractures at L3/L4. Z-scores at the lumbar spine and left femoral neck were -3.0 and -2.9 respectively, suggesting an underlying pathological cause.

Secondary screening revealed florid Graves' disease (TSH <0.05mU/L, T4 61pmol/L, T3 28.9pmol/L), with markedly elevated thyroid stimulating immunoglobulin 110U/L (200x upper limit of normal). Surprisingly his symptom burden was mild, and there were no features of Graves' orbitopathy or dermopathy. He was commenced on carbimazole 15mg tds.

He was vitamin D replete with adequate dietary calcium intake. The remainder of his secondary osteoporosis screen was unremarkable. His only other risk factor for osteoporosis was smoking (~20 pack-year history) and there was no family history of premature osteoporosis/fracture.

Six months later he had an exacerbation of back pain with a new L2 vertebral fracture, despite achieving near biochemical euthyroidism. He remains on carbimazole 15mg bd and definitive management has been discussed.

Denosumab was inadvertently commenced by his GP in-between clinic visits. Ongoing management options include antithyroid medication alone, transitioning to bisphosphonate therapy or an anabolic agent. DEXA is due to be repeated.

## Discussion

Multiple vertebral fractures as the presenting feature of Graves' disease in a young male is unusual. Whilst bone loss (cortical>trabecular) is a uniform feature of overt hyperthyroidism, studies examining the impact of Graves' disease on BMD and vertebral fractures in men are scarce, especially in younger populations<sup>1,2</sup>.

Furthermore, studies examining reversibility of bone loss with treatment of hyperthyroidism have yielded variable results, and have largely focused on post-menopausal women. Additional studies are required to evaluate the impact of antithyroid medication and/or antiresorptive therapy in young males with Graves' disease to further inform management.

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## A pheochromocytoma crisis presenting with a severe takotsubo-like cardiomyopathy treated with ECMO

**Emma Boehm<sup>1</sup>, Joshua Hawson<sup>2</sup>, Subodh Joshi<sup>2</sup>, Spiros Furlanos<sup>1</sup>, Christopher J Yates<sup>1</sup>**

1. Dept Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, VIC, Australia

2. Dept Cardiology, Royal Melbourne Hospital, Melbourne, Vic, Australia

**Introduction:** Pheochromocytoma crises can be precipitated by medications and can rarely present as a takotsubo-like cardiomyopathy that poses challenging haemodynamic management issues.

**Case:** A 39-year-old female presented with severe headache. Her initial blood pressure was 180/90mmHg. She was treated for a presumed migraine with morphine, metoclopramide, and phenothiazines. Following an episode of syncope, an ECG showed changes concerning for ST-elevation myocardial infarction. Urgent coronary angiography showed normal coronary arteries and a reverse takotsubo cardiomyopathy with ejection fraction of 5-10% (normal >50%). The patient developed cardiogenic shock refractory to inotropic support and was commenced on extracorporeal membrane oxygenation (ECMO). Plasma metanephrines were sent, however in the interim an abdominal ultrasound revealed an 8cm mass abutting the right upper renal pole. The patient was commenced on a phentolamine infusion for a presumptive diagnosis of a pheochromocytoma crisis, potentially precipitated by metoclopramide. She was transitioned to phenoxybenzamine and metoprolol and ECMO ceased on day 9. A repeat echocardiogram showed normal left ventricular function. A Gallium Octreotate PET/CT revealed a large avid left adrenal mass with no metastasis. A left adrenalectomy was performed 6 weeks later with histopathology confirming a pheochromocytoma.

**Discussion:** Commonly prescribed medications can exacerbate pheochromocytoma crises, which typically present with paroxysms of headache, palpitations and chest pain. A takotsubo-like acute catecholamine-mediated cardiomyopathy has also been described<sup>1</sup>. Stunning of myocardial fibres occurs as catecholamine excess drives massive concentrations of calcium into the sarcoplasmic reticulum, reducing mitochondrial energy production<sup>1</sup>. ECMO is a solution to the unique haemodynamic challenges of providing alpha- and then beta-adrenoceptor blockade to a patient with cardiogenic shock to allow for recovery of myocardial function<sup>2</sup>.

**Conclusion:** Pheochromocytoma crisis should be considered in the differential diagnosis for a takotsubo-like cardiomyopathy. In this case ECMO was successfully used to overcome the challenge of providing adrenoceptor blockade to a patient in cardiogenic shock.

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## Renal dysgenesis and delayed puberty: a case of renal and gonadal dysgenesis in a patient with severe chronic kidney disease-mineral and bone disorder (CKD-MBD)

**Genevieve L Calder<sup>1</sup>, Dilan Seneviratne Epa<sup>1</sup>, Richard J MacIsaac<sup>1</sup>**

1. Department of Diabetes and Endocrinology, St Vincent's Hospital Melbourne, Fitzroy, Victoria, Australia

Denys-Drash Syndrome (DDS) is a rare condition of renal and gonadal dysgenesis. Typically diagnosed in early childhood, it is associated with end stage kidney disease, intersex disorders with risk of gonadoblastoma; and Wilms' tumour. We report a case involving a delayed diagnosis of DDS in a 23 year old woman with severe CKD-MBD and delayed puberty.

The patient had a history of congenital renal dysplasia. She had required peritoneal dialysis from age eight months, live renal transplant at age three years and haemodialysis since graft failure at age 20 years. The CKD-MBD involved severe secondary hyperparathyroidism (PTH 172pmol/L, corrected serum calcium 1.84-2.17mmol/L, ALP 1200UL) and rapidly evolving osteomalacia with debilitating skeletal deformities. She was also found to have primary amenorrhoea, characterised by

hypergonadotrophic hypogonadism, absent thelarche, a hypoplastic rudimentary uterus on pelvic imaging, and a karyotype of 46,XY. Genetic testing for DDS with Wilm's tumour-1 (WT-1) suppressor gene mutation is still pending. Management has been multi-disciplinary. To attenuate her secondary hyperparathyroidism and osteomalacia, phosphate binders and calcitriol doses have been increased. Puberty induction has also been commenced with topical estradiol 25mcg/24hr. Dysmorphic gonadal tissue will need resection if found on laparoscopic exploration to mitigate gonadoblastoma risk. The long-term plan is for a second renal transplant.

This case is an important reminder that while disorders of pubertal development are commonly associated with CKD in adolescent patients, puberty should not be absent. As such, primary amenorrhoea warrants thorough investigation. The consequences of the delay in diagnosis of gonadal dysgenesis are significant for this patient: the hypogonadism is likely to have potentiated the bone loss associated with her CKD-MBD which has resulted in debilitating skeletal deformities; and her renal transplant has had to be deferred until the DDS is sufficiently optimised.

## A Hairy Conundrum

**Thora Y Chai**<sup>1,2</sup>, **Teresa Lam**<sup>1,3</sup>

1. Department of Diabetes and Endocrinology, Westmead Hospital, Westmead, NSW, Australia

2. Sydney Medical School, The University of Sydney, Sydney, NSW, Australia

3. School of Medicine, University of New South Wales, Sydney, NSW, Australia

### Introduction:

Androgen-secreting ovarian tumours are rare, accounting for <5% of ovarian neoplasms. We report a post-menopausal female with severe hyperandrogenisation from a Leydig cell tumour, identified after bilateral salping-oophorectomy and was undetected on multiple imaging scans.

**Case:** A 52-year-old female developed facial hirsutism six months after reaching menopause. Her hirsutism worsened six months later, with her face, chest and abdomen affected. New symptoms of virilisation also occurred (enhanced libido and voice deepening). She had a prior diagnosis of polycystic ovarian syndrome in her twenties and had never experienced hyperandrogenisation.

She had elevated serum androgens (total testosterone level 7.4nmol/L (RR 0.4-1.4nmol/L); free androgen index (FAI) 26%; free testosterone level 196pmol/L (RR 4-21pmol/L)). Her DHEAS level was 1.4µmol/L (RR 1.3-6.2µmol/L) and her adrenal CT did not identify any adrenal lesions. Congenital adrenal hyperplasia was excluded with a 17-hydroxy progesterone level at 2.7nmol/L (RR < 1.3nmol/L) and a negative ACTH stimulation test. Cushing's disease was excluded with a 24-hour urine free cortisol level at 183nmol/24hr (RR 100-330nmol/24hr) and a serum cortisol level at 40nmol/L (RR < 137nmol/L) after 1mg dexamethasone suppression. A transvaginal pelvic ultrasound did not identify any ovarian lesions or hyperthecosis.

Six months on, her plasma testosterone levels elevated to 11.5nmol/L and FAI was 35.9%. A CT chest/abdomen/pelvis and MRI abdomen did not identify any masses suggestive of androgen-secreting tumours. She was referred for a bilateral salping-oophorectomy, given ongoing suspicion of an androgen-secreting ovarian tumour. A right ovarian Leydig cell tumour was identified on histopathology with positive immunostaining for calretinin, inhibin and melan A (Ki-67 proliferation index <1%). Her serum androgens normalised post-surgery (total testosterone level 0.4nmol/L; free testosterone level 8.0pmol/L)

**Conclusions:** Leydig cell tumours frequently occur in post-menopausal women and should be suspected if sudden virilisation occurs. Imaging modalities do not always detect these tumours, making diagnosis a challenging affair.

## Investigation of an adrenal incidentaloma leads to an unsuspected diagnosis

**Thomas Chesterman**<sup>1,2</sup>, **Lachlan Angus**<sup>1</sup>, **Jui Ho**<sup>1,2</sup>, **Morton Burt**<sup>1,2</sup>

1. Flinders Medical Centre, Bedford Park, SA, Australia

2. College of Medicine and Public Health, Flinders University, Bedford Park, South Australia, Australia

### Introduction:

Phaeochromocytomas and paragangliomas are rare catecholamine producing tumours arising from the adrenal medulla or extra adrenal autonomic paraganglia. Biochemically, they are characterised by elevated plasma or urinary normetanephrine levels. Patients with end stage renal failure typically have higher plasma levels which may make diagnosis more challenging in this setting.

### Case:

A 76 year old woman with end stage renal failure was referred for further evaluation of a 25 mm adrenal mass with indeterminate washout characteristics. Initial plasma metanephrines, normetanephrines and 3-methoxytyramine levels were elevated approximately two, nine and four times the upper limit of normal respectively. On repeat testing plasma normetanephrines remained elevated >4 times the upper limit of normal. Surprisingly, Gallium DOTATATE PET scan revealed that the adrenal lesion was not avid, however intense tracer uptake was seen in a nodule in the left carotid sheath. The patient was medically managed with prazosin but declined further surgical or genetic investigation.

### Discussion:

Studies consistently demonstrate elevation of plasma normetanephrine and metanephrine levels in patients with renal disease. The kidneys are only responsible for about 15% of the clearance of free metanephrines, therefore the elevated levels are hypothesised to be related to increased sympathetic activation and analytic interference, rather than reduced renal clearance. Adjusted limits of twice the upper limit of normal for metanephrines and at least three times the upper limit of normal for metanephrines have been proposed to account for this increase.

### Conclusion:

It is important for clinicians involved in the diagnosis of phaeochromocytomas and paragangliomas to be aware of how renal failure may affect their biochemical investigations.

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## Primary thyroid lymphoma: an uncommon cause of compressive goitre

**Kay Hau Choy<sup>1</sup>, Jane Zhang<sup>2</sup>**

1. *Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia*

2. *Department of Endocrinology, Wollongong Hospital, ISLHD, Wollongong, NSW, Australia*

**Case:** An 85-year-old female presented with stridor on a background of enlarging neck mass over six weeks. Apart from lethargy, there were no symptoms of hypothyroidism. She reported a family history of hypothyroidism and thyroid cancer. Her stridor resolved following a dose of nebulised adrenaline. Blood tests showed profound hypothyroidism with thyroid-stimulating hormone of 170 mIU/L (reference range, 0.2–4.2), undetectable thyroid hormone levels and raised thyroglobulin antibodies, in keeping with Hashimoto's thyroiditis. CT scan revealed a large goitre with mass effect on the pharynx at the level of the hyoid, leading to narrowing of the trachea. Ultrasound-guided core biopsy of the thyroid gland and flow cytometry showed features consistent with a B-cell non-Hodgkin's lymphoma. A subsequent whole-body CT scan demonstrated paratracheal lymphadenopathy with no lymphadenopathy or malignancy elsewhere. She was started on levothyroxine as well as R-CHOP chemotherapy which she tolerated well. She became biochemically euthyroid 3 months later and continued to have regular endocrinology and haematology follow-ups.

**Discussion:** Primary thyroid lymphoma (PTL) is a rare cause of malignancy, accounting for only 5% of all thyroid malignancies and <2% of extranodal lymphomas, with an annual incidence of 1-2 cases per million.<sup>1,2</sup> PTL usually occurs in the 7<sup>th</sup>-decade age group and is four times more prevalent in women. Patients with Hashimoto's thyroiditis have a 40-80-fold increased risk of developing PTL.<sup>2,3</sup> The most common presentation is a rapidly enlarging painless goitre. Ultrasonography is the initial diagnostic modality and cross-sectional imaging is indicated in the presence of compressive symptoms. Fine needle aspiration cytology, using flow cytometry and immunohistochemistry, remains the key modality used for diagnostic confirmation. Treatment is dictated by histological classification. Multimodal treatment with rituximab and combination chemoradiation therapy provide the highest overall survival rates. Prognosis is generally excellent but may vary due to the heterogeneous nature of PTL.<sup>1-3</sup>

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## Ectopic adrenocorticotrophic hormone syndrome secondary to prostate carcinoma: an uncommon cause of Cushing's syndrome

**Kay Hau Choy<sup>1</sup>, Alexia Pape<sup>2</sup>**

1. *Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia*

2. *Department of Endocrinology, Wollongong Hospital, ISLHD, Wollongong, NSW, Australia*

A 67-year-old male with hormone-refractory metastatic prostate carcinoma was admitted with back pain due to pathological vertebral fractures, recent onset weight gain and increasing pedal oedema. His admission was complicated by profound hypokalaemia, metabolic alkalosis, new onset insulin-requiring diabetes, resistant hypertension requiring multiple antihypertensives and worsening thrombocytopaenia. Cushing's syndrome (CS) due to ectopic adrenocorticotropin secretion (EAS) was confirmed (table), as evidenced by failure of cortisol suppression following low-dose dexamethasone administration, with significantly raised adrenocorticotrophic hormone, markedly elevated urinary free cortisol and absence of pituitary adenoma on neuroimaging. However, he lacked the typical signs of CS. Computed tomography demonstrated bilateral adrenal gland hypertrophy. Investigation for thrombocytopaenia revealed bone marrow infiltration by neuroendocrine tumour with prostatic markers, reflecting neuroendocrine differentiation of the pre-existing prostate carcinoma. Hypercortisolaemia and its consequent metabolic derangements were temporarily controlled with metyrapone. He was a poor surgical candidate and subsequently received palliative-intent chemotherapy. He died two months later from respiratory sepsis.

Table – Biochemistry results	
Test	Result (reference interval)
Venous blood gas	
pH	7.511 (7.38 – 7.44)
pCO <sub>2</sub>	44.5 mmHg (35 – 45 mmHg)
Bicarbonate	35.4 mmol/L (22 – 29 mmol/L)
Base excess	11.5 mmol/L (-3.0 – +3.0 mmol/L)
Serum potassium	2.8 mmol/L (3.5 – 5.2 mmol/L)
Platelet count	
On admission	96 x10 <sup>9</sup> /L (150 – 400 x10 <sup>9</sup> /L)
Nadir	22 x 10 <sup>9</sup> /L (150 – 400 x10 <sup>9</sup> /L)
Morning cortisol after overnight 1mg dexamethasone administration	1,747 nmol/L (167 – 507 nmol/L)
Adrenocorticotrophic hormone	566 ng/L (7.2 – 63.3 ng/L)
24-hour urinary free cortisol	>6,105 nmol (<166 nmol)
Midnight salivary cortisol	13.8 nmol/L (<3.2 nmol/L)

Prostate carcinoma is a rare cause of EAS.<sup>1</sup> Neuroendocrine differentiation in prostate cancer can result in concomitant ectopic hormone production and is associated with tumour progression and poor prognosis.<sup>2</sup> Several cases of EAS in prostate cancer have been reported and the common features included metabolic alkalosis, hypokalaemia, hypertension and lack of classic somatic signs of CS.<sup>1-4</sup> Treatment modalities of EAS are limited, as definitive management is surgical, either with excision of primary tumour, bilateral adrenalectomy or both. Despite previous reports showing temporary biochemical control of hypercortisolism with adrenal steroidogenesis inhibitors, there is limited evidence that medical therapy is effective in lengthening survival of affected individuals.<sup>2-5</sup> Of cases where the cause of death was reported, most patients died from sepsis, likely due to uncontrolled hypercortisolaemia.<sup>1-4</sup> Our case highlights the clinical presentation of EAS in prostate cancer and illustrates its diagnostic and treatment challenges.

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## Diabetic ketoacidosis, hypertriglyceridaemia and acute pancreatitis: a case series of the enigmatic triad

**Kay Hau Choy<sup>1</sup>, Tegan van Gemert<sup>2</sup>, Zoran Apostoloski<sup>2</sup>, Jane Zhang<sup>2</sup>**

1. Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia
2. Department of Endocrinology, Wollongong Hospital, ISLHD, Wollongong, NSW, Australia

**Background:** The triad of diabetic ketoacidosis (DKA), hypertriglyceridaemia and acute pancreatitis is a rare phenomenon. Herein, we present a case series of two patients with this unique clinical scenario.

**Results:** Two male patients, aged 34 and 19 years, with no known history of diabetes mellitus or dyslipidaemia presented with acute epigastric pain. Radiological assessment revealed acute pancreatitis, with biochemistry showing concurrent diabetic ketoacidosis (DKA) and hypertriglyceridaemia in all cases. Our first patient—who had a triglyceride level of 91mmol/L (reference interval, <2.5mmol/L), HbA1c of 14.1%, pancreatic necrosis and multi-organ failure—received plasmapheresis. The other patient had a triglyceride level of 27mmol/L with HbA1c of 12.7% and achieved rapid resolution of metabolic derangements with intravenous insulin infusion. Both patients were managed in intensive care unit. All patients were discharged on combination lipid-lowering therapy and required ongoing subcutaneous insulin. Their triglycerides and glycaemic control were monitored regularly at clinical follow-up and no patients have had recurrence of pancreatitis or DKA.

**Discussion:** Acute pancreatitis and hypertriglyceridaemia can both be a cause of or a consequence of DKA.<sup>1</sup> DKA is a state of profound insulin deficiency associated with dysregulated glucose and lipid metabolism. The resulting hypertriglyceridaemia may induce pancreatitis with direct toxicity to the acinar cells and pancreatic capillaries.<sup>2,3</sup> Hypertriglyceridaemic-pancreatitis can precipitate beta-cell dysfunction, leading to transient insulin deficiency and potentially triggering DKA. Infusions of insulin, heparin and plasmapheresis have been used in addition to aggressive fluid repletion to lower triglycerides in different subsets of patients. Fibrates are the first-line long-term pharmacotherapy in patients with hypertriglyceridemia who are at risk of pancreatitis.<sup>1-3</sup>

**Conclusion:** A diagnosis of DKA in hypertriglyceridaemic-pancreatitis and vice versa is challenging due to the complex causal-effect relationship of the three concurrent conditions. Awareness of this uncommon triad is vital for early recognition and appropriate intervention.

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## Linagliptin-associated generalised skin eruption: a case report and review of the literature

**Kay Hau Choy<sup>1</sup>, Avinash Suryawanshi<sup>1,2</sup>**

1. *Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia*

2. *Sydney Medical School, University of Sydney, Sydney, NSW, Australia*

**Introduction:** Dipeptidyl peptidase-4 inhibitors (DPP4-i) are widely used for treatment of type 2 diabetes mellitus (T2DM) due to their favourable tolerability profile; however, severe dermatological side effects have been reported. Herein, we present a rare case of generalised skin eruption in association with linagliptin therapy.

**Case:** A 54-year-old gentleman with T2DM was started on linagliptin as an add-on therapy to metformin. He also had a background of Susac syndrome for which he received 2-monthly intravenous immunoglobulin. 3 weeks after he was commenced on linagliptin, he developed an intensely pruritic vesicular eruption with no associated bullae on his scalp, trunk and limbs, with prominent involvement of the arms. There was no evidence suggestive of an infectious aetiology. He was treated with topical betamethasone dipropionate and linagliptin was discontinued. His rash and itch subsided over days following linagliptin withdrawal. He was continued on metformin monotherapy for management of his T2DM.

**Discussion:** DPP4-i increase incretin levels, which decrease glucagon level and stimulate insulin secretion. DPP4 is also present in other body parts including the skin and inhibiting it can have wide-ranging effects beyond those on the pancreas.<sup>1</sup> There have been reports of cutaneous adverse effects in association with different types of DPP4-i, albeit uncommon. The time-to-onset of cutaneous drug reactions in these cases range from 1 day to 37 months, with most following the first dose.<sup>2,3</sup> Case reports on linagliptin-associated cutaneous reactions are much more limited, with all cases in the form of bullous eruption.<sup>1,4</sup> In view of the plausible temporal relationship and the dramatic improvement observed following drug withdrawal, linagliptin was the most probable culprit in the development of cutaneous eruption in our patient. To our knowledge, this case of linagliptin-associated non-bullous skin reaction is the first of its kind. Our case highlights the rare association of severe cutaneous reaction with DPP4i use.

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## Severe ketoacidosis in association with a low-carbohydrate ketogenic diet

**Kay Hau Choy<sup>1</sup>, Mawson Wang<sup>1</sup>, Avinash Suryawanshi<sup>1,2</sup>, Nic Kormas<sup>1,2</sup>**

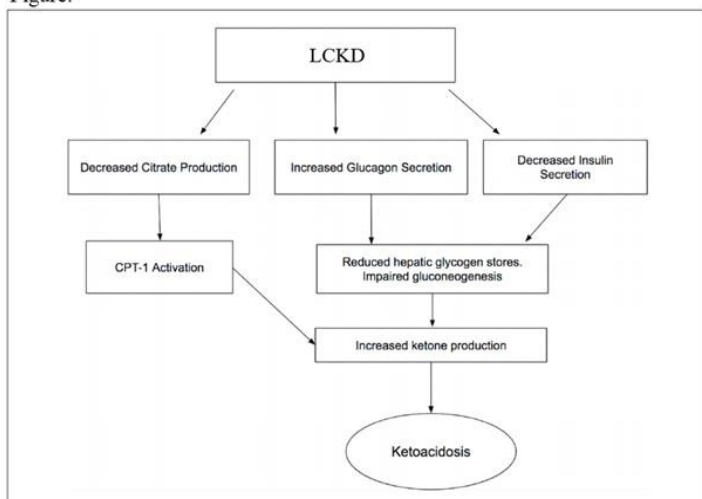
1. *Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia*

2. *Sydney Medical School, University of Sydney, Sydney, NSW, Australia*

**Background:** Low-carbohydrate ketogenic diet (LCKD) has become increasingly popular over the past decades. We describe a case of LCKD-associated severe ketoacidosis in an adult with normal glucose metabolism.

**Case:** A 37-year-old woman with a BMI of 28.0kg/m<sup>2</sup> presented with three days of persistent vomiting and epigastric pain without symptomatic hyperglycaemia. She had started on a LCKD with ketone supplements for weight loss two weeks prior, with an estimated daily carbohydrate intake of <20g. She had no history of diabetes, took no regular medications and reported no alcohol consumption or toxin ingestion. She had a previous history of anorexia nervosa with a nadir weight of 27 kg. Investigations revealed severe ketoacidosis: pH, 7.09; pCO<sub>2</sub>, 19mmHg; bicarbonate, 6mmol/L; point-of-care ketone, 7.0mmol/L; glucose, 23.2mmol/L; anion gap 39mmol/L; and lactate, 3.7mmol/L. She was treated for presumed diabetic ketoacidosis with insulin and glucose infusion, achieving swift biochemical resolution. HbA1c was normal, with no beta-cell dysfunction or autoimmunity. She remained euglycaemic without any glucose-lowering therapy. The patient was discharged with dietary advice to avoid LCKD and ketone supplements.

Figure.



**Discussion:** LCKD induces ketosis via various mechanisms. In carbohydrate-rich states, citrate produced by glycolysis inhibits carnitine palmitoyltransferase complex-1 (CPT-1), limiting the beta-oxidation of fatty acids and thereby reducing ketogenesis.<sup>1</sup> However, the opposite occurs in low-carbohydrate states. LCKD enhances alpha cell secretion of glucagon and lower insulin concentrations, resulting in a high glucagon-to-insulin ratio which promotes ketosis. Lactate—which increases during starvation—induces hepatic ketogenesis. The ketotic state progresses to ketoacidosis once usual physiologic-buffering mechanisms are exceeded (figure). Carbohydrate-restricted ketogenic diet stimulates release of counterregulatory hormones, promoting hepatic gluconeogenesis and glycogenolysis and subsequent hyperglycaemia.<sup>2,3</sup> Severe carbohydrate-restricted diets with ketone supplements may induce ketoacidosis with hyperglycaemia in predisposed individuals, highlighting the importance of taking a detailed dietary history during an atypical presentation of what is thought to be diabetic ketoacidosis.

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## Severe hyponatraemia as the presenting manifestation of empty sella syndrome

Kay Hau Choy<sup>1</sup>, Tegan van Gemert<sup>2</sup>

1. Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia

2. Wollongong Hospital, ISLHD, Wollongong, NSW, Australia

**Case:** An 85-year-old male—with a history of laryngeal cancer previously treated with radiotherapy—was admitted with lethargy and confusion. He was diagnosed with severe hyponatraemia with a nadir sodium level of 107 mmol/L (range: 135-145mmol/L). Serum and urine chemistries were consistent with syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH). Investigation for hyponatraemia revealed a low morning cortisol of 133 nmol/L (range: 167-507nmol/L), with subnormal synacthen stimulation and inappropriately normal adrenocorticotropin hormone. Prolactin and IGF-1 levels were low. Thyroid function tests showed low free T4 and free T3 with a normal TSH on a background of pre-existing hypothyroidism, consistent with inadequate thyroid hormone replacement. His pituitary profile was in keeping with panhypopituitarism. A pituitary MRI demonstrated findings consistent with empty sella syndrome, which was suspected to be a consequence of previous radiotherapy. His hyponatraemia gradually normalised following initiation of hydrocortisone, hypertonic saline and fluid restriction. Thyroxine dose was increased. He was discharged home on low-dose hydrocortisone. His sodium level remained stable two months post-discharge.

**Discussion:** Hyponatraemia is the most common electrolyte abnormality encountered in clinical practice, with an estimated incidence of 15–30%.<sup>1</sup> Appropriate management of hyponatraemia is vital as in its severe form it has a high morbidity and mortality. Rapid correction of hyponatremia risks causing cerebral demyelination which is often fatal.<sup>2</sup> Hyponatremia as the presenting manifestation of empty sella syndrome is uncommon. Its clinical presentation is similar to SIADH, but fluid restriction alone is unable to fully correct hyponatraemia. The cause of ADH secretion in hypopituitarism-associated hyponatraemia is due to adrenal insufficiency. The glucocorticoid deficit is not an osmotic, but a physiological stimulus for ADH secretion.<sup>3</sup> Glucocorticoid replacement has been shown to reverse the impaired water diuresis of this disorder by increasing the renal excretion of solute free water and is therefore the mainstay of treatment in this context.<sup>3-5</sup>

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## FGF23-mediated hypophosphataemia following intravenous iron administration in a patient with multiple sclerosis

**Lucy Collins<sup>1</sup>, Rebecca J Foskey<sup>2</sup>, Mark S Stein<sup>1</sup>, Paul Wraight<sup>1</sup>, Spiros Furlanos<sup>1,3</sup>**

1. Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

2. Department of Endocrinology, Western Hospital, Melbourne, Victoria, Australia

3. Royal Melbourne Hospital Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

Fibroblast growth factor 23 (FGF23)-mediated hypophosphataemia is a potentially under-recognised complication following intravenous iron infusion. We present a case of a 53-year-old woman with relapsing remitting multiple sclerosis who developed symptomatic severe hypophosphataemia following an iron infusion. At presentation, biochemistry revealed: serum phosphate 0.32 mmol/L (0.75-1.50 mmol/L), corrected calcium 2.33 mmol/L (2.10-2.60 mmol/L), creatinine 67  $\mu$ mol/L (45-90  $\mu$ mol/L), albumin 37 g/L (35-50 g/L), 25-hydroxyvitamin D 124 nmol/L (sufficiency > 50 nmol/L), 1,25-dihydroxyvitamin D 48 pmol/L (50-190 pmol/L) and parathyroid hormone 17.1 pmol/L (1.7-10.0 pmol/L). The patient was treated with intravenous and oral phosphate replacement. Urinary phosphate fractional excretion was calculated to be 88% (a value of > 5% indicates renal phosphate wasting). In this case serum FGF23, assayed externally using a Diasorian Liaison XL (Vercelli, Italy), was significantly elevated at 136 pg/mL (23.2-95.4 pg/mL). Following a re-admission to hospital for nausea and vomiting, presumed secondary to high dose oral phosphate, our patient was commenced on calcitriol and cholecalciferol replacement. We found this approach to be well tolerated and efficacious. Cholecalciferol and calcitriol were weaned over 18 weeks with maintenance of serum phosphate.

Intravenous iron is administered to patients across a range of medical specialities. There is limited awareness of the effect of iron infusions on serum phosphate, and no guidelines regarding the management of consequent hypophosphataemia. We hope our case raises awareness amongst health professionals of the potential deleterious outcome of symptomatic hypophosphataemia following iron administration; likely mediated by a transient elevation of FGF23. The risk of hypophosphataemia may be further potentiated by risk factors such as malnutrition, use of Receptor activator of nuclear factor kappa-B (RANK) ligand inhibitors and possibly, multiple sclerosis given their higher basal FGF23 (1). We recommend patients at risk of hypophosphataemia receiving iron infusions undergo measurement of serum phosphate at one- and three-weeks post treatment.

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## Carbimazole-induced hepatotoxicity in the treatment of Graves' disease

**Jessica Disler<sup>1</sup>, Jessica Triay<sup>1</sup>**

1. Department of Diabetes and Endocrinology, Bendigo Health, Bendigo, Victoria, Australia

### Case presentation

A 73-year-old female presented with symptomatic hyperthyroidism characterised by tachycardia, exertional dyspnoea and nocturnal pruritus. Her past medical history included osteopaenia and treatment with cholecalciferol supplements. Biochemical hyperthyroidism was confirmed with an elevated free thyroxine (fT4) of 40.8 pmol/L (10.0-19.0), elevated free triiodothyronine (fT3) of 12.6 pmol/L (3.5-6.5) and a suppressed thyroid stimulating hormone (TSH) of < 0.03 mIU/L (0.50-4.00). Thyroid peroxidase antibodies were elevated at > 1300 kIU/L and a corresponding thyroid pertechnetate study showed diffuse symmetrical uptake consistent with Graves' disease. Oral carbimazole was commenced at 20 mg twice daily with improvement in biochemistry. However, she developed abdominal pain shortly after and investigations were consistent with diagnosis of cholestatic hepatitis given an elevated alanine aminotransferase (ALT) of 201 U/L (5-30), alkaline phosphatase (ALP) of 301 U/L (30-115) and gamma-glutamyltransferase (GGT) of 220 U/L (5-35). Her bilirubin remained normal at 13  $\mu$ mol/L (3-15) despite reports of dark urine. Her liver function improved with cessation of carbimazole therapy temporally supporting a diagnosis of thionamide-related hepatotoxicity.

### Discussion

Thionamide is a mainstay treatment of hyperthyroidism, often offered as first-line to patients who wish to defer or avoid definitive therapy. Mild elevations in serum aminotransferases are common following treatment initiation, particularly in association with propylthiouracil (1), however the occurrence of severe cholestatic hepatotoxicity is a rare phenomenon. Histopathological changes consistent with cholestasis are most common, with isolated hepatitis occurring infrequently (2). The mechanism of injury remains elusive although an immunological reaction has been proposed. A clinical diagnosis can be complicated as hyperthyroidism alone can cause liver dysfunction, although this is expected to normalise with effective treatment (3). Rapidly developing liver function derangement or persistently elevated liver enzymes despite euthyroidism should lead to clinical consideration of thionamide-related hepatotoxicity and exploration of definitive therapy options.

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## Secondary hyperparathyroidism and severe hypercalcaemia due to vitamin D deficiency

**Jessica Disler<sup>1</sup>, Mark Savage<sup>1</sup>, Jessica Triay<sup>1</sup>**

*1. Department of Diabetes and Endocrinology, Bendigo Health, Bendigo, Victoria, Australia*

### Case presentation

An 18-year-old male of African and Caucasian ethnicity presented with two-years of fatigue and myalgia. Biochemistry revealed hypercalcaemia (corrected calcium 3.06mmol/L) and concomitant severe vitamin D deficiency (20nmol/L). Parathyroid hormone (PTH) level was markedly elevated (31.5pmol/L) with a high-normal alkaline phosphatase (137U/L). There was no clinical evidence of a granulomatous or lymphoproliferative disease. His prior medical history included a poorly healing wrist fracture and there was no relevant family history. Calcium:creatinine clearance ratio was reduced (0.0079) but vitamin D was low at 37nmol/L.

Cholecalciferol 25,000IU was administered with normalisation of vitamin D level (70nmol/L) but an associated rise in serum calcium (3.37mmol/L) and PTH (47.5pmol/L); after an interval, recommenced at 2000IU daily. Intravenous zoledronic acid (4mg) was administered with good effect. Imaging investigations included a parathyroid sestamibi scan, 4D computed tomography, ultrasound, magnetic resonance imaging, and whole-body fluorodeoxyglucose-positron emission tomography scan. Extensive surgical neck exploration failed to identify eutopic or ectopic parathyroid tissue. Despite persistent PTH elevation, he remained normocalcaemic. A final diagnosis of secondary hyperparathyroidism from severe vitamin D deficiency was made.

### Discussion

Vitamin D deficiency is common in the young adult Australian population (31%) (1) with even higher rates in those with African ethnicity (2). Secondary hyperparathyroidism is a physiological response to maintain normocalcaemia and leads to osteomalacia (3). Whilst vitamin D deficiency is recognised to cause hypercalcaemia, this is typically mild (4) and a severity of this degree has not been previously reported. This case highlights the necessity to correct vitamin D deficiency to eliminate secondary hyperparathyroidism even in extreme cases of hypercalcaemia prior to further investigation or intervention. Replacement of vitamin D deficiency in the presence of hypercalcaemic hyperparathyroidism can be challenging but is generally considered safe and may ameliorate the severity of hypercalcaemia (5). An investigation algorithm for hyperparathyroidism will also be covered.

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## Graves' disease following radioactive iodine ablation: an unusual occurrence of Marine-Lenhart syndrome

**Audrey SY Eer<sup>1,2</sup>, Mark A Ng Tang Fui<sup>1,2</sup>**

*1. Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia*

*2. Department of Medicine, The University of Melbourne (Austin Health), Parkville, VIC, Australia*

Publish consent withheld

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## A precarious ptosis in pregnancy

**Prishila Fookeerah<sup>1</sup>, Sally Newsome<sup>2</sup>, Carolyn Petersons<sup>2</sup>**

*1. Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia*

*2. Department of Endocrinology and Diabetes, The Canberra Hospital, Canberra, ACT, Australia*

## Case

A 36 year old female, with no past medical history, presented to her general practitioner at 25 weeks' gestation with retro-orbital pressure and left eye ptosis. Ophthalmology assessment at 30 weeks' gestation confirmed a 3mm left upper eyelid ptosis but was otherwise unremarkable. Magnetic resonance imaging (MRI) performed at 33 weeks' gestation because of worsening symptoms demonstrated a 22x11x11mm pituitary lesion, extending into the left cavernous sinus and encasing the left internal carotid artery. Examination findings were in keeping with a partial third cranial nerve palsy with no visual threat. Prolactin concentration was elevated at 4440mIU/L, consistent with the third trimester of pregnancy and cortisol 612nmol/L (100-540), an expected physiological response in pregnancy. IGF-1, TSH, FT4 and 24 hour urinary free cortisol were in the normal range.

In the following weeks, her third nerve palsy progressed. Poor eye adduction and new anisocoria were observed despite a trial of dexamethasone initiated by the neurosurgical team. There had been no evidence of foetal compromise. A caesarean section was performed at 35 weeks' gestation due to deterioration of maternal neurological symptoms and the lack of effective medical therapy.

There were no perioperative complications. Symptoms improved after delivery. Prolactin concentration initially dropped to 941 mIU/L but later peaked at 2422mIU/L, due to breastfeeding which is now being weaned after 12 months. Repeat MRI has shown no change in tumour size.

## Discussion

New diagnosis of nonfunctioning pituitary macroadenomas in pregnancy is rare.<sup>1</sup> Lactotroph hyperplasia caused by the stimulatory effect of oestrogen can infrequently result in clinically significant tumour expansion.<sup>2</sup> Mean prolactin concentration of up to 4092 mIU/L may be observed in the third trimester.<sup>3</sup> There are no guidelines to assist with management of these tumours in pregnancy. This case highlights the challenges faced when managing clinically worsening non-functioning pituitary adenomas in pregnant women.

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## Severe gestational hypertriglyceridaemia complicated by acute pancreatitis

**Rebecca J Foskey<sup>1</sup>, Aimee Khoo<sup>1</sup>, Iniyaval Thevathasan<sup>2</sup>, Christopher J Yates<sup>1,3</sup>, Rinky Giri<sup>1</sup>, Dev Kevat<sup>1,4</sup>**

1. *Endocrinology and Diabetes, Western Health, Sunshine, Victoria, Australia*

2. *Obstetrics and Gynaecology, Western Health, Sunshine, Victoria, Australia*

3. *Department of Medicine (RMH), The University of Melbourne, Parkville, Victoria, Australia*

4. *School of Public Health, Monash University, Clayton, Victoria, Australia*

Publish consent withheld

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## Recurrent thyroiditis after early pregnancy loss

**Lavanya Gupta<sup>1</sup>, Josie McCarthy<sup>1</sup>, Ducan Topliss<sup>1</sup>**

1. *Department of Endocrinology, Alfred Hospital, Melbourne, Victoria, Australia*

Post-partum thyroiditis is the commonest endocrine disorder associated with pregnancy, occurring in 8%<sup>1</sup> of the post partum population during 1st year after childbirth. It is an autoimmune disorder precipitated by the postpartum immunological rebound that follows the immunosuppression during pregnancy. After one episode of postpartum thyroiditis, there is a 70%<sup>2</sup> chance of recurrence with subsequent pregnancies. Though there have been case reports of postpartum thyroiditis after spontaneous abortion, here we present a case of three documented episodes of recurrent postpartum thyroiditis following early pregnancy loss, which we believe has not been reported previously.

A 32-year-old female presented with palpitations and lethargy three months after her first pregnancy loss. Laboratory examination revealed transient hyperthyroidism followed by hypothyroidism at 6 months. Within a month whilst she was hypothyroid, she became pregnant. She continued to remain euthyroid whilst on treatment. TPO antibody was not detected. She had an uncomplicated delivery and three months after the delivery, she again had symptoms of palpitations, hot flushes and weight loss due to hyperthyroidism, which resolved within three months. Eighteen months later, she had a second miscarriage and within a month developed hyperthyroidism followed by hypothyroidism, and then recovered. Radionuclide scans revealed complete absence of thyroïdal pertechnetate accumulation.

Two episodes of painless thyroiditis within few months of pregnancy loss in this woman suggest that the immunological changes of a short-term gestation may be sufficient to lead to thyroiditis. Moreover these women are as prone to recurrence as woman with post-partum thyroiditis after childbirth.

As these women may be attempting to conceive again it is important to advise avoidance of conception until normalization of thyroid function. Evaluation of thyroid function is routinely warranted in women with a history of post partum thyroid dysfunction to optimize maternal thyroid status during early pregnancy, before the development of fetal thyroid function.

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## An endocrine cause of reversible cardiac failure- acute catecholamine cardiomyopathy in patients with a pheochromocytoma

**Lavanya Gupta<sup>1</sup>, Anneke Graf<sup>1</sup>, Ducan Topliss<sup>1</sup>**

*1. Department of Endocrinology, Alfred Hospital, Melbourne, Victoria, Australia*

Pheochromocytoma can cause a myriad of cardiovascular issues including cardiogenic shock which is associated with high mortality. Early diagnosis and prompt treatment are critical but often delayed because of the diagnostic challenge. We present two cases of adrenergic cardiomyopathy associated with cardiogenic shock as the initial presentation of pheochromocytoma.

**Case 1:** A 39-year-old female with a history of hypertension, anxiety and palpitations presented with severe hypertension, headache and rapid deterioration into cardiogenic shock. An echocardiogram demonstrated severely impaired left ventricular function with basal akinesis. Inotrope therapy worsened oscillatory periods of tachycardia, hyper- and hypotension. Extracorporeal membrane oxygenation (ECMO) was required for circulatory support. Because of the possibility of a catecholamine associated cardiomyopathy, a bedside abdominal ultrasonography was performed and showed an 8 cm diameter left adrenal mass. Plasma metanephrines were elevated (give data). A 68-Gallium DOTATATE positron emission tomography (PET) scan showed a large left adrenal pheochromocytoma which was successfully removed after a month of preparation with phenoxybenzamine.

**Case 2:** A 46-year-old female presented with cardiogenic shock and multi-organ failure. An echocardiogram showed regional cardiac wall motion abnormality consistent with Takotsubo's. She was haemodynamically unstable with markedly varying vasopressor requirements and fever. ECMO was commenced. Bedside ultrasonography incidentally detected a 4.3 cm diameter left adrenal mass. Raised plasma metanephrines 660pmol/L (<500) and MIBG Nuclear Imaging indicated this was a avid left adrenal pheochromocytoma. After phenoxybenzamine preparation left adrenalectomy was performed. Histopathology confirmed pheochromocytoma.

Initially a diagnostic dilemma, in both cases the identification of an adrenal mass was delayed. Alpha- and subsequent beta-blockade therapy markedly improved left ventricular function. Excision of the tumour was safely done in a non-emergency situation. Both patients are now well, and normotensive.

In patients with severe cardiomyopathy with unstable haemodynamic status, acute catecholamine cardiomyopathy associated with pheochromocytoma should be considered in the differential diagnosis and adrenal imaging performed.

1. Diaz Et Al. international Journal of Surgery 2019

## A quintessential of hypokalaemia

**Lavanya Gupta<sup>1</sup>, Josie McCarthy<sup>1</sup>, Ducan Topliss<sup>1</sup>**

*1. Department of Endocrinology, Alfred Hospital, Melbourne, Victoria, Australia*

Hypokalaemia is one of the commonly encountered fluid and electrolyte abnormalities in clinical medicine, especially in the elderly population. In young adults with persistent hypokalaemia, however, it is important to rule out inherited tubular disorders. The most frequent inherited tubulopathy is Gitelman's syndrome, an autosomal recessive trait with incidence of 25 per million people. It is due to mutation of SLC12A3 gene, which encodes thiazide sensitive sodium chloride co-transporter (NCC) expressed in the distal convoluted tubule. It is characterized by significant hypomagnesemia, low urinary calcium excretion, and secondary aldosteronism, which is responsible for hypokalemic metabolic alkalosis.

A 25 year old male with a history of gout and traumatic fractures was referred for ongoing issues with headache, fatigue and persistent hypokalaemia in spite of being on a potassium supplement. He was on no potassium depleting agents. Serum potassium was 2.9 mmol/L one year previously. Serum potassium was 3.0 with serum magnesium 0.67 mmol/L (0.7-0.95mmol/L) serum bicarbonate 35 mmol/L (22-26mmol/L) and urinary calcium excretion xx (RR?). ECG showed sinus rhythm and T-wave flattening. His brother was also found to have hypokalaemia.

A diagnosis of Gitelman syndrome was established. He was treated with oral potassium supplementation (64 mmol/day) and magnesium sulphate 146mg/day. Liberal dietary salt intake was encouraged. of Serum potassium had normalized on 3 months follow-up (3.8 mmol/L).

Gitelman syndrome should be considered in the differential diagnosis of for persistent hypokalaemia, especially in an otherwise healthy young adult.

## Osteopetrosis: A wide spectrum of disease

**Rabbia Haider<sup>2,1</sup>, Jenny Gunton<sup>2,1</sup>**

*1. Westmead Clinical School, Sydney University, Sydney, NSW, Australia*

*2. Endocrinology, Westmead Hospital, Sydney, NSW, Australia*

**Introduction:** Osteopetrosis is a heterogeneous group of heritable disorders with a defect in osteoclast-mediated bone resorption. We present two cases which demonstrate the variability in phenotypes.

**Cases:** Case 1 is a 19-year-old man with a strong family history of osteopetrosis who was diagnosed at birth. He had sustained 10 upper and lower limb fractures, and has mild sensorineural hearing loss on audiometry. He was vitamin C and vitamin D deficient with a normocytic anaemia. He was given vitamin C and cautious vitamin D replacement. Case 2 is a 54-year-old female who was diagnosed at six weeks of age. She has sustained >40 pathological fractures, is blind despite surgical optic nerve decompression at a young age, has pancytopenia and suffered from recurrent osteomyelitis and osteonecrosis of the jaw. She was managed with dexamethasone and interferon gamma.

**Discussion:** Osteopetrosis occurs as a result of a loss of function mutation, which impairs osteoclast acidification at the ruffled border through trafficking or fusion of lysosome-related organelles. Severe cases may have pancytopenia, hepatosplenomegaly, and cranial nerve dysfunction including blindness. Patients have high fracture risk. Radiologically, they often have characteristic 'bone within bone' appearance and 'sandwich vertebrae'. Bone marrow transplant can be considered for osteoclast-intrinsic defects when diagnosed young (usually <1year). Corticosteroids are second line therapy and interferon gamma remains experimental, with case reports showing clinical improvement. A current randomised trial is running in adults.

**Conclusions:** While rare, osteopetrosis has a wide clinical spectrum. Less severe cases have normal life expectancy. Severe disease has high risk of fractures, pancytopenia, deafness and blindness and risk of recurrent osteomyelitis. While early diagnosis is important, there are still few management options available for these patients.

## Bilateral acute Charcot neuroarthropathy in a young woman with type 1 diabetes, with concomitant osteoporosis and vitamin D deficiency

**Kate Hawke<sup>1</sup>, Anna McLean<sup>1</sup>, Ashim Sinha<sup>1</sup>**

*1. Cairns Hospital, Cairns City, QLD, Australia*

Charcot neuroarthropathy (CN) is a potentially devastating condition requiring early identification to reduce progression to long-term disability. The mean age in diabetes cohorts is 60 years, with male predominance (McEwen 2013). We describe a case of a young woman with acute CN with rapid sequential involvement of both feet.

A 32-year-old woman with type 1 diabetes mellitus presented with 24 hours of atraumatic left foot pain, swelling and bruising. Her background included a low-trauma radial head fracture.

The left foot had loss of longitudinal arch and diffuse swelling. Monofilament sensation was absent at the plantar 5<sup>th</sup> metatarsophalangeal joint and the lateral midfoot. The left foot was warmer at medial midfoot and lateral forefoot by 2.4 and 2.1 degrees Celsius, respectively.

Left foot Xray and MRI demonstrated past fracture of calcaneus and 1<sup>st</sup> and 2<sup>nd</sup> metatarsals.

We diagnosed acute CN, Brodsky 3b Eichenholtz 1 changes. Management was immediate left foot offloading. After four days, the contralateral foot developed bruising and pain. Right foot Xray showed old fractures of the 1<sup>st</sup> to 4<sup>th</sup> metatarsals.

Bone mineral density (BMD) was reduced for age. 25-hydroxy-vitamin D was 24nmol/L (reference 50-150nmol/L). This was treated with intramuscular vitamin D. Faecal pancreatic elastase was in the severe exocrine insufficiency range at 52ug/g (reference > 200ug/g). Bone markers were consistent with healing fractures, with elevated P1NP.

We report a case of bilateral acute CN with an underlying fracture pattern, in a patient with diabetes and osteoporosis. The relationship between BMD and CN is unclear (Rogers 2011, Zhao 2017). Reduced femoral neck BMD has been demonstrated in patients presenting with CN (Young 1995), but others have found no difference in baseline BMD between CN and matched non-CN patients (Jansen 2018). Fracture pattern CN (but not dislocation pattern) has been associated with a peripheral deficiency of BMD (Herbst 2004).

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## Denosumab-associated spontaneous vertebral fractures: A case-series

**Madhuni Herath<sup>1</sup>, Phillip Wong<sup>1, 2, 3</sup>, Frances Milat<sup>1, 2, 3</sup>**

1. Department of Endocrinology, Monash Health, Melbourne, VIC

2. Department of Medicine, Monash University, Melbourne, VIC

3. Hudson Institute of Medical Research, Melbourne

Publish consent withheld

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## An uncommon cause of hypertension

**Elise Hickey<sup>1</sup>, Rakesh Iyer<sup>2</sup>**

1. The Canberra Hospital, Turner, ACT, Australia

2. Calvary Hospital, Canberra, ACT, Australia

A 32 year old previously well man of Indian descent presented with headaches and hypertension (BP 190/140). Additional features of metabolic syndrome included newly diagnosed type 2 diabetes mellitus, dyslipidaemia and hepatic steatosis. Low-dose dexamethasone test was abnormal on screening for secondary hypertension, with an elevated ACTH. Despite history of weight gain, clinical signs of cortisol excess were not immediately obvious, with only pale abdominal striae noted. Subsequent shaving of facial hair revealed previously unappreciated rounded facies, acne vulgaris and mild facial hyperpigmentation.

Widened mediastinum on chest x-ray prompted a CT angiography of the chest to exclude aortic dissection. This demonstrated a 63x40mm soft tissue anterior mediastinal mass. Biopsy revealed atypical carcinoid tumour with intense activity on DOTATATE-PET, consistent with a well-differentiated neuroendocrine tumour.

An incidental pituitary microadenoma on CT brain necessitated inferior petrosal sinus sampling, which confirmed an ectopic source of ACTH. Thymectomy was subsequently performed, with post-operative improvement in blood pressure, glycaemic control and lipid profile. 3 month post-operative dexamethasone suppression test had normalised.

Neuroendocrine tumours of the thymus are exceedingly rare, biologically aggressive neoplasms with an estimated incidence of 1 per 5 million people. They account for just 0.4% of all neuroendocrine tumours (1). Although the majority are non-functioning, they are capable of producing a range of biogenic amines predisposing patients to paraneoplastic syndromes (1). There is limited data to guide management. Despite surgical resection and neoadjuvant therapy, >70% develop loco-regional or distant metastases within five years of diagnosis (1), with poor overall five-year survival ranging from 28–68% (1,2,3,4).

The diagnostic challenge in this case was the subtle clinical features of Cushing's syndrome with an asymptomatic occult primary tumour. Care should be taken to evaluate classical signs of cortisol excess in the context of increased skin pigmentation and obscuring elements such as facial hair.

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## Severe hypocalcemia from osteoblastic bone metastases successfully managed with steroids

**Jeremy Hoe<sup>1</sup>, Jaime Lin<sup>1</sup>, Sheng Song Gan<sup>1</sup>, Delwyn Lim<sup>1</sup>, Ester Yeoh<sup>1</sup>**

1. Endocrinology, Khoo Teck Puat Hospital, Singapore

### Introduction

Severe hypocalcemia due to avid osteoblastic bone metastases is a rare occurrence that has been reported to occur in less than 1% of patients with metastatic prostate carcinoma.<sup>1</sup> We report a case of severe refractory hypocalcemia due to osteoblastic bone metastases that was successfully managed with steroids.



### Case Description

A 73 year old male with osteoblastic metastases from advanced prostate carcinoma was admitted for lower limb weakness due to spinal cord compression from bony metastases. During admission, he had severe symptomatic hypocalcemia, a low ionised serum calcium of 0.9 mmol/l, low serum phosphate of 0.76 mmol/L and elevated intact parathyroid hormone of 21.8 pmol/L. Serum creatinine, magnesium and 25-hydroxy vitamin D levels were normal. His alkaline phosphatase (ALP) was markedly elevated at 5214 U/L. His hypocalcemia persisted despite a continuous calcium infusion and high doses of oral calcium carbonate and calcitriol. Eventually, the use of intravenous methylprednisolone (1mg/kg) normalized the patient's serum calcium after two doses.

### Discussion

In patients with osteoblastic bone metastases, an increase in bone formation leads to increased influx of calcium into osteoblastic bone. This results in development of hypocalcemia.<sup>2</sup> Hypocalcemia due to osteoblastic bone metastases is frequently refractory to usual treatment with calcium, vitamin D and vitamin D analogues<sup>3</sup> because of ongoing calcium uptake by osteoblastic metastases and parathyroid hormone which stimulates proliferation of osteoblastic bone metastases.

Given the action of glucocorticoids on reducing osteoblast number and function, methylprednisolone was used in our patient to treat hypocalcemia associated with osteoblastic prostate carcinoma. A rapid response to intravenous methylprednisolone was seen in our patient. To our knowledge, this is the second case report on successful use of methylprednisolone in management of a patient with severe, refractory hypocalcemia due to osteoblastic bone metastases.<sup>4</sup>

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## Medical management of tumour-induced osteomalacia after elusive radiological localisation: A Case Report

SHAO FENG MOK<sup>1</sup>, Caroline Hoong<sup>1</sup>

1. National University Hospital, Singapore

### Introduction

Tumour-induced osteomalacia (TIO) is a rare paraneoplastic syndrome characterised by renal phosphate wasting due to excess fibroblast growth factor-23 (FGF23) production from a phosphaturic mesenchymal tumour (PMT). While surgery is potentially curative, localisation is often challenging.

### Case report

This case describes a 53-year-old lady who had recurrent fragility fractures in the spine, hip and pelvis, associated with lower limb pain and weakness developing over 2 years. Biochemistry revealed hypophosphataemia, hyperphosphaturia, inappropriately suppressed 1,25(OH)<sub>2</sub>D and raised FGF23. Localisation for the PMT was unsuccessful, despite multiple investigations including 68-Gallium-DOTANOC PET-CT, bilateral lower limb MRI for non-specific inguinal lymph nodes and various ultrasonographic evaluation of soft tissue lesions (including a benign breast tumour which was biopsied). Selective venous sampling was not performed due to uncertain utility and lack of local expertise. She was treated medically with oral phosphate, cholecalciferol and calcitriol, with a view to perform interval surveillance DOTA-peptide scan. Phosphate level was kept in the low-normal range and alkaline phosphatase reduced from baseline with an aim for normalisation as a marker of disease activity. Development of secondary hyperparathyroidism improved with up-titration of calcitriol. There was no hypercalcaemia on routine monitoring of urinary calcium excretion. Symptoms of generalised body pain and weakness, as well as her bone mineral density have improved over 21 months with medical therapy.

### Conclusion

This is a challenging case of TIO which has failed to localise despite best efforts. One must consider pursuing FGF23-independent and dependent causes of osteomalacia with hyperphosphaturia when patients present with severe frailty and hypophosphatemia as substantial morbidity results from delayed diagnosis and treatment. TIO-related PMTs can unfortunately be difficult to localise, even with a combination of functional and anatomical imaging. With medical therapy, bone mineralisation and symptoms can improve significantly. Patients need to be monitored for complications of long-term phosphate and calcitriol replacement.

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## Acute transient painful thyroid swelling post fine needle aspiration biopsy of a thyroid nodule

Thaw Dar Htet<sup>1</sup>, Jacqueline Centre<sup>1</sup>, Angela Sheu<sup>1</sup>

1. Endocrinology department, St Vincent's Hospital, Sydney, NSW, Australia

A 24-year-old female presented with a palpable neck lump. Background significant for essential thrombocytopenia, treated with aspirin 100 mg daily. Ultrasound (US) showed a normal sized thyroid gland (right lobe 9 cc and left lobe 5 cc) and a dominant nodule in the right lower pole measuring 18x17x11 mm. She underwent US-guided fine needle aspiration biopsy (FNAB) of the nodule, and 3 hours following, she developed painful neck swelling and dysphagia. On examination, there was a tender diffuse

goitre. No local bruising was noted at the FNAB site. Neutrophil count was raised at  $12.2 \times 10^9/L$  (2.0-7.5) with normal inflammatory markers and thyroid functions. Repeat thyroid US showed bilateral enlargement (right lobe 27 cc and left lobe 21 cc) and mixed echogenicity around the biopsied nodule, suggestive of small subcapsular haematoma. Computed tomography of her neck showed heterogeneous enhancement of thyroid gland suggestive of inflammation. She was treated with daily prednisolone weaned over one week during which the symptoms subsided. Repeat US 3 months later showed resolution of the acute changes with normal sized thyroid gland. Unfortunately, the biopsy result was inconclusive. Repeat FNAB 6 months later with prednisolone cover was uncomplicated and revealed benign follicular cytology.

Thyroid nodule FNAB is commonly performed and is generally safe<sup>1-3</sup>. Common complications include local pain and minor bleeding<sup>4</sup>. Rarely, acute painful goitre can occur<sup>4-6</sup>. Onset varies from within minutes to 4 hours post FNAB<sup>5-7</sup>. The mechanism is unclear but it is thought to be due to the action of inflammatory-vasoactive substances and local vasodilation due to minor skin injury<sup>7-8</sup>. Most reported cases were treated with steroids with symptoms settling within one week<sup>5-7</sup>. Clinicians should be aware of this rare complication so that appropriate management can be undertaken. It is important to discuss this potential complication with patients to prevent unnecessary distress.

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### Blue sclerae and joint dislocations without clinical fractures in a rare and mild variant of osteogenesis imperfecta

**Olivia Jarrett<sup>1</sup>, Benjamin Kwan<sup>2,3</sup>, Kirtan Ganda<sup>2,3</sup>, Bruce Hopper<sup>4</sup>, Bronwyn Crawford<sup>2,3</sup>**

1. NBMLHD, Nepean Hospital, Kingswood, NSW, Australia

2. Department of Endocrinology, Concord Repatriation General Hospital, Sydney, NSW, Australia

3. University of Sydney, Sydney, NSW, Australia

4. Hunter New England LHD, Forster, NSW, Australia

Osteogenesis Imperfecta (OI) is a group of genetically heterogeneous disorders leading to variable phenotypes. It is the most common inherited form of bone fragility and affects 1 in 10,000 to 20,000 births [1]. Most cases are inherited in an autosomal dominant manner and are caused by mutations in the COL1A1 or COL1A2 genes, which encode type 1 collagen [2]. The hallmark of OI is fragility fractures [2]. OI type 1 is the mildest form and is characterised by decreased bone density, increased fractures predominantly in long bones, joint laxity, blue sclerae and hearing loss [3]. Dentinogenesis imperfecta may also occur. We present a 56-year-old male (height 167cm) with blue sclerae and severe osteoporosis (FN T-score -4.0 SD). He had a history of frequent joint dislocations in his youth (knees and ankles with rugby, shoulders with sneezing). There was no history of symptomatic fragility fractures. He had reduced hearing and normal dentition. He had been treated with alendronate for 5 years and denosumab for 2 years without improvement in bone density. There was no history of fractures or blue sclerae in his parents or two siblings, and he had no children. A thoraco-lumbar spine xray showed mild thoracic kyphoscoliosis and 40% loss of height of T7. Secondary osteoporosis screen was negative. Genetic screening of the Brittle Bone panel of 19 genes revealed a heterozygous c.380G>A variant in the COL1A2 gene, which results in a Gly127Asp substitution in the triple-helix domain. There are only 2 previous reports of this mutation in the OI database [4,5]. This patient exhibits some features commonly associated with OI type 1. However, the history of frequent dislocations without increased clinical fractures, despite a low bone density, is unusual. This rare gene variant of OI may represent a mild and unique phenotype of type 1 OI.

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### A rare cause of secondary hypertension in an Indigenous female with primary amenorrhea: 17-alpha Hydroxylase Deficiency

**Melissa Katz<sup>1</sup>, Sheila Cook<sup>1</sup>, Durgesh Gowda<sup>1</sup>**

1. Endocrinology and Diabetes, Toowoomba Hospital, Toowoomba, QLD, Australia

Congenital adrenal hyperplasia (CAH) is a rare cause of hypertension with an incidence in Australia between 1:15 000 and 1:18 000 births.[1] 17-alpha hydroxylase deficiency accounts for only 1% of cases of CAH.[2] The classic presentation in phenotypic females is hypertension, hypokalaemic alkalosis and amenorrhoea.[3]

We describe a case of 17-alpha hydroxylase deficiency in an 18-year-old Indigenous female with recurrent presentations of severe symptomatic hypertension and hypokalaemia. This occurred on a background of primary amenorrhoea and minimal secondary sexual development with breast buds only. Investigations revealed a markedly elevated corticotropin (ACTH) and gonadotrophins while cortisol, dehydroepiandrosterone (DHEA) and testosterone levels were low. The urinary steroid profile showed undetectable levels of steroid and androgen metabolites. A karyotype was 46XY. Genetic testing confirmed a homozygous mutation of CYP17A1 resulting in a complete combined deficiency of 17 $\alpha$ -hydroxylase/17,20-lyase activity.

Glucocorticoid therapy was commenced with rapid normalization of serum potassium and blood pressure.

Data from Western Australia found the incidence of CAH was two and a half times higher in the Indigenous population than the non-Indigenous population.[4] This is the first time that a case of 17-hydroxylase deficiency has been reported in an Indigenous Australian. Its identification in this case has important implications when screening family members for secondary causes of hypertension.

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### ACTH assays in Cushing's Syndrome - the need for caution and communication.

Sarah Qian<sup>1</sup>, Claire McSwiney<sup>1</sup>, Joel Smith<sup>1</sup>, Jason Tan<sup>1</sup>, Nilika Wijeratne<sup>2</sup>, Dev Kevat<sup>3,1</sup>

1. *Western Health, Melbourne*
2. *Dorevitch Pathology, Melbourne*
3. *School of Public Health, Monash University, Melbourne, VIC, Australia*

Adrenocorticotrophic hormone (ACTH) concentration levels are key in determining the cause of Cushing's syndrome, however the assay is vulnerable to interference. This report discusses the case of a 40 year old woman with cortisol excess and unilateral adrenal lesion but elevated ACTH concentration suggestive of ACTH-dependent Cushing's syndrome. Through close collaboration with the chemical pathology team, it was determined that assay interference was likely leading to a falsely elevated ACTH concentration. The patient underwent a successful unilateral adrenalectomy and avoided unnecessary testing.

Ms M is a 40 year old woman referred due to an adrenal mass found incidentally, and a raised serum cortisol level. She had two year history of lethargy, central adiposity and easy bruising, as well as three months of amenorrhea.

CT findings showed a 3cm left adrenal adenoma with heterogenous contrast enhancement. Random cortisol level was 656nmol/L (NR 145-619) and failed to suppress with 1mg dexamethasone suppression test. ACTH (using Siemens immulite assay throughout) was detectable throughout 4mg Dexamethasone suppression test, with a peak of 3.0 pmol/L at 4 hours.

This picture suggested ACTH-dependent hypercortisolaemia. However, this is discordant with clinical picture and known adrenal lesion. ACTH testing on two other platforms (Roche & Diasorin Liasion) showed undetectable and low levels, prompting a successful left adrenalectomy rather than invasive inferior petrosal sinus sampling. Histology showed a 35mm cortical benign adenoma. ACTH levels measured on the Roche assay rose in the 8 hours post surgery as would be expected with the removal of a cortisol secreting adenoma. The patient was subsequently placed on a weaning dose of hydrocortisone with clinical improvement.

In addition to a review of the literature, we review the attributes of the three platforms used to measure ACTH in Australia, and techniques to manage potential assay interference.

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### Pituitary Lymphoma – a rare case of hypopituitarism.

Albert Kim<sup>1,2</sup>, David R Chipps<sup>1,2</sup>

1. *Department of Diabetes and Endocrinology, Westmead Hospital, Westmead, NSW, Australia*

### Case

60-year-old Caucasian man presented with an acute, unilateral third nerve palsy in the context of functional decline over 8 weeks. Imaging revealed a pituitary mass with parasellar extension into the cavernous sinus. Bloods revealed panhypopituitarism— TSH 0.54mIU/L(RR 0.4-4.0), T4 8.2pmol/L(RR 9.0-19.0), T3 2.1pmol/L(RR2.6-6.0), ACTH 2.4pmol/L(RR0-12), cortisol 198nmol/L(RR100-540), IGF-1 3.0nmol/L(RR6.3-28.1), FSH 4.7IU/L(RR<9.0), LH 2.4IU/L(RR<9.0), total testosterone 3.5nmol/L(RR>10) and prolactin 868mIU/L(RR<400).

Hydrocortisone, thyroxine and testosterone was commenced. Inflammatory markers were elevated, and blood counts revealed anaemia and leukopaenia. Investigations for vasculitis, tuberculosis, sarcoidosis and syphilis were negative. Pituitary biopsy was planned though not performed as FDG-PET revealed numerous avid lesions throughout the liver, spleen, skeleton and intra-abdominal lymph nodes. Liver lesion biopsy confirmed the diagnosis of diffuse large B-cell lymphoma and chemotherapy was commenced with resolution of the third nerve palsy, as well as radiological remission of disseminated disease. He remains on hydrocortisone and thyroxine replacement currently and is continuing to receive chemotherapy.

### Discussion

Systemic malignancies infrequently metastasise to the pituitary with an estimated incidence of 5.1%. The incidence of systemic lymphoma involving the pituitary is very rare with lymphomas accounting for 0.5% of tumour metastases to the pituitary.(1)

Pituitary metastases represent an uncommon complication of systemic malignancy. Most commonly, these patients present with diabetes insipidus(45.2%of pituitary metastases), highlighting the predominance of metastasis to the posterior pituitary via direct arterial supply. Pituitary metastases may present atypically with anterior pituitary insufficiency(23.6%) or ophthalmoplegia(21.6%). Constitutional symptoms are less common but should raise concern for atypical aetiology.

Anterior pituitary involvement is more frequently seen in pituitary lymphoma compared to solid organ tumours.(2) Pituitary function may recover with chemotherapy for the underlying lymphoma.(3, 4)

### Conclusion

Our case highlights the importance of considering a broad range of differential diagnosis in the investigation and management of hypopituitarism. The absence of diabetes insipidus does not exclude pituitary metastases.

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## Severe Salt and Water Imbalance in a Woman with Primordial Dwarfism

Jasmine Wintour<sup>1</sup>, Eleanor Warwick<sup>2</sup>, Johanna Kuehn<sup>1</sup>, Thida Myint<sup>2,3</sup>, Himanshu Goel<sup>2,4</sup>, Katie Wynne<sup>1,2</sup>

1. Department of Diabetes & Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

2. School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

3. Department of Nephrology, John Hunter Hospital, New Lambton Heights, NSW, Australia

4. Hunter Genetics, Waratah, NSW, Australia

A 53-year old woman of height 101cm and weight 15kg, presented with confusion, and serum sodium level 107mmol/l. She had a generalized seizure and was given two 25ml boluses of hypertonic 3% saline. She was transferred to ICU and received an infusion of 3% saline at 5mls/hour, calculated using the Adrogue-Madias equation.<sup>1</sup> She required two boluses of 100mls 0.9% saline when hypotensive (70/40mmHg) with low-normal urine output (5-10mls/hour). Collateral history revealed IgM paraproteinaemia and cold agglutinin disease.

Investigations demonstrated potassium 3.8mmol/l, urea 2.3mmol/l, creatinine 47umol/l, ACTH 4.9pmol/l, cortisol 278nmol/l, TSH 1.77mIU/l, T4 9.5pmol/l, LH 5.7IU/l, FSH 23.4IU/l, estradiol <100pmol/l, prolactin 29mU/l, GH 0.4mU/l, IGF-1 28ug/L. She commenced hydrocortisone 20mg intravenously three-times daily for anterior pituitary deficiency. Hydrocortisone triggered a transient diuresis of 30-40mls/hour. She received 100mls of 5% dextrose over two hours to prevent sodium overcorrection. Her sodium level reached 115mmol/l on day 2 and 125mmol/l on day 3.

MRI pituitary confirmed a hypoplastic gland with preserved posterior bright spot. Despite stress dose hydrocortisone 8mg three times daily, renal excretion of sodium remained inappropriately elevated, with excess loss of potassium, phosphate, calcium, glucose and protein consistent with tubular dysfunction. Transabdominal ultrasound demonstrated a small uterus and ovaries with two asymptomatic renal calculi. She was discharged day 7 on Hydrocortisone 4mg waking, 2mg noon, 2mg 4pm, oral sodium 2.4g/day and potassium chloride 20mmol/day, with a sodium level of 136mmol/l and normal serum potassium

A diagnosis of DNA Polymerase Epsilon Deficiency<sup>2</sup> due to biallelic pathogenic genetic variants in *POLE* caused her extreme growth disorder starting in utero.<sup>2,3</sup> Clinical features include short stature, microcephaly, immune dysfunction, micrognathia, a thin nose, wide neck and posteriorly rotated ears. Pituitary failure is reported and in this case became clinically apparent later in life.<sup>2</sup> Profound hyponatraemia was complicated by an accompanying renal tubular defect.

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## Want to be a boy or girl?

**Theingi Kyaw<sup>1</sup>, SAI THIHA TUN<sup>2</sup>, THEIN SOE TUN<sup>2</sup>, KHIN AUNG HTUN<sup>3</sup>**

1. *Endocrine and Metabolic Diseases, DSMA, Yangon, Myanmar*
2. *Endocrinology and Metabolic diseases, DSMA, Yangon, Myanmar*
3. *Surgical Department, DSMA, YANGON, MYANMAR*

**Background:** 46, XY disorder of sexual development (DSD) is a heterogeneous group of pathologies characterized by a wide spectrum of phenotypes and aetiologies. While advances in biochemical, hormonal tests and molecular genetics, the diagnosis still remains uncertain for most patients with 46, XY DSD.

**Case Summary:** A 17-year-old person, reared as female and later raised as a boy after his aunt noticed his genitalia, presented with bilateral gynecomastia, small phallus with hypospadias, non-palpable gonads and lack of secondary sexual characters, but without short stature. Karyotype analysis revealed 46 XY karyotype. Testosterone level was raised at 10.31 ng/ml (2.84-8.0 ng/ml), dihydrotestosterone level was lower limit of normal at 1.06 nmol/L (0.85-3.37 nmol/L) and testosterone-to-dihydrotestosterone ratio is elevated at 34.6. Luteinizing hormone (LH) was 48.92 mIU/ml (1.1-7 mIU/ml) and follicle-stimulating hormone (FSH) was 34.48 mIU/ml (1.5-12.4 mIU/ml). Ultrasound (abdomen) revealed undescended testis at right upper inguinal area with no ovary or uterine slit. CT (Abdomen) showed mild hepatomegaly, no testes in scrotal sac and no undescended testes, uterus or prostate. MRI (Abdomen) was unable to be done at that time. Based on these results, he was diagnosed as partial androgen insensitivity syndrome with the main differential diagnosis of 5-alpha reductase type 2 deficiency. Bilateral mastectomy, diagnostic laparoscopy with left orchidectomy and right orchidopexy were done to him. Right testicle could be brought to deep inguinal ring level. Right inguinal canal was explored again and testicle was further brought down. He was also treated with IM Testosterone 250 mg every 3 weeks for 8 months and thereafter, testosterone dose was increased to 250 mg every week. Significant improvement in phallus size was seen at 3 months follow up. The patient is being managed with multidisciplinary approach (General surgeons, Urologists and Endocrinologists).

## Multiple Endocrine Neoplasia Type 2B Diagnosed Early by Conjunctival Neuroma: a Case Report

**Dong-Mee Lim<sup>1</sup>, Won-Min Hwang<sup>1</sup>, Keun-Young Park<sup>1</sup>, Jong-Dai Kim<sup>1</sup>, Ju-Young Hong<sup>1</sup>**

1. *Konyang University Hospital, Daejeon, Chungnam, South Korea*

Multiple endocrine neoplasia type 2B (MEN 2B) is an autosomal dominant disorder characterized by medullary thyroid cancer, pheochromocytoma, neuroma and Marfanoid feature. Medullary thyroid cancer occurs in more than 95% patients of MEN 2B and increases mortality. So, the early diagnosis of multiple endocrine neoplasia is very important, because in the early diagnosed and treated medullary thyroid cancer, the prognosis is excellent. This is a case of multiple endocrine neoplasia type 2B that diagnosed early by conjunctival neuroma. A 15-year-old female patient was presented with both conjunctival masses that occurred 6 months ago. The excisional biopsy revealed conjunctival neuroma. The multiple endocrine tumor was suspected, further evaluation was performed. Medullary thyroid cancer was confirmed by thyroid ultrasound and fine needle aspiration. Finally, MEN type 2B was confirmed by a RET mutation genetic testing.

## Severe hyperparathyroidism – primary hyperparathyroidism vs parathyroid carcinoma

**Jaime Lin<sup>1,2</sup>**

1. *Woodlands Health Campus, Singapore*
  2. *Endocrinology, Tan Tock Seng Hospital, Singapore*
- Background

The most common presentation of Primary hyperparathyroidism (PHPT) is asymptomatic hypercalcaemia. Parathyroid hormone (PTH) levels are generally mildly elevated or inappropriately normal given the hypercalcaemia. Parathyroid carcinoma, in contrast presents with severe hypercalcaemia and PTH elevation between 3 and 10 times of the normal ranges<sup>1</sup>. Metabolic bone and renal complications are more common.

We describe 2 cases of profound hyperparathyroidism (HPT), suspicious for parathyroid carcinoma with varying clinical and biochemical complications.

### Case 1

K.K, a 55-year-old female referred for elevated Alkaline Phosphatase (ALP) of 627U/L (RR:40-120U/L)<sup>+</sup>, with corrected calcium (cCa) of 2.72mmol/L (RR:2.15–2.50mmol/L)<sup>+</sup>, hypophosphatemia of 0.6mmol/L (RR:0.8-1.4mmol/L)<sup>-</sup>, markedly raised intact PTH (iPTH) of 182pmol/L (RR:0.8-6.8pmol/L)<sup>^</sup> and Vitamin D of 9ug/L (RR:20-50ug/L)<sup>#</sup>. Bone mineral densitometry (BMD) revealed severe osteoporosis. Thyroid ultrasound (TUS) and MRI neck showed a left parathyroid mass of 3.6x0.6x1.4cm with features suspicious for oesophageal wall involvement.

Hypercalcaemia remained stable with oral hydration. K.K underwent parathyroidectomy 2 months later.

## Case 2

J.S, a 40-year-old female with 4-year history of recurrent renal calculi, was referred for severe hypercalcaemia of 3.34mmol/L<sup>-</sup> and iPTH of 150pmol/L<sup>^</sup>, phosphate of 0.5mmol/L<sup>-</sup>, and vitamin D of 17ug/L<sup>#</sup>. ALP was 125U/L<sup>+</sup>. TUS showed a 4.8cm well-circumscribed parathyroid nodule. BMD revealed osteopenia.

Hypercalcaemia was managed inpatient with intensive intravenous fluids, Calcitonin, Bisphosphonate and Cinacalcet. A transient improvement was achieved with a nadir cCa of 2.7mmol/L<sup>+</sup>, which subsequently rise within days to a peak of 3.22mmol/L<sup>+</sup>. Inpatient parathyroidectomy was performed.

In both cases, no hypocalcaemia or hungry bone syndrome was evident post operatively. Histologies were consistent with parathyroid adenoma.

## Conclusion

- PHPT can be associated with severely raised iPTH greater than 100pmol/L.
- Despite severely raised iPTH, hypercalcaemia can be mild or resistant.
- Patients with metabolic bone disease and/or recurrent renal calculi should be screened for HPT.
- Parathyroid Carcinoma, should be suspected in patients with high PTH levels.

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## Pembrolizumab-induced diabetes presenting as severe diabetic ketoacidosis - A case report

**Phillip K Lo<sup>1</sup>, Lisa Raven<sup>1</sup>, Krupali Bulsari<sup>1</sup>**

*1. Endocrinology, Campbelltown Hospital, Campbelltown, NSW, Australia*

### Introduction:

Pembrolizumab is an immune checkpoint inhibitor (ICI) of the programmed cell death 1 (PD-1) receptor. The resultant dysregulation in immunologic tolerance is responsible for ICI-related adverse events including endocrinopathy.

### Case:

Our patient is a 55-year-old male with a medical history of only an unresectable right pleural mesothelioma treated with chemotherapy followed by three cycles of pembrolizumab 200mg three weekly. He presented with an altered level of consciousness on a background of intermittent vomiting and poor oral intake as well as a one week history of polyuria, polydipsia and 8kg weight loss. He had no focal infective symptoms. His family history is significant for a brother with type 1 diabetes mellitus. On examination, his Glasgow Coma Score was 11 /18. He was tachypnoeic with a respiratory rate of 45/min but was afebrile and normotensive. His cardiorespiratory examination was unremarkable. His venous blood gas confirmed the presence of diabetic ketoacidosis and acute kidney injury. His initial pH was 7.04 with serum bicarbonate 5 mmol/L, serum glucose 87.3 mmol/L, capillary ketone 6.8 mmol/L, serum creatinine 422 umol/L and eGFR 13ml/min/m<sup>2</sup>. HbA1c was 10.8% (95 mmol/mol) and he had negative insulin, glutamate decarboxylase and islet cell antibodies. His thyroid function and pituitary profile was normal. He was treated with intravenous insulin-dextrose infusion and subsequently discharged on basal/bolus subcutaneous insulin therapy.

### Summary:

ICI-induced diabetes is a rare complication with an incidence varying from 0.2 % - 2.2%<sup>1,2</sup>. There are eight case reports of pembrolizumab-induced diabetes presenting with diabetic ketoacidosis<sup>1</sup>. It is crucial to monitor patients on ICI therapy for potential life threatening complications.

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2. Immune checkpoint inhibitors: an emerging cause of insulin-dependent diabetes. Kotwal et al. BMJ Open Diab Res Care 7: e000591, 2019

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## Retrospective case series on use of tolvaptan for inpatient management of hyponatremia

**Nuttaradee Lojanapiwat<sup>1</sup>, Mina Mohammad Ebrahim<sup>1</sup>, Christopher Gilfillan<sup>1</sup>**

*1. Endocrinology, Eastern Health, Melbourne, Victoria, Australia*

### Introduction:

Hyponatremia is the most common electrolyte disorder in clinical practice which affects 15-20% of hospitalised patients with significant clinical and economic burden. The most common cause of hyponatremia is syndrome of inappropriate ADH (SIADH), characterised by euvolesmia with relatively high urine osmolality compared to serum osmolality. Treatment of hyponatremia is controversial with fluid restriction being the first line treatment for SIADH. Tolvaptan is an antagonist to

vasopressin type 2 receptors in the renal collecting duct which has been recommended as the second line treatment for euvolemic or hypervolemic hyponatremia.

**Aim:**

The aim is to determine the efficacy and cost effectiveness of inpatient tolvaptan use and help guide future treatment of hyponatremia.

**Material and methods:**

This is a retrospective case series of patient who received tolvaptan for treatment of hyponatraemia at Eastern Health. The medical records of patients were used to obtain data.

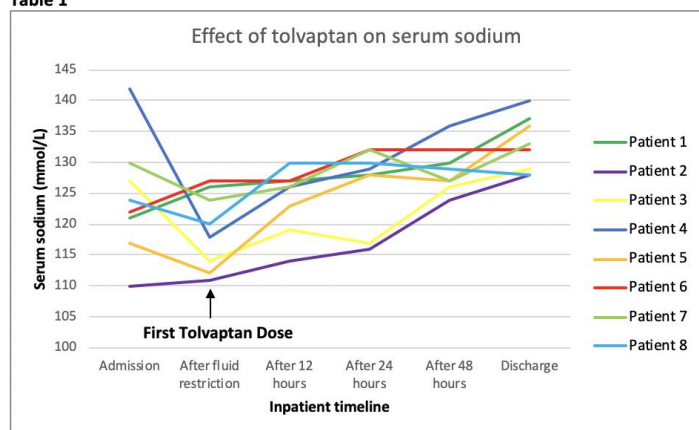
**Results:**

Eight patients received tolvaptan at Eastern Health in 2018 and 2019. The average age of patients was 85 years with average length of stay of 19 days. Three patients needed ICU admission due to severe hyponatremia. Treatment with 7.5mg or 15mg of tolvaptan was started after a trial of fluid restriction for five days on average. The serum sodium increased from an average of 124mmol/l on admission to 133mmol/l on discharge after an average of two days of tolvaptan (table 1). Two patient had sodium overcorrection of more than 10mmol/l over 24 hours and one received IV dextrose. All patients were symptomatic with their hyponatremia, and one remained symptomatic with residual fatigue after tolvaptan (table 2).

**Conclusion**

Tolvaptan is an effective treatment of hyponatremic patients. At the cost of \$16.21 per 15mg dose, it could be potentially cost saving due to reduced length of hospital stay and avoidance of ICU admissions in these complex patients.

**Table 1**



**Table 2**

Key: Each number indicates an individual patient

Symptoms	Before tolvaptan	After tolvaptan
Asymptomatic		1 2 3 4 6 7 8
Nausea		
Confusion	3 4 5	
Unsteady gait		
Fatigue	1 5 6 7	5
Drowsiness	2 4 5 8	
Low mood	8	
Seizures		
Respiratory distress		
Coma		

**Medical management of a patient with ectopic ACTH syndrome**

**Stephen Ludgate<sup>1</sup>, Kenneth Ho<sup>1</sup>**

1. Department of Endocrinology, Ryde Hospital, Sydney, NSW, Australia

We present 78-year-old man who presented to Ryde hospital with two weeks of generalised malaise, breathlessness and lower limb oedema. On presentation, serum potassium was 2.1 mmol/L, falling to 1.8 mmol/L despite IV replacement. Fasted morning bloods showed ACTH 242ng/L (0 – 47), and cortisol 1,590nmol/L (140 – 630). Overnight dexamethasone suppression test (DST) showed no reduction in cortisol. 24-hour urinary free cortisol was 4,818nmol (60–270). High-dose DST with 2mg QDS for 2 days returned cortisol of 1,230nmol and 1,360nmol. Abdominal ultrasound US showed three echogenic lesions in the liver. Subsequent triple phase CT scan revealed a right upper lobe primary bronchogenic neoplasm with hepatic and bilateral adrenal metastases. Biopsy of a liver lesion was consistent with small-cell lung cancer. Immunohistochemistry stained positive for synaptophysin, chromogranin, INSM1, CD 56, TTF1 but additional stain for ACTH was negative.



The patient required daily 90 mmol IV potassium and 84 mmol oral potassium for maintenance. He commenced 11 $\beta$ -hydroxylase inhibitors to block cortisol biosynthesis and control symptoms, initially with metyrapone 250 mg BD and subsequently, ketoconazole 200 mg BD. He responded with greatly reduced potassium replacement requirements. The patient tolerated the treatment well. Through extensive consultations with Endocrinology and Oncology services, he eventually declined definitive management with bilateral adrenalectomy or chemotherapy and was managed palliatively with continued medical management of hypercortisolism and remained comfortable throughout his end-of-life-care.

Endogenous Cushing's syndrome is rare, with an incidence of 0.7–2.4 per million population per year. Ectopic ACTH producing tumours are responsible for approximately 5–10% of these cases. The prognosis for ectopic ACTH syndrome depends on the source of ACTH production however outcomes are shown to be favourable when curative treatment is undertaken. When curative treatment is not possible, medical management is highly effective at managing the symptoms and improving the quality of life of the patient.

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## Cyproterone Related Meningioma In A Trans-sexual Female And Regression Following Its Discontinuation

**Danish Mahmud<sup>1</sup>, Richard Gauci<sup>1</sup>**

1. Fiona Stanley Hospital, Murdoch, WA, Australia

### Introduction

Meningiomas are hormone sensitive tumours known to express progesterone, androgen and estrogen receptors (1). They are more common in women, particularly during pregnancy and reproductive years. Cyproterone acetate is used for androgen suppression in male to female transsexual patients, but also has anti-gonadotropic and progestational effects (2).

### Case Report

A 55-year-old male to female transsexual patient presented with impairment of vision in the left eye (temporal field defect and colour vision changes) after being on hormone management (Estradiol 12-18mg/day and Cyproterone acetate 100mg BD) therapy for twenty years, managed in the general practice setting. MRI showed multiple meningiomata with the left frontal meningioma measuring 9x13x11mm causing compression of the optic nerve. Additional lesions identified on MRI included a 10x10mm right petroclinoid meningioma, a 11x17mm left paraclinoid meningioma, and a 15x15mm right tentorial meningioma.

Based on the symptomatic nature of left frontal meningioma, neurosurgical management was recommended which the patient declined. Cyproterone acetate was weaned and ultimately ceased and replaced with Spironolactone. Oestradiol dose was reduced, though negotiating a dose with the patient was difficult. Significant improvement in visual symptoms and reduction in size of meningiomata ensued over 12 months following discontinuation of Cyproterone acetate.

### Discussion

Our case report adds to the previously established association linking long term high dose Cyproterone acetate (50mg/day) exposure to meningioma(3). There are also few case reports of regression in size of meningiomata with discontinuation of cyproterone acetate and oestradiol (4, 5). Our patient had significant clinical and radiological improvement with discontinuation of Cyproterone only (oestradiol was continued) avoiding the need for neurosurgery.

### Key message:

A period of discontinuation of Cyproterone acetate may be reasonable first step in patients with symptomatic meningioma on long term Cyproterone acetate treatment.

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## Hypogonadotropic Hypogonadism

**Phyo Maung Maung Min<sup>1</sup>, Chan Nyein Thu<sup>1</sup>, KYAW MYINT OO<sup>1</sup>, THEINGI KYAW<sup>1</sup>**

1. *Endocrinology and Metabolic diseases, DSMA, Yangon, Myanmar*

**Background:** Isolated hypogonadotropic hypogonadism (IHH), called idiopathic or congenital hypogonadotropic hypogonadism (CHH) is a condition due to deficiency or insensitivity to gonadotrophin releasing hormone (GnRH).

**Case Summary:** A 25 yr old graduated man presented with gynaecomastia and poor development of secondary sexual characteristics. He had no experience of shaving since adolescence and easy fatigue, palpitation and dyspnoea on exertion. Examination revealed 5' 8" tall, arm span- 70 inches, BMI 24.3 kg/m<sup>2</sup>, BP – 110/80 mmHg, HR- 68/min, clinically Heart & Lungs - normal, absence of male pattern hairs, gynaecomastia on both sides, small male secondary sex characters.

Laboratory tests showed CBC normal, ESR 27, U&C, sugar, lipid profile normal, FT3 5.42, FT4 17.38, TSH 2.14, Cortisol 592.6, FSH 2.49, LH 2.71, Testosterone 0.26, Prolactin 99.13, Semen analysis could not be done, chromosome study 46XY. USG(Breast) showed no definite breast lump. USG(Scrotum) revealed Rt Testes 3.4x1.4x2.1cm size, vol 5.2 ml, Lt Testes 3.1x1.2x2.1cm size, vol 4.3ml. Normal outlines and echo texture with tiny calcified foci in both. Echocardiogram revealed normal valves and chambers, EF 70%. NECT and CECT of head, neck and chest showed normal. No suprasella and intrasella calcification. Pituitary gland was normal. No mass and calcification. Both thyroids were normal. NECT and CECT of abdomen and pelvis showed Liver - normal. No SOL. Few small intrahepatic bile duct stones or calcifications in right hepatic lobe. MRI brain scan revealed no abnormality in brain and pituitary. On Bone Densitometry, T score was - 0.5 (Osteopenia). ECG was NAD.

The goals of therapy are to replace or restore sex steroid hormones and to induce and maintain normal reproductive function. He was given IM Testosterone enanthate 250 mg every 3 weeks. If fertility is desired, pulsatile GnRH therapy or gonadotropin is necessary.

## A case of a calcitonin secreting malignant insulinoma

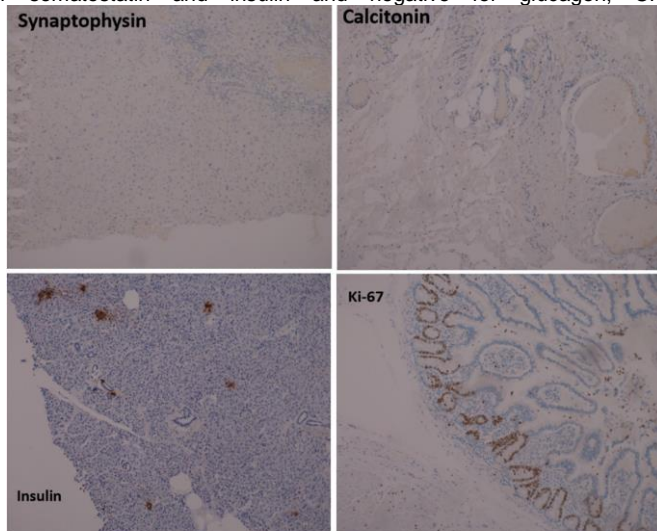
**Mina Mohammad Ebrahim<sup>1</sup>, Rosemary Wong<sup>1</sup>, Christopher Gilfillan<sup>1</sup>**

*1. Department of Diabetes and Endocrinology, Eastern Health, Box Hill, VIC, Australia*

Background: Neuroendocrine tumors (NETs) are a heterogeneous tumors that express general biomarkers and specific peptide hormones.<sup>1</sup> Insulinomas are rare NETs causing hyperinsulinemic hypoglycaemia and can be malignant in 10% of cases.<sup>1,2,3</sup>

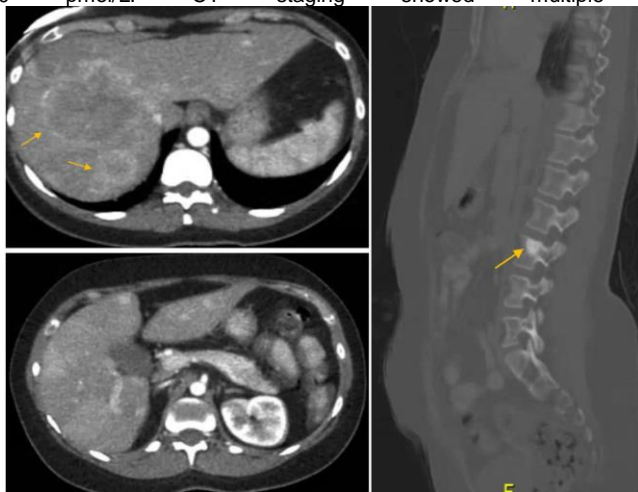
Case: A woman aged 34 lives in supported accommodation, found incidentally to have deranged liver function tests and multiple liver lesions on CT scanning. She had history of Fragile X syndrome and severe intellectual impairment.

Further liver biopsy showed neoplastic cells strongly positive for synaptophysin, chromogranin and calcitonin, weakly positive for somatostatin and insulin and negative for glucagon, CK7, CK20, TTF-1 and PAX-8; Ki-67 10% (Fig



1). Figure 1. Immunostaining of the liver biopsy

Histology was highly suggestive of metastatic medullary thyroid carcinoma (MTC). Serum calcitonin was elevated with peak of 400 pmol/L. CT staging showed multiple liver and skeletal metastases (Fig



2). Figure 2. CT chest, abdomen and pelvis

She was treated with vandetanib which was stopped three months later due to worsening of liver metastases and development of symptomatic hypoglycaemia. The C-peptide and insulin levels were elevated (Table 1).

Fasting		Normal range
Blood Glucose	2.8 mmol/L	
C-Peptide	4.11 pmol/mL	0.33-1.47 pmol/mL
Insulin level	133 mU/L	2.6-24.9 mU/L

Table 1. Paired fasting serum glucose, C-peptide and insulin

The PET GaTATE scan confirmed a metastatic neuroendocrine tumour (Fig 3).

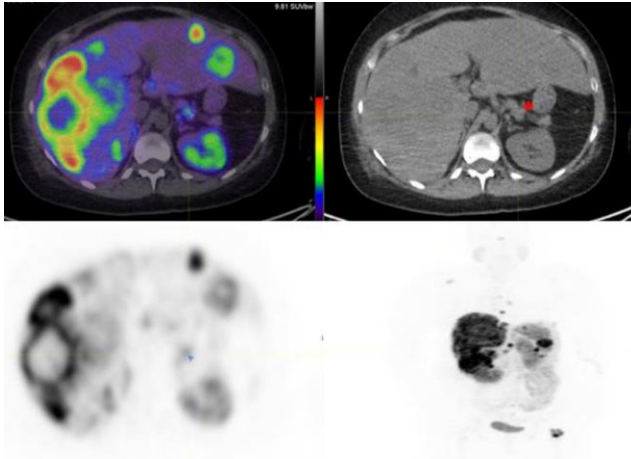


Figure 3. PET GaTATE scan (in 7 months since first presentation) showed uptake in pancreatic body as well as metastatic disease in liver, bones and mediastinal nodes with no abnormal uptake in thyroid.

A palliative treatment was chosen with the main challenge being severe hypoglycaemia. She was treated with continuous dextrose infusions, prednisolone, diazoxide and cornstarch. Due to limited success, octreotide and everolimus were added with good effect.

Discussion: Calcitonin is a marker of medullary thyroid carcinoma (MTC) but can be rarely secreted by other NETs as in our patient.<sup>4,5</sup> The treatment objectives are tumoral and hormonal control. As with our case, at metastatic stage the treatment aim is palliative with hypoglycaemia remaining the main challenge.<sup>3</sup> In addition to conventional treatment like diazoxide, dietary changes and debulking strategies, somatostatin analogs and mTOR inhibitors can successfully control severe hypoglycaemia.<sup>3</sup> Octreotide, lanreotide, everolimus and sunitinib, a multitargeted tyrosine kinase inhibitor, have all also been shown to improve progression-free survival in pancreatic NETs.<sup>6,7,8,9</sup>

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## Primary adrenal lymphoma and adrenal insufficiency

**Ashish Munsif<sup>1</sup>**

*1. Endocrinology, Nepean Hospital, Sydney, NSW, Australia*

**Background:** Primary adrenal lymphoma (PAL) is a rare, aggressive malignancy that can cause adrenal insufficiency with bilateral lesions. Fewer than 200 cases of this condition have been reported worldwide, of which a small proportion are unilateral [1, 2].

**Clinical case:** A 66 year old gentleman with a background of hypertension, diverticular disease and obesity presents to emergency with nonspecific abdominal pain during an interstate motorbike trip. Examination does not reveal any cushingoid features or hypertension. Abdominal computed tomography imaging demonstrates a 72x64x68mm ill-defined left adrenal mass suspicious for primary adrenal cortical carcinoma. Due to intractable pain despite oral analgesia, the patient represents to emergency a few days later and is admitted for pain management. Functional testing for adrenal insufficiency, subclinical Cushing's syndrome, pheochromocytoma and hyperaldosteronism are requested during this admission, all of which are ultimately negative. Mass related pain is hypothesised to be the primary cause for the symptoms, with haemorrhage into the mass a less likely differential. Repeat computed tomography and PET imaging reveals an enlarging mass with intense uptake (SUVmax 36.9) and development of extensive lymphadenopathy, including para-aortic and left internal mammary uptake (SUVmax 41.9). A left adrenal mass biopsy is subsequently performed that identifies an aggressive, triple hit, Diffuse Large B Cell Lymphoma (DLBCL). The patient is currently undergoing R-CHOP chemotherapy with mild improvement.

#### **Conclusion:**

1. PAL typically present with bilateral adrenal lesions and have concurrent adrenal insufficiency.
2. The most common subtype is Non-Hodgkin's Lymphoma, specifically DLBCL.
3. The presence of a unilateral lesion and adrenal preservation make this case extremely rare
4. PAL can mimic endocrine pathologies and therefore should be considered as a differential for adrenal lesions, especially if these are bilateral with associated adrenal insufficiency
5. Biopsy of the suspected lesion following exclusion of a functioning mass (particularly pheochromocytoma) greatly aids with the diagnostic dilemma

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#### **Now you see. Now you don't. Pituitary stalk transection syndrome, a misnomer.**

**Divya Namboodiri<sup>1</sup>, Andrew Davidson<sup>1</sup>, Bernard Champion<sup>1</sup>, Martina Preda<sup>2</sup>, Veronica Preda<sup>1</sup>**

1. *Endocrinology, Macquarie University Hospital, Sydney, NSW, Australia*

2. *Radiology, I-Med Radiology, Sydney, NSW, Australia*

Pituitary Stalk Transection Syndrome is a rare form of hypopituitarism described in the literature. It requires long-term follow up as hormonal deficiencies can manifest at different stages in life<sup>1</sup>. The name seems to be a misnomer, as most patients have no history of surgery or trauma.

Our patient, a 34 year old woman, with multiple pituitary hormonal deficiencies (MPHD), presented at 14 years of age with primary ovarian failure and short stature. She was subsequently noted to have MPHD and commenced on glucocorticoid, thyroxine and sex hormone replacement. She recently presented to our multidisciplinary pituitary service for review of her diagnosis in the context of ongoing fatigue and lethargy, impacting her day-to-day function. Physical examination revealed short stature and myxoedema, with biochemistry demonstrating panhypopituitarism. MRI demonstrated the pathognomonic features of hypoplastic adenohypophysis, absent stalk and ectopic posterior pituitary bright spot (EPPBS) at the median eminence<sup>2</sup>. There was no history of perinatal, childhood or adult trauma. Structural pituitary abnormalities have been reported in about two thirds of patients with hypopituitarism and are more common in MPHD<sup>2</sup>. The absence of stalk in our patient is an important indicator of MPHD.

Postulated mechanisms for "pituitary stalk transection syndrome" include, perinatal trauma and congenital hypoplasia or dysplasia with early foetal maldevelopment of midline structures, resulting in the failure of complete descent of the neurohypophysis and its investing vascular plexus into the sella turcica<sup>1</sup>. This syndrome is an important differential in patients presenting with hypopituitarism and should prompt a full pituitary evaluation including MRI. With the recent change in funding for growth hormone replacement in Australia, evaluation of and replacement for growth hormone deficiency is an important consideration in our patient due to the potential to improve quality of life.

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#### **Proactive about Proinsulinoma, a rare neuroendocrine tumour.**

**Divya Namboodiri<sup>1</sup>, Tamara Preda<sup>2</sup>, Koroush Haghghi<sup>3</sup>, Bernard Champion<sup>1</sup>, Diana Learoyd<sup>4</sup>, Veronica Preda<sup>1</sup>**

1. *Endocrinology, Macquarie University Hospital, Sydney, NSW, Australia*

2. *Surgery, St Vincent's Hospital, Darlinghurst, NSW, Australia*

3. *Surgery, St Vincent's Hospital, Darlinghurst, NSW*

4. *Endocrinology, Kolling Institute, Northern Clinical School, Royal North Shore Hospital, St Leonards, NSW, Australia*

Proinsulinomas are extremely rare pancreatic neuroendocrine tumours (NETs) that can be a diagnostic dilemma. These may be missed without methodical, extensive work up and specialised imaging is necessary to arrive at a final diagnosis. We present, a 34 year old gentleman, misdiagnosed with an anxiety disorder for two years before seeing an endocrinologist.

Our patient reported episodes of severe neuroglycopenic symptoms rectified on eating. He reported low capillary glucose levels, down to 1.1 mmol/L. To prevent episodes of hypoglycaemia, he was consuming carbohydrates every two hours, with over 10kg of weight gain. Physical examination revealed a high body mass index of 32 kg/m<sup>2</sup> but no features suggestive of an endocrinopathy. Biochemistry revealed, normal fasting insulin, C-peptide and beta hydroxybutyrate levels following a 12 hour fast, but a raised proinsulin level of > 99.9pmol/L (<13.3). A CT of his abdomen did not demonstrate focal pancreatic lesions. MRI along with DOTATE-PET localised a small circumscribed lesion within the neck of pancreas. He proceeded to have a Whipple's procedure, and now two years following surgery, he remains well, with no suggestion of recurrence clinically or radiologically.

Pancreatic NETs are a heterogeneous group of islet-cell tumours with a tendency for hormonal production. Among this group, proinsulinomas are very rare. Whilst insulinomas and proinsulin-secreting NETs have many parallels it is important to understand their differences. Proinsulinomas have elevated proinsulin levels with low or normal insulin levels, as opposed to insulinomas, which mostly presents with elevated insulin levels. Diagnosis of organic hypoglycaemia can be challenging in the absence of elevated fasting insulin levels, hence the need to include proinsulin levels in the work up<sup>1</sup>. Tumour localisation is often challenging and may require multiple sequential imaging modalities<sup>2</sup>. Surgery is the mainstay of treatment<sup>1</sup>. Long-term follow up is important due to potential for recurrence and metastasis.

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## Differentiated papillary thyroid cancer with pancreatic solid organ metastases and slowly progressive disease with medical management.

**Divya Namboodiri<sup>1</sup>, Veronica Preda<sup>1</sup>, Diana Learoyd<sup>2</sup>**

1. *Endocrinology, Macquarie University Hospital, Sydney, NSW, Australia*

2. *Endocrinology, Kolling Institute, Northern Clinical School, Royal North Shore Hospital, St Leonards, NSW, Australia*

A 75 year old man with symptomatic multinodular goiter and Bethesda category 4 dominant nodule underwent total thyroidectomy and lymph node dissection. He was diagnosed with a pT3, N1b, MX, poorly differentiated thyroid carcinoma, with papillary, follicular, oncocytic and tall cell foci, and evidence of vascular invasion. Following surgery, radioactive iodine (RAI) (6GBQ) was administered. Stimulated thyroglobulin (Stim-Tg) was elevated with no radiological evidence of distant metastases. Over the following 3 to 4 years, he has required additional two doses of ablative RAI (6GBQ and 8GBQ) due to ongoing rise in Unstim-Tg levels. There was also evidence of new bony (sacrum, humerus, femur) and pancreatic metastases with histopathology from biopsy of pancreatic lesions showing metastatic thyroid carcinoma. The pancreatic lesions and sacral lesion showed good clinical and radiological response to external beam radiotherapy (EBRT).

Unfortunately, more recently, there has been a further rise in the Unstim-Tg, evidence of new liver metastases on FDG/PET and slow growth of the femoral lesion, but without any clinical manifestations. This raises the question of, the most appropriate course of management in this gentleman with slow progression of disease but remains asymptomatic; Tyrosine Kinase Inhibitor (TKI) therapy versus active surveillance and symptomatic management. With complete metabolic response of pancreatic lesion to local EBRT, he has avoided debilitating surgery and the potentially toxic side effects of TKI therapy, which can occur in up to 60% of patients.

This case emphasizes that, the natural history of non-medullary thyroid cancer, can be indolent and unpredictable; and metastases to intra-abdominal organs are rare<sup>1,2</sup> and present unique challenges. Multimodality Imaging is the key in staging and management should be guided by clinical manifestations. Local therapies, such as EBRT to isolated bony metastases, remain useful and in the setting of oligo-metastatic bone disease, may be all that is needed.

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## A Difficult Case of Tumoral Calcinosis

**Kirsten Neal<sup>1,2</sup>, Roderick Clifton-Bligh<sup>1</sup>**

1. *Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia*

2. *Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia*

Hyperphosphataemic tumoral calcinosis (HTC) is a rare condition associated with chronic hyperphosphataemia leading to abnormal calcium deposits in soft tissue causing deformity, pain and immobility. It is more commonly associated with conditions such as chronic renal disease, and less commonly related to dysregulation of Fibroblast Growth Factor 23 (FGF23), a hormone responsible for phosphate homeostasis. Autosomal recessive HTC is associated with inactivating mutations in *FGF23*, *GALNT3* (enzyme responsible for full FGF23 bioactivity), or *KLOTHO* (co-receptor for FGF23). There is one reported case in

the literature of an auto-immune aetiology; an 8-year-old boy with HTC who subsequently developed Type 1 diabetes prompting an autoimmune screen<sup>1</sup>. We report the case of a 54-year-old female who presented with diffuse, painful HTC with normal renal function, low FGF-23 and homozygosity around the FGF-23 gene on chromosomal array. The unusual late age of onset led us to consider an auto-immune aetiology: positive chromatin antibodies were detected, and her symptoms and biochemistry responded to prednisolone. Further investigations are in progress including direct *FGF23* sequencing and testing for antibodies directed against FGF23. Our case highlights the importance of considering FGF23 deficiency or resistance in the diagnosis of HTC.

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## A challenging case of an ectopic ACTH-producing tumour

**Kirsten Neal<sup>1</sup>, Ann McCormack<sup>1</sup>**

1. *Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia*

Bronchial carcinoid tumours are very rare tumours but are the most common tumour associated with ectopic ACTH production leading to Cushing's syndrome. If feasible, the mainstay of treatment is surgical resection, granted the tumour is locatable. We present a case of a 26-year-old male with Cushing's syndrome who had a difficult-to-locate bronchial carcinoid tumour. Investigations including high-dose dexamethasone suppression test, inferior petrosal sinus sampling and CRH stimulation were suggestive of ectopic ACTH production. CT and SPECT/CT revealed no tumours and MRI pituitary showed a 3.5mm lesion consistent with microadenoma. <sup>68</sup>Ga-DOTATE-PET/CT demonstrated mild DOTATE avidity in a small right hilar lymph node, most consistent with inflammation. Endoscopic bronchial ultrasound was not feasible. All investigations were performed in a tertiary centre with pituitary specialisation. The primary tumour was seemingly unidentifiable and the patient underwent transsphenoidal exploration which was non curative; histopathology revealing a Rathke's cleft cyst. The patients' Cushing's syndrome continued to worsen and after repeat investigations a cardiothoracic surgeon explored the right hilar inflammatory lesion with a mini-thoracotomy, revealing low grade neuroendocrine tumour in four of sixteen lymph nodes; the primary tumour still not identified. Post-operatively the patient experienced partial recovery lasting several months but soon his symptoms worsened and cortisol levels rose again. Medical treatment was commenced. Eighteen months post mini-thoracotomy a repeat <sup>68</sup>Ga-DOTATE-PET/CT identified a subtle right upper lobe lung lesion leading to a second, albeit difficult, thoracotomy where the primary tumour was resected. This resulted in a dramatic improvement in the patient's symptoms and cortisol levels. This case highlights the difficulty that often arises trying to locate ectopic ACTH producing tumours and that when high quality investigations have been performed the results should be taken with great heed.

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## An interesting case of malignant hypertension and thyroid malignancy

**Foong Kien Newk-Fon Hey Tow<sup>1</sup>, Mina Mohammad Ebrahim<sup>1</sup>, Rosemary Wong<sup>1</sup>, Christopher Gilfillan<sup>1</sup>**

1. *Eastern Health, Wantirna South, VICTORIA, Australia*

An 80 year old female who presented with headaches, blurry vision and GTC seizures post starting Lenvatinib.

She has a history of a 75mm papillary thyroid cancer with stage T4aN1aM0. She had a total thyroidectomy followed by Iodine 131 RAI ablation. She had two RAI remnant ablations with 3 withdrawal scans. She developed multiple lungs nodules up to 19mm which were metastatic thyroid papillary carcinoma. She preferred to start on the best available treatment (Lenvatinib) due to deteriorating general health. Her other history includes high blood pressure.

Her CTB showed extensive posterior cerebral oedema. Her MRI brain showed extensive increased signal in the occipital lobe regions consistent with widespread changes of PRES. She was managed with a hydralazine infusion in ICU. Amlodipine was added and Lenvatinib was stopped. She was started on levetiracetam 1g BD for her multiple seizures. 5 weeks later, her repeat MRI brain showed resolution of her PRES changes.

Metastatic papillary thyroid cancer is an indolent malignancy with a median survival of 3 to 6 years. There is no drug therapy that has demonstrated an overall survival benefit in RAI refractory metastatic differentiated thyroid cancer.<sup>1</sup> Kinase inhibitor therapy is considered in RAI refractory DTC patients with metastatic, rapidly progressive and symptomatic disease. Lenvatinib is an oral multikinase inhibitor that mainly targets VEGFR 1-3 and FGFR 1-4. It delays time to disease progression by 14.7 months.<sup>2</sup>

The risk of hypertension is high when starting Lenvatinib with 73% of patients on the drug having high blood pressure. PRES is a neuro-radiological phenomenon that can progress to seizures, coma and death. High blood pressure induces vasoconstriction to reduce cerebral perfusion pressures, resulting in cerebral oedema. Treatment is usually supportive. PRES is reversible with signs and symptoms resolving within days to weeks although in one case series, 27% were fatal.<sup>3</sup>

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## Tale of Two Thyrotropinomas

**Hui Yi Ng<sup>1</sup>, Divya DN Namboodiri<sup>1</sup>, Andrew AD Davidson<sup>2</sup>, Bernard BC Champion<sup>1</sup>, Veronica VP Preda<sup>1</sup>**

1. *Endocrinology, Macquarie University Hospital, Macquarie University, NSW, Australia*

2. *Neurosurgery, Macquarie University Hospital, Macquarie University, NSW, Australia*

Thyrotropinomas are rare pituitary tumours and comprise 0.5-3% of all pituitary adenomas. There is no gender or ethnic predilection and patients tend to present at 40-50 years. A quarter of thyrotropinomas co-secrete other anterior pituitary hormones; the most common being GH (17.9%), prolactin (10.2%), and gonadotropins (1.8%)(1).

Thyrotropinomas present with secondary hyperthyroidism and a sellar mass. Three quarters are macroadenomas and are associated with increased alpha subunit (aSU); microadenomas usually have normal aSU levels (2). Thyrotropinomas are derived from the Pit-1 cell lineage, and may be locally invasive. (3) The main differential diagnosis is thyroid hormone resistance. (1) The gold standard of therapy is surgery, which should restore euthyroidism in 75-85%.

We report 2 cases of thyrotropinomas. Case one was a 51-year-old man diagnosed with a pituitary macroadenoma after investigation of headache and palpitations. Biochemistry showed secondary hyperthyroidism, elevated growth hormone (GH), failure of GH suppression, and elevated aSU. He underwent curative endoscopic transphenoidal hypophysectomy. Histopathology showed a Pit-1 plurihormonal adenoma positive for TSH, GH and prolactin, with elevated Ki-67 (5.2%).

Case two was a 35-year-old woman diagnosed with a pituitary macroadenoma after investigation of headache. Biochemistry showed secondary hyperthyroidism, with normal other anterior pituitary hormones and normal aSU. Planned transphenoidal hypophysectomy was deferred when it was discovered that she was pregnant. She was monitored clinically throughout pregnancy; visual fields were normal, and she remained mildly biochemically hyperthyroid. She suffered significant first trimester hyperemesis. Labour was induced at 38 weeks for gestational diabetes. Six weeks post-partum she underwent curative endoscopic transphenoidal hypophysectomy, which was complicated day 7 by symptomatic hyponatraemia due to presumed rapid alteration from hyperthyroidism to hypothyroidism. Histopathology showed a Pit-1 plurihormonal adenoma and a co-existent mixed prolactin, TSH and FSH staining adenoma, with normal Ki-67 (1%).

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## Plasmacytoma presenting as an invasive pituitary mass

**Bianca Nightingale<sup>1</sup>, Thomas Robertson<sup>2</sup>, Emma Duncan<sup>1</sup>**

1. *Endocrinology Department, Royal Brisbane and Women's Hospital, Herston, Qld, Australia*

2. *Queensland Pathology, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia*

A 74-year old male presented with a left sixth cranial nerve palsy and three month history of headache and diplopia but no weight loss, or symptoms of anterior pituitary insufficiency or diabetes insipidus. Past medical history included Crohn's disease, hypertension, ischaemic heart disease and permanent pace maker for symptomatic bradycardia. Left cranial nerve six palsy with no visual field defect was evident on examination. Pre-operative pituitary function and blood chemistry was within normal limits. An enhancing sellar lesion with suprasellar extension and skull base involvement measuring 28 x 34 x 35 mm was evident on neuroimaging. Pituitary histopathology reported sheets of atypical plasma cells, positive for CD79a, CD138 with light chain restriction, consistent with plasmacytoma. Extensive investigation did not reveal underlying multiple myeloma and he commenced treatment with radiation to the sellar region for the isolated sellar plasmacytoma. Follow-up revealed the eventual development of systemic multiple myeloma, 13 months after his initial presentation.

Isolated plasmacytoma presenting as a pituitary mass is rare. Headache, diplopia and visual field defects are the most frequent presenting symptoms. Cranial nerve palsy is common, mainly affecting the abducens nerve. Most patients have intact anterior pituitary function and imaging typically portrays an invasive mass. Radiotherapy is usually the initial treatment, with chemotherapy or autologous stem cell transplant utilised for systemic multiple myeloma. Solitary plasmacytomas have been reported to progress to multiple myeloma in 30-60% of cases.

Sellar plasmacytomas are rare and are often mistaken for non-functioning pituitary adenomas. An aggressive, destructive sellar mass with cranial nerve palsy but minimally disturbed anterior pituitary function should raise suspicion of a plasmacytoma. As treatment and prognosis differ, it is important to detect underlying multiple myeloma in patients presenting with sellar plasmacytoma.

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### **Sporadic pheochromocytoma, pancreatic neuroendocrine tumour and a sacral hibernoma: a case report**

**Ravind Pandher<sup>1</sup>, Ashish Munsif<sup>1</sup>, Emily Hibbert<sup>1</sup>**

*1. Endocrinology, Nepean Hospital, Kingswood, NSW, Australia*

Most pheochromocytomas and pancreatic neuroendocrine tumours are sporadic in nature however the presence of multiple neuroendocrine tumours raises the suspicion of a hereditary endocrinopathy. Hibernomas, benign tumours that morphologically resemble brown fat, do not possess a clear aetiology and a link with other neuroendocrine tumours remains unclear. We report an unusual case of a concurrent sporadic pheochromocytoma, pancreatic neuroendocrine tumour and a sacral hibernoma.

A 61 year old female with a 3 month history of abdominal pain which led to the discovery of a lesion in her right adrenal gland and a soft tissue mass at the pancreatic tail on a CT Abdomen. The adrenal lesion was biochemically suggestive of a pheochromocytoma (plasma normetanephrine 4930 pmol/L, plasma 3-methoxytyramine 580 pmol/L, urinary noradrenaline 5564 pmol/day, urinary dopamine 4720 nmol/day).

A <sup>68</sup>Ga-DOTATATE-PET-CT scan revealed DOTATATE avid lesions in the right adrenal gland, tail of pancreas and right sacral ala. Following preoperative medical therapy, the patient underwent a right adrenalectomy and a resection of the distal pancreatic lesion.

Histopathology confirmed a pheochromocytoma with no conspicuous mitotic activity, and the pancreatic tail lesion was consistent with a well-differentiated neuroendocrine tumour (NET) (Ki-67 score <3%). Following normalisation of the serum catecholamines, a biopsy of the sacral lesion was undertaken, which returned positive for a hibernoma. Genetic testing revealed no identifiable genetic mutations.

This case reports the synchronous presence of a pheochromocytoma, pancreatic NET and sacral hibernoma with no identifiable genetic mutation. To date, the association between hibernomas and neuroendocrine tumours has not been fully established, but a few case reports suggest a possible association between MEN1 and hibernomas.

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### **Ectopic adrenocorticotrophic hormone syndrome in a patient with metastatic medullary thyroid carcinoma**

**Ravind Pandher<sup>1</sup>, Sharon Yeoh<sup>1</sup>**

*1. Endocrinology, Nepean Hospital, Kingswood, NSW, Australia*

Ectopic adrenocorticotrophic hormone (ACTH) syndrome in a patient with medullary thyroid carcinoma is a rare complication, and is associated with a significant mortality risk. We report the case of a gentleman with established metastatic medullary thyroid carcinoma who presents with features of ectopic ACTH syndrome.

A 61 year old gentleman presented with a syncopal episode and was found to be severely hypokalaemic (K<sup>+</sup> 1.0mmol/L) with an associated metabolic alkalosis and hypochloraemia. He had a total thyroidectomy and central neck dissection followed by radioactive iodine the year prior for metastatic medullary thyroid carcinoma with evidence of pulmonary metastases. He had an elevated urinary free cortisol of 3944nmol/24hrs (N:50-250) and an elevated ACTH of 48.8 pmol/L (N:0-12).

Bilateral adrenalectomy was considered in view of the risk of infections associated with being in the immunosuppressed state however the patient was deemed too unwell for surgery and was commenced on medical blockade therapy with Metyrapone. The patient subsequently developed *Streptococcus Pneumoniae* pericarditis with a pericardial effusion requiring drainage, along with a *Klebsiella Pneumoniae* bacteraemia. He continued to deteriorate and eventually passed away.

This case highlights the high morbidity and mortality associated with ectopic ACTH syndrome from medullary thyroid cancer, which is reported in 0.6% of cases.



## Getting to the heart of hyperthyroidism

**Vicka Poudyal<sup>1</sup>, Anthony Zimmermann<sup>1</sup>, Peak Mann Mah<sup>1</sup>**

*1. Lyell McEwin Hospital, Elizabeth Vale, SA, Australia*

### Introduction

We present a case of hyperthyroxinemia, decompensated idiopathic heart failure, and pituitary adenoma to discuss the diagnostic and management challenges in differentiating between thyroid stimulating hormone (TSH) producing pituitary adenoma and thyroid hormone resistance (RTH).

**Case:** A 58-year-old man with a three-year history of idiopathic non-ischaemic dilated cardiomyopathy with moderate to severe left ventricular failure, was brought into the emergency department with NYHA class IV heart failure. Family history remarkable for brother treated for Graves' disease with radioactive iodine over 20 years ago, subsequent reassessment for elevated TSH while on thyroxine replacement was found to have heterophile antibodies.

Our patient presented frail BMI 18 kg/m<sup>2</sup>, relative tachycardia (85 beats per minute on beta blockade), with a small palpable goitre. Laboratory investigations without iodinated contrast or biotin revealed elevated thyroid hormone levels (free T4: 44 pmol/L, free T3: 9.2 pmol/L) with an inappropriately normal TSH: 3.19 mIU/L (Roche). The result was reproducible on an alternative platform (Siemens), heterophile antibodies studies and dilution test negative. Elevated sex hormone binding globulin: 133 nmol/L (10-45 nmol/L). Alpha subunit: TSH ratio increased to 1.14 (<1). MRI revealed a 5mm right pituitary adenoma. TSH receptor antibody negative and other anterior pituitary hormones normal. Dynamic testing with a T3 suppression test was not appropriate due to his cardiac decompensation. SHBG, alpha subunit: TSH ratio and MRI were suggestive of a diagnosis of thyrotropin-secreting tumor (TSHoma).

Carbimazole was started due to concern that hyperthyroidism may be contributing to his decompensating cardiac failure while waiting for further investigation. Thyrotropin-releasing hormone stimulation test done withholding carbimazole for 10 days and family history, both suggestive of RTH.

THRB gene pathogenic mutation (c.1357c>a) found and he was diagnosed with RTH. Unfortunately, our patient within a day of discharge to a nursing home died suddenly. A limited post-mortem request has been requested.

## Hypercalcaemia in pregnancy: a diagnostic dilemma

**Liesl Altus<sup>1</sup>, Linda Watson<sup>1</sup>, Vicka Poudyal<sup>1</sup>**

*1. Lyell McEwin Hospital, Elizabeth Vale, SA, Australia*

Introduction: Primary hyperparathyroidism (PHPT) in pregnancy is rare but associated with significant maternal and fetal risks.

Clinical case: A 25 yo female, G2P0, was referred with hypercalcaemia at 22 weeks' gestation. She was diagnosed with hypertension and nephrotic-range proteinuria earlier in the pregnancy. Corrected calcium was elevated 2.9 mmol/L with non-suppressed parathyroid hormone (PTH) 4.7 pmol/L. Vitamin D was 47 nmol/L. Urinary fractional excretion of calcium was unexpectedly low at 0.85%. Calcium levels in 3 first degree relatives were normal and genetic testing for genes implicated in MEN and familial hypocalciuria hypocalcaemia (FHH) were negative. Thyroid imaging suggested an inferior adenoma which, when biopsied, had elevated PTH levels on saline washout. A diagnosis of primary hyperparathyroidism was made and she proceeded to minimally invasive parathyroidectomy at 29 weeks gestation. Parathyroid adenoma was confirmed histologically. She is now 31 weeks gestation and remains normocalcaemic.

This case highlights the diagnostic challenges associated with hypercalcaemia in pregnancy. Pregnancy hormones drive an increase in calcitriol, which in turn leads to increased intestinal calcium absorption. The fetal calcium requirements are predominantly in the 3<sup>rd</sup> trimester, thus to prevent hypercalcaemia prior there is normally a compensatory increase in urinary calcium excretion.

Hyperparathyroidism is linked with increased maternal and fetal complications in particular hyperemesis, pre-eclampsia, nephrolithiasis and pancreatitis in the mother, and fetal growth restriction, neonatal hypocalcaemia and tetany in the infant due to chronic hypercalcaemia suppressing the fetal parathyroid.

Adequate hydration with close monitoring is required for all patients, with the gold standard remaining parathyroidectomy in the second trimester for moderate to severe cases.

Conclusion: Although rare, hypercalcaemia in pregnancy presents as a diagnostic challenge due to the normal physiological changes that occur. Correct diagnosis is important to allow appropriate and timely surgery for those with PHPT to reduce the associated risks.

## Potassium losing aldosterone oozing adrenocortical carcinoma- rare disease, rarer presentation

**Smitha Rao<sup>1</sup>, Zahir S Hussain<sup>1</sup>**

*1. Madras medical college, Chennai, Tamilnadu, India*

Background: Adrenocortical carcinomas (ACC) are rare malignancies with an incidence of one to two per million every year. 60% of these tumors are functional, most of which are Cushing's syndrome. ACC's are generally a postoperative diagnosis, often detected by immunohistochemistry. Aldosterone producing ACC's (APAC) are extremely rare out of these with an incidence less than 7%.

Aim: To report a rare case of aldosterone producing ACC.

Materials and methods: 24 year old gentleman presented with episodic lower limb weakness with headache of 3 months duration and was referred to us.

Results: A young man presented to us with lower limb weakness with headache and was found to be normotensive on evaluation. He had a vague mass palpable in the right upper quadrant on palpation. On further investigation, he was found to have hypokalemia, which persisted despite serial corrections. A detailed biochemical evaluation revealed elevated aldosterone levels and the ratio of plasma aldosterone concentration to plasma renin assay. 24 hour urine fractionated metanephrines and cortisol were within normal limits. Contrast imaging of the abdomen showed a right adrenal mass of 9.7x7.7cm, which was lipid poor with decreased adrenal wash out, suspicious of adrenal malignancy. He underwent a right open adrenalectomy revealing a large right adrenal tumor of 12x10cm with no surrounding invasion. Postoperative histopathology was suggestive of adrenocortical carcinoma with a Weiss score of 3. Complete surgical resection normalized his potassium levels postoperatively.

Conclusion: Hypokalemia evaluation can be laborious and can lead us to rare diagnosis. Normotensive patients with persistent hypokalemia should raise a suspicion for adrenal tumors. Aldosterone producing adrenocortical tumors are a possibility to be remembered in the workup for adrenal tumors. Complete surgical excision is the cornerstone of treatment with long term follow up with imaging and hormonal levels.

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## Casting a wide "NET": Multiple Endocrine Neoplasia type 1 Phenotype endocrinopathy manifesting in the 7th decade with coexisting primary hyperparathyroidism and a pleurihormone secreting enteropancreatic neuroendocrine tumour

**Dhanya Sanjeev<sup>1</sup>, Alexia Pape<sup>1</sup>**

*1. The Wollongong Hospital, Figtree, NSW, Australia*

Multiple endocrine neoplasia type 1 (MEN1) is defined clinically as the occurrence of two or more primary MEN1 tumour types, or in family members of a patient with a clinical diagnosis of MEN1, the occurrence of one of the MEN1-associated tumours.

A 70 years old man was incidentally diagnosed with hypercalcaemia in blood tests. Further investigations confirmed primary hyperparathyroidism. While awaiting parathyroid surgery, he was found to have a positive faecal occult blood test. Gastroscopy revealed duodenal ulcers, a biopsy from the ulcer diagnosed a low grade neuroendocrine tumour.

This is on the background of obesity, type 2 DM, gastro oesophageal reflux symptoms and atrial fibrillation.

Proton pump inhibitors (PPI) were ceased for blood tests for hormonal overproduction. He became symptomatic with episodes of flushing and dizzy spells and falls at home requiring hospital admission. He had severe orthostatic hypotension. While in hospital he developed upper GI bleeding and duodenal obstruction, which required HDU admission, but resolved with reinstatement of PPI.

Biochemical tests showed secretion of multiple hormones, including gastrin, glucagon, and pancreatic polypeptide. Dotatate PET scan showed intense uptake in second part of duodenum with multiple foci of tracer uptake within the pancreas. MRI abdomen- did not show any lesions other than the pancreatic and duodenal lesion.

Curative Whipple's surgery was planned in view of the localised disease. At laparotomy, unexpectedly two liver lesions were found, which were confirmed to be metastatic neuroendocrine tumour, and surgery was abandoned.

He was commenced on Somatostatin analogues with improvement in symptoms, with ongoing follow-up.

Parathyroid glands were removed successfully. Genetic testing for MEN -1 is pending.

We briefly discuss the epidemiology and complications of MEN-1 to highlight how unusual the first presentation in this age group is.

## Primary adrenal insufficiency due to heparin-induced thrombotic thrombocytopenia syndrome (HITTS): a case report

Matthew Sawyer<sup>2,1</sup>, Andy Lim<sup>1,3</sup>, Aditya Tedjaseputra<sup>4</sup>

1. General Medicine, Monash Health, Melbourne, VIC, Australia

2. Endocrinology, Eastern Health, Melbourne, VIC, Australia

3. Department of Medicine, Monash University, Melbourne, VIC, Australia

4. Haematology, Monash Health, Melbourne, VIC, Australia

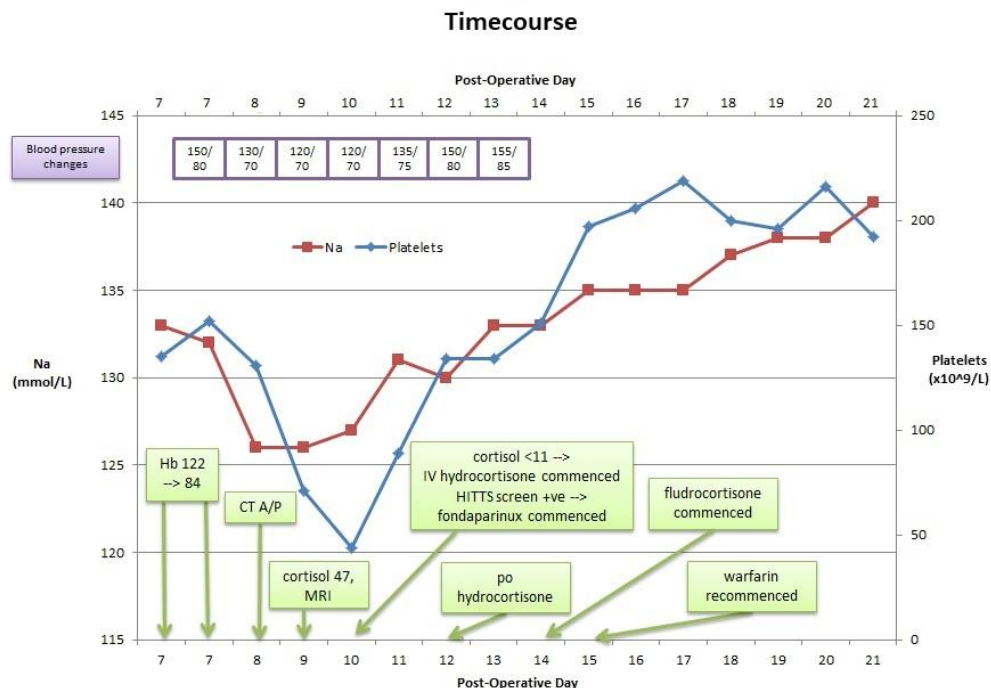
An 83-year-old woman underwent an elective total hip joint replacement. Warfarin for atrial fibrillation was withheld perioperatively and her admission was complicated by pulmonary embolism (PE) diagnosed on CTPA on day two post-operatively. She was recommenced on warfarin and discharged with bridging therapeutic enoxaparin. She presented to a different hospital the following day with pleuritic chest pain and became hypoxic. Repeat CTPA did not demonstrate PE, however left lower lobe consolidation was evident.

Her haemoglobin was noted to acutely decline from 122 to 84g/L (110-160) in a period of six hours without an overt source of bleeding, however remained stable thereafter. CT A/P identified ill-defined hypodensities in the expected region of the adrenal glands, suspicious for haemorrhage.

On review, the patient reported generalised abdominal pain and was drowsy. Morning cortisol was 47 nmol/L (185-625) and ACTH was 162 pmol/L (<10). She became progressively hyponatraemic from 133 to 126 mmol/L, but not hyperkalaemic. Her systolic blood pressure trended down from 150-160mmHg but was maintained at above 120mmHg. MRI of the adrenals supported the diagnosis of bilateral adrenal haemorrhage. Repeat cortisol the following day was undetectable (<11) and IV hydrocortisone was commenced.

Her platelet count was concurrently noted to have declined to  $44 \times 10^9/L$  (150-450) from 135 three days earlier. A high pre-test probability for heparin induced thrombotic thrombocytopenia syndrome (HITTS) was reflected in a positive ELISA, confirmed on serotonin release assay, and she was commenced on fondaparinux. Following the resolution of thrombocytopenia without further thrombotic sequelae she was discharged on hydrocortisone, fludrocortisone and warfarin.

This case highlights an atypical presentation and evolution of primary adrenal insufficiency due to adrenal haemorrhage in the context of HITTS. Notably this occurred in the absence of shock, which is near universal, but with a relative hypotension, and thus may be difficult to recognise



clinically.

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## Metastatic pheochromocytoma, pituitary adenoma, pulmonary chondrosarcoma and lung adenocarcinoma in a patient with a novel MAX germline mutation.

**Amanda Seabrook**<sup>2,1,3</sup>, **Catherine Luxford**<sup>1</sup>, **Judy Kirk**<sup>4</sup>, **Trish Dwight**<sup>1,3</sup>, **Roderick Clifton-Bligh**<sup>2,1,3</sup>

1. Cancer Genetics, The Kolling Institute of Medical Research, Sydney, NSW, Australia

2. Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

3. The University of Sydney, Sydney, NSW, Australia

4. Familial Cancer Service, Westmead Hospital, Sydney, NSW, Australia

**Context:** Pheochromocytoma and paraganglioma (PPGL) are rare neuroendocrine tumours that arise from the chromaffin cells of the adrenal medulla or paravertebral autonomic ganglia. They are the most highly heritable cancer, with germline mutations having been identified in 14 driver mutations. Individuals harbouring one of these mutations are at risk of developing PPGL at a young age, with more aggressive features such as multifocal or metastatic disease<sup>1</sup>.

With the advent of whole exome sequencing, MAX (MYC-associated factor X) was the 10<sup>th</sup> germline driver mutation identified to cause hereditary PPGL. It is located on the long arm of chromosome 14 at position 23.3 (14q23.3). MAX is the most highly conserved dimerisation component of the MYC-MAX-MXD1 complex, which is involved in the regulation of cell proliferation, differentiation and apoptosis. These loss of function mutations are characterised by an autosomal dominant pattern of inheritance with a parent-of-origin effect<sup>2-3</sup>. There is mounting evidence MAX mutations have a role in pituitary tumorigenesis given the notable co-existence of pituitary adenoma in this cohort of individuals<sup>4</sup>.

**Case Description:** We describe the case of 67 year-old female who underwent a bilateral adrenalectomy aged 21 for bilateral pheochromocytoma. Recent functional imaging with <sup>68</sup>Ga-DOTATATE demonstrated metastatic disease with intense uptake localised to an abdominal para-aortic lymph node and multiple lung lesions. Pertinent medical history includes a non-functioning pituitary adenoma and two additional lung pathologies; adenocarcinoma (FDG-avid) and pulmonary chondrosarcoma. Next generation sequencing identified a novel MAX pathogenic variant (class IV, written c.22G>T). It was not found in population or variant databases. Analysis via *in silico* tools predicted a truncated protein product due to a premature translational stop signal at codon 8.

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## Positive Thyroid Receptor Blocking Antibodies identified in patients with severe hypothyroidism- Case report.

**Julie Sherfan**<sup>1</sup>, **Joel Lasschuit**<sup>3,2</sup>, **Nimalie Perera**<sup>1,4,5</sup>, **David Sullivan**<sup>1,2</sup>

1. Department of Chemical Pathology, NSW Health Pathology, Royal Prince Alfred, Camperdown, NSW, Australia

2. Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia

3. Endocrinology, St Vincent's Hospital, Darlinghurst, NSW, Australia

4. Department of Chemical Pathology, Sydney Children's Hospital Westmead, Westmead, NSW, Australia

5. Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

### Introduction:

Graves' disease is traditionally thought to be caused by 'stimulatory' thyroid receptor autoantibodies (TRAB), resulting in hyperthyroidism. However, TRAB differ in function (stimulating-TSI, blocking-TBI or neutral), and result in variable thyroid dysfunction and orbitopathy<sup>1</sup>.

We present a patient with hypothyroidism and elevated TRAB, and another patient with initial hyperthyroidism and elevated TRAB requiring treatment, who then developed progressive hypothyroidism despite therapy cessation.

### Case 1:

A 76-year-old female was referred for advice, due to difficulty stabilising thyroxine replacement for primary hypothyroidism. Comorbidities included treated diffuse large B-cell lymphoma, rheumatoid arthritis in remission, and dilated cardiomyopathy.

She initially presented with heart failure, hyponatraemia and hypercholesterolemia, with TSH 67.4mIU/L, fT4 6.3pmol/L, fT3 1.4pmol/L, anti-TPO antibody <5 IU/mL, thyroglobulin antibody 699IU/mL, and TRAB 14IU/L. Thyroid was small volume on ultrasound.

Of note, TRAB remained elevated since diagnosis (even after cessation of biotin supplements). Possibility of TBI was investigated.

### Case 2:

A 39-year-old female presented with signs and symptoms of severe hyperthyroidism. TSH was <0.01mU/L, fT4 >100pmol/L, fT3 >50pmol/L, thyroglobulin 11.9ug/L, thyroglobulin antibody 864IU/mL and TRAB 41IU/L. She was treated with Carbimazole (8-weeks), but treatment was stopped due to hypothyroidism with TSH 57.5 mU/L, fT4 6.7 pmol/L and fT3 4.0 pmol/L, that progressed despite stopping therapy. Possibility of TBI was investigated.

**Methods:**

Both serums were tested on the Thyretain® TSI/ TBI bioassays (Quidel, San Diego, USA).

**Results:**

Serum of both cases negative for TSI, but TBI in case 1 was 95% (RI<34% Inhibition) and TBI in case 2 was 54% (RI<34% Inhibition).

**Conclusion:**

These cases illustrate the clinical utility of the of the Thyretain® TSI / TBI bioassay to distinguish subclasses of TRAB by 'functional' measurement of cAMP produced in transfected cell culture. This creates a two-dimensional picture and will be useful in managing and monitoring patients with complex thyroid dysfunction.

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2. Alemtuzumab-Induced Thyroid Dysfunction Exhibits Distinctive Clinical and Immunological Features, (J Clin Endocrinol Metab 103: 3010–3018, 2018)

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### Addisonian crisis secondary to altered prednisolone metabolism following cessation of rifampicin.

**Nicholas Shoung<sup>1</sup>, Christine Rowland<sup>1</sup>**

1. *Endocrinology, Greenslopes Private Hospital, Brisbane, QLD, Australia*

Rifampicin is a potent inducer of the cytochrome p450 oxidative system, responsible for the metabolism of Prednisolone. Previous studies have shown a reduction in the half life of cortisol in patients treated with simultaneous Rifampicin. In these studies, the half-life of cortisol returned to normal when Rifampicin was ceased. There are minimal studies available describing the effect after cessation of Rifampicin. We present a patient with Addisonian crisis 13 days after cessation of Rifampicin likely due to sustained induction of cytochrome p450 and therefore recommend close monitoring up to 2 weeks after cessation of Rifampicin in patients taking daily Prednisolone.

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### Intractable Postural Hypotension: Brittle Carcinoid vs Brittle Diabetes

**Alanna Tan<sup>1,2</sup>, Michael Michael<sup>2</sup>, Grace Kong<sup>2</sup>, Simon Forehan<sup>1,2</sup>, Cherie Chiang<sup>1,2</sup>**

1. *Diabetes & Endocrinology, Royal Melbourne Hospital, Parkville, VIC, Australia*

2. *Peter MacCallum Cancer Centre, Melbourne, VIC, Australia*

A 78-year-old man with metastatic ileal neuroendocrine tumour (NET) previously controlled on octreotide LAR 20mg monthly presents with sudden onset flushing, diarrhoea, postural dizziness and syncope. His relevant medical history includes poorly controlled T2DM, diabetic neuropathy and essential hypertension.

Compared to 12 months prior, his chromogranin A (CgA) level was 34,000 µg/L (from 263 µg/L; reference range 27-94) and urine 5-HIAA was 475 µmol/d (from 65 µmol/d; reference range <50). CT abdomen demonstrated progressive, small volume hepatic metastases with mild uptake on GaTate scan. He was changed to lanreotide 120mg monthly which improved his diarrhoea and flushing. Due to deteriorating severe postural hypotension (60 mmHg drop), peptide receptor radionuclide therapy (PRRT) was administered with initial post therapy improvement. Three weeks post PRRT, postural hypotension recurred and he had further syncopal episodes despite improvement in the frequency of flushing and resolution of diarrhoea. Fludrocortisone and midodrine were commenced with minimal effect. Tilt table test demonstrated significant postural drop without reflex tachycardia (baseline BP 200/90, HR 71; 11 mins BP 70/50, HR 86). His heart rate variability was reduced at 4.5% (normal >10%), consistent with autonomic neuropathy. Anti-neuronal antibodies were negative.

**Discussion**

Our subject's lack of heart rate variability on deep breathing and postural change is highly suggestive of cardiac autonomic neuropathy (CAN). CAN has been reported in 34.3% of patient with T2DM. Risk factors include poor glycaemic control, disease duration, age related neuronal attrition and elevated baseline blood pressure. However, unlike carcinoid syndrome, diabetic autonomic neuropathy is not accompanied by flushing and diaphoresis. Furthermore, the time-course of postural hypotension was rapidly progressive over one month and coincided with the progression of his NET. This raises the possibility of paraneoplastic neuropathy which involved autoimmune responses to onconeural antigens shared by the cancer and the peripheral or central nervous system.

## A difficult case of adrenal Cushing's syndrome in a young woman: issues in diagnosis and management

**Tunn Lin Tay<sup>1</sup>, Shui-Boon Soh<sup>1</sup>**

1. Changi General Hospital, Singapore

A 25 year old Chinese young lady was referred to Endocrinology clinic in view of subclinical hyperthyroidism (fT4 12.4, TSH 0.054) picked up incidentally by her dermatologist who was seeing her for oily skin and alopecia. Although she had no symptoms or signs of hyperthyroidism, she was noted to be Cushingoid in appearance - she had rounded facies, supraclavicular adipose tissue, thin limbs and thin skin with easy bruising. Her BMI was however only 19 kg/m<sup>2</sup> and did not have abdominal striae, hirsutism or proximal myopathy. She was hypertensive with a blood pressure of 150/110 mmHg. In view of these features, screening for Cushing's syndrome was done: 1mg overnight dexamethasone suppression test (ONDST) revealed cortisol was not suppressed (615nmol/L), and 24h urine free cortisol (UFC) was raised at 2233 nmol/day (5.4x upper limit of normal). Corresponding ACTH was low (2.8 ng/L), confirming ACTH-independent Cushing's. However, both CT and MRI adrenals showed normal adrenals with no masses.

Suspicion at this point was that of primary pigmented nodular adrenal hyperplasia (PPNAD) in view of her young age, ACTH independent Cushing's syndrome and lack of adrenal mass found on imaging. Liddle's test was done:

## Unilateral gynecomastia as an initial presentation of hyperthyroid Graves' disease

**Yotsapon Dr. Thewjitcharoen<sup>1</sup>, Soontaree Ms. Nakasatien<sup>1</sup>, Siriwan Ms. Butadej<sup>1</sup>, Thep Prof. Himathongkam<sup>1</sup>**

1. Theptarin Hospital, Klongtoey, Bangkok, Thailand

**Background:** Gynecomastia had been developed infrequently in male patients with hyperthyroidism as a result of the increase in the physiologically active estrogen to androgen ratio. However, its occurrence as the initial presentation in patients with undiagnosed hyperthyroidism is extremely uncommon. We report an unusual case of hyperthyroid Graves' disease presented with unilateral gynecomastia.

**Clinical Case:** A 24-year-old Thai man complained of non-painful enlargement of left breast with palpitation, excessive sweating and weight loss 3 kilograms in 1 month. Physical examination revealed non-tender, palpable glandular tissues, measuring about 3 cm beneath and around left areola. Moderately diffuse thyroid goiter with bruit was also found. He had no obvious thyroid-associated eye signs. Hyperthyroidism Graves' disease was confirmed biochemically with highly elevated anti-TSH receptor antibody. Further laboratory investigations showed an elevated serum estradiol of 88 pg/ml (normal range in male 10-40 pg/ml) and highly elevated serum total testosterone at > 15 ng/ml (normal range in male 2.5-8.4 pg/ml). Treatment of hyperthyroidism with methimazole resulted in resolution of the gynecomastia within 2 months and patient was received radioiodine treatment as a definitive treatment for Graves' disease.

**Conclusion:** Although the association of gynecomastia and hyperthyroidism was described for more than one century, the frequency and severity of this manifestation vary widely among affected patients, and in rare cases gynecomastia could be an initial presentation of hyperthyroidism in male patients. The balance between free testosterone and estrogen in patients with hyperthyroidism is affected by elevated serum levels of sex hormone binding globulin. The clinical manifestations of hyperthyroidism are multi-system, mainly caused by the acceleration of various physiological processes in all organs. This case highlights the importance of considering hyperthyroidism in male patients presented with unilateral gynecomastia.

## Familial dysalbuminaemic hyperthyroxinaemia from a mutation in the ALB gene as a cause for discordant thyroid function tests

**Matthew JM Ting<sup>1</sup>, Rui Zhang<sup>2</sup>, Bryan K Ward<sup>1</sup>, Scott G Wilson<sup>1,3,4</sup>, John P Walsh<sup>1,5</sup>**

1. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

2. Pathwest Laboratory Medicine, Nedlands, WA, Australia

3. School of Biomedical Sciences, University of Western Australia, Crawley, WA, Australia

4. Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom

5. Medical School, University of Western Australia, Crawley, WA, Australia

**Background:** Thyroxine protein binding abnormalities are a rare cause of discordant thyroid function tests in otherwise clinically euthyroid individuals. We report a mother and daughter pair with Familial Dysalbuminaemic Hyperthyroxinaemia causing inappropriately high free T4 levels as measured by immunoassay. **Case report:** A 69 year old female was referred to the thyroid clinic for assessment of discordant thyroid function tests. Biochemistry dating back to July 2015 showed persistently elevated free T4 levels by immunoassay ranging between 25 pmol/L to 34 pmol/L with normal or slightly decreased TSH values ranging between 0.05 mU/L to 1.90 mU/L. The patient was taking thyroxine for Hashimoto's thyroiditis and had been on a stable dose of 100 mcg daily for the past 30 years without any symptoms suggestive of thyrotoxicosis. Previously, dosage reduction of thyroxine to 75 mcg daily resulted in symptoms of hypothyroidism. Measurement of free T4 using Liquid Chromatography - Tandem Mass Spectrometry (LCMS/MS) gave a value of 19.5 pmol/L. Exome sequencing (confirmed by Sanger sequencing) detected a guanine to adenine substitution at residue 725 in exon 7 of the ALB gene, which predicts an arginine to histidine amino acid change (NM\_000477; exon7; hg38: c.G725A:p.R242H). This variant has previously been

reported as associated with dysalbuminaemic hyperthyroxinaemia, and is classified as pathogenic. Subsequent testing of the patient's daughter revealed a similar pattern of thyroid function tests on immunoassay and LCMS/MS as well as the same genetic variant. Comparison of results obtained by different free T4 assays showed the Roche and Centaur platforms to be most affected by the variant, whereas the Abbott Architect gave the best agreement with LCMS/MS. **Conclusion:** Familial Dysalbuminaemic Hyperthyroxinaemia can result in elevated free T4 levels on immunoassay methods and should be considered in the differential diagnosis of discordant thyroid function tests.

## The challenges of post-bariatric surgery hypocalcaemia in pre-existing hypoparathyroidism

**Annabelle M Warren<sup>1</sup>, Annabel S Jones<sup>1</sup>, Leon A Bach<sup>1</sup>, Shoshana Sztal-Mazer<sup>1</sup>**

*1. The Alfred Hospital, Melbourne, VIC, Australia*

**Background** Conventional treatment for hypoparathyroidism relies on gastrointestinal absorption of oral calcium and calcitriol. Challenges in managing post-thyroidectomy hypocalcaemia in patients with a history of bariatric surgery and malabsorption have been described (1, 2), but postoperative management of bariatric surgery in patients with established hypoparathyroidism has not.

**Case A** 46-year-old woman underwent elective sleeve gastrectomy. Past history included iatrogenic hypoparathyroidism and hypothyroidism following total thyroidectomy 20 years earlier in 1999 for multinodular goitre, and psoriatic arthritis on immunosuppression. The operation was complicated by multiple gastric perforations necessitating emergency Roux-en-Y gastric bypass. The patient developed abdominal sepsis and was transferred to a tertiary centre. Four days post-operation, having remained nil orally, the ionised calcium level was 0.78 mmol/L (1.11-1.28 mmol/L) without seizure or arrhythmia, and continuous intravenous calcium infusion via central line was necessary to achieve normocalcaemia. Intravenous calcium gluconate 4.4 mmol 6-hourly was continued for 6 months during sepsis and debridement until oral intake was restored. Intravenous calcitriol twice-weekly reduced calcium requirement and provided relative bone protection. Euthyroidism was achieved with intravenous levothyroxine.

**Discussion** Calcium replacement is complicated when enteral absorption is impaired. Available non-oral options for treatment of hypoparathyroidism will be presented alongside cost analysis. Subcutaneous recombinant human PTH 1-34 (teriparatide) and PTH 1-84 (natpara) are now approved in the USA for hypoparathyroidism, and are recommended in cases of malabsorption (3-5). Strategies to improve enteral absorption of calcium and calcitriol post bariatric surgery such as escalating doses, gastrostomy tube insertion and pancreatic enzyme supplementation have been successfully trialled (6, 7).

**Conclusion** We propose careful consideration be given before bariatric surgery in patients with pre-existing hypoparathyroidism, due to difficulty in managing hypocalcaemia with impaired gastrointestinal absorption, which is exacerbated when complications occur. Approval of subcutaneous recombinant PTH for hypoparathyroidism in Australia will alter future management.

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## Case Study: The clinical utility of Tg doubling time in the management RAI-Refractory follicular thyroid cancer.

**Ayanthi Wijewardene<sup>1</sup>, Benjamin Kwan<sup>1,2</sup>, Robert Russo<sup>3</sup>, Bronwyn Crawford<sup>1,2</sup>**

*1. Department of Endocrinology, Concord Hospital, Concord, NSW, Australia*

*2. University of Sydney, Sydney, NSW, Australia*

*3. Department of Nuclear Medicine, Concord Hospital, Sydney, NSW, Australia*

Thyroid cancer guidelines recommend multi-tyrosine kinase inhibitors(TKI) for radioactive iodine (RAI)-refractory disease<sup>1,2</sup>. In clinical practice initiating TKI therapy remains contentious given significant treatment toxicity. We present a 69-year-old man who, in 2007, had a 30 mm follicular thyroid carcinoma abutting surgical margins and several foci of vascular invasion. A scan 6 months after RAI(3.7 GBq) showed no iodine-avid disease and stimulated Tg was undetectable. He was lost to follow up for five years.

In 2013, he represented with serum Tg 42.1 ug/L (TSH 2.24 mIU/L). A second dose of I-131(4.2 GBq) showed uptake in the thyroid bed only(stimulated Tg 110 ug/L). On suppressive thyroxine Tg remained elevated (29 ug/L). In 2015, a third dose of I-131(4.0 GBq) showed no abnormal uptake (stimulated Tg 173 ug/L) and no FDG avid metastatic focus was seen on subsequent PET imaging.

In 2016 the suppressed Tg doubled in 6 months from 42 to 80 ug/L. Non-specific findings were seen on repeat FDG-PET imaging, including mild FDG avidity(SUV 3.2) at the L. pulmonary hilum and, on low dose CT, 5 subcentimetre nodules in L. lung. A diagnostic chest CT confirmed 5 small pulmonary nodules, 4-7 mm, with no hilar/mediastinal lymphadenopathy, which were not apparent following the fourth dose of I-131(6.2 GBq) . A Ga-68 Dotatate PET scan did not show octreotide avid disease.

Suppressed Tg level increased 10-fold(111 to 1122 ug/L) between Feb 2018 and May 2019. A progress CT chest showed 16 subcentimetre nodules throughout both lungs with a new hilar lymph node metastasis 28x23mm. FDG-PET imaging showed marked FDG avidity at the L hilar mass(SUV 10.3) and several pulmonary nodules were FDG avid.

Although he remained asymptomatic(WHO performance score 0) Lenvatinib was commenced. Our case illustrates the clinical utility of Tg kinetics, in addition to imaging, as an objective measure to guide treatment.

## Come dry spells or high water

**Jovitta William<sup>1</sup>, Sumathy Perampalam<sup>1</sup>**

1. *The Canberra Hospital, Garran, ACT, Australia*

Diabetes insipidus (DI) in pregnancy is a rare condition with an incidence of 1/30,000 (1). The classic clinical picture is that of increased thirst, polydipsia and polyuria, however presentations of oligohydramnios have also been described (2).

As the physiological changes seen in pregnancy closely resemble the pathological appearance of DI and primary polydipsia, differentiating these states clinically and biochemically can be challenging. While water deprivation tests are routinely completed in the non-pregnant population, it is seldom used in the evaluation of DI in pregnancy due to maternal and fetal risk with dehydration and hypernatraemia (3).

We present the case of LG, a 36 year-old G4P3 female presenting at 22 weeks gestation with new thirst and polyuria >8L/day. Given her complex social and psychiatric history, a high degree of suspicion remained for primary polydipsia, necessitating the completion of multiple investigations including MRI brain and a modified water deprivation test to definitively diagnose DI in pregnancy.

Gestational diabetes insipidus (GDI), secondary to increased vasopressinase production or decreased metabolism, is a transient condition of pregnancy, usually appearing in third trimester and resolving within two weeks post-partum (3) at which time desmopressin can be weaned. Surprisingly, LG's excessive polyuria and thirst persisted post-partum, with repeat water deprivation testing at four months demonstrating persistent DI.

LB's early onset of DI in second trimester, absence of symptoms in previous pregnancies, antibody-positive thyroid disease and failure of her DI to resolve post-partum, are suggestive of an acquired decrease in ADH reserve rather than GDI. Our case typifies the difficulties encountered by endocrinologists and obstetricians alike in identifying and formally diagnosing DI in pregnancy, especially when primary polydipsia is suspected. It also demonstrates the importance of considering other causes of DI in pregnancy such as subclinical posterior pituitary pathology, which takes a different course post-partum.

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## A unique case of atezolizumab-induced autoimmune diabetes

**Mimi Wong<sup>1,2</sup>, Nirjhar Nandi<sup>2</sup>, Ashim Sinha<sup>2,3</sup>**

1. *School of Medicine, University of Queensland, Brisbane, QLD, Australia*

2. *Department of Diabetes and Endocrinology, Cairns Hospital, Cairns, QLD*

3. *Department of Medicine, James Cook University, Cairns, QLD*

**Background:** Immunotherapy is a novel treatment which is associated with autoimmune side effects. The risk though of autoimmune diabetes is quite low, occurring in only 0.2-1.0% of patients, and most cases occur following PD-1 rather than PD-L1 inhibitor use. To date there are no prior reports of autoimmune diabetes developing in the setting of immunotherapy in a patient of Indigenous background whom are most susceptible to type 2 diabetes. We report a unique case following atezolizumab use, which is a PD-L1 inhibitor.

**Case Presentation:** A 55 year old Indigenous female was commenced on atezolizumab for recurrent squamous cell lung carcinoma. There was no history of diabetes, thyroid disease and autoimmune disease. Prior to its commencement random blood sugar levels (BSLs) ranged between 4.8 – 7.9 mmol/L and HbA1c was 5.1%. Two months following atezolizumab use, there was the onset of fatigue, polyuria, polydipsia and new hyperglycaemia with random BSLs up to 10.9 mmol/L. Subsequently she was found to have borderline low C-peptide levels of 0.6 nmol/L (0.5 – 1.0 nmol/L), and positive zinc transporter 8 (ZnT8) antibodies with undetectable glutamate acid decarboxylase and islet tyrosine phosphatase 2 antibodies. Following the diagnosis of autoimmune diabetes, she was commenced on low dose lantus which maintained BSLs between 5 –



7 mmol/L and resolved her symptoms of hyperglycaemia. Although atezolizumab is thought to have led to the development of autoimmune diabetes, it was initially continued with close oncology and endocrinology follow-up.

**Conclusion:** There are few case reports of atezolizumab-induced autoimmune diabetes, and we present the first case associated with ZnT8 antibodies. In addition this case is also unique in that it occurred in a patient of Indigenous background.

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## Nesidioblastosis in an adult with short gut syndrome and type 2 diabetes

**Mimi Wong<sup>2,1</sup>, Luke Conway<sup>1</sup>, Caroline Cooper<sup>2,3</sup>, Ashim Sinha<sup>1,4</sup>, Nirjhar Nandi<sup>1</sup>**

1. Department of Diabetes and Endocrinology, Cairns Hospital, Cairns, QLD

2. School of Medicine, University of Queensland, Brisbane, QLD, Australia

3. Department of Pathology, Princess Alexandra Hospital, Woolloongabba, QLD

4. Department of Medicine, James Cook University, Cairns, QLD

**Background:** Adult nesidioblastosis is characterised by endogenous hyperinsulinemia typically causing post-prandial hypoglycaemia, and most commonly occurs following bariatric surgery. We report a unique case of nesidioblastosis in a 67 year old female.

**Case Presentation:** A 5 year history of symptomatic hypoglycaemia occurred in a patient with short bowel syndrome and with a history of type 2 diabetes mellitus. In 2012 she was diagnosed with type 2 diabetes and was managed with a glucagon-like peptide (GLP) 1 agonist (*Exenatide* 10mcg twice a day) for 18 months. Since 2013 she had episodes of symptomatic hypoglycaemic, which were particularly worst in the last 12 months causing neuroglycopenic symptoms. Continuous glucose monitor captured 42 hypoglycaemia episodes in a two week period, with her lowest blood sugar level of 2.0 mmol/L. There was no suggestion of a medical illness or medication contributing to her hypoglycaemia, with a negative sulphonylurea screen and insulin antibody of 0.4 units (<0.7 units). There however was the suspicion of a hyperinsulinemia state following a venous fasting sample where her glucose was 8.1 mmol/L, insulin 210 mU/L (<25 mU/L) and C-peptide 3.8 nmol/L (0.2 – 1.2 nmol/L). Localizing studies did not identify a focal pancreatic lesion, and included magnetic resonance imaging, PDOPA positron emission tomography and endoscopic ultrasound. She was managed with an open distal pancreatectomy, and her operative specimen has confirmed the diagnosis of nesidioblastosis. Post-operatively she had ongoing issues with hypoglycaemia, which necessitated the use of octreotide to maintain euglycaemia.

**Conclusion:** We present the first case of nesidioblastosis occurring in a patient with short gut syndrome and pre-existing type 2 diabetes, managed in the past with a GLP-1 agonist, all of which may have been a predisposing factor for islet cell hyperplasia.

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## When the stakes are high but the calcium is higher

**Linda Wu<sup>1</sup>, Mark Sywak<sup>3,2</sup>, David Marshman<sup>4</sup>, Sean Seeho<sup>3,5</sup>, Jeremy Hoang<sup>1,6</sup>, Tang Wong<sup>7</sup>, Venessa Tsang<sup>1,3</sup>**

1. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Department of Endocrine Surgery, Royal North Shore Hospital, St Leonards, New South Wales, Australia

3. Northern Clinical School, University of Sydney, Sydney, NSW, Australia

4. Department of Cardiothoracic Surgery, Royal North Shore Hospital, St Leonards, NSW, Australia

5. Department of Obstetrics and Gynaecology, Royal North Shore Hospital, St Leonards, New South Wales, Australia

6. Department of Nuclear Medicine, Royal North Shore Hospital, St Leonards, New South Wales, Australia

7. Department of Endocrinology, Prince of Wales Hospital, Randwick, NSW, Australia

A 33 year old primigravid woman was referred for endocrine review for asymptomatic hypercalcaemia at 8 weeks gestation. Serum corrected calcium was 2.66 mmol/L with an inappropriately raised parathyroid hormone (PTH) 76ng/L (10-65ng/L) and raised 24hr urine fractional excretion of calcium (FECa) of 0.05. Calcium progressively rose to 2.9 mmol/L by 22 weeks gestation. Neck ultrasound showed multiple thyroid nodules with the dominant nodule having Bethesda category IV cytology however no parathyroid adenoma visible. Neck exploration and right hemithyroidectomy was undertaken at 24 weeks gestation with 4 glands visualised and 2 parathyroid glands resected. Histopathology revealed 2 fat replete parathyroid glands, 23mm follicular carcinoma and a focus of papillary microcarcinoma. Calcium and PTH transiently normalised but by 25 weeks gestation cCa was 2.96mmol/L and PTH 85ng/L. A low dose Sestamibi scan was performed and localised a posterior mediastinal parathyroid adenoma at T4 level. A multidisciplinary decision was made to proceed with hemisternotomy at 26 weeks gestation with successful resection of a 975mg fat deplete parathyroid adenoma. Post-operative PTH was low initially and subsequently normalised and calcium levels remained within normal limits.

### Discussion

Primary hyperparathyroidism in pregnancy carries significant risks, with a recent case series reporting preeclampsia in 30% of medically managed patients and preterm delivery in 66%<sup>1</sup>. Key considerations for surgical resection of parathyroid adenoma in pregnancy include gestational age, localisation modalities with radiation exposure in pregnancy and in the case of ectopic adenoma, surgical feasibility. This is the second case ever published where sternotomy has been successfully performed for treatment of ectopic parathyroid adenoma in pregnancy<sup>2</sup>.

### Conclusion

Ectopic parathyroid adenoma in pregnancy are a high risk situation where risks of surgery need to be carefully weighed with risks of hypercalcaemia and maternal and fetal wellbeing.

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## Growing concerns: an unusual case of acromegaly

Linda Wu<sup>1</sup>, Benjamin Harris<sup>3,2</sup>, Lyndal Tacon<sup>1,3</sup>

1. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Department of Respiratory Medicine, Royal North Shore Hospital, St Leonards, New South Wales, Australia

3. Northern Clinical School, University of Sydney, Sydney, NSW, Australia

A 41 year old lady presented with menstrual irregularities, increasing shoe size and snoring. Examination revealed prognathism, macroglossia, thickened skin and acral enlargement. Blood pressure and visual fields were normal. Investigations confirmed elevated growth hormone (GH) 55.8 mIU/L (0-15) that failed to suppress after 75g glucose load (74.7 mIU/L). IGF-1 was elevated, 69 nmol/L (11-35). The remainder of the pituitary profile, serum calcium and parathyroid hormone, were normal. MRI revealed diffuse enlargement of the pituitary with a possible 2-3 mm microadenoma. Background history included a 10 year history of slowly enlarging left lower lobe pulmonary nodule. FDG-PET/CT scan revealed the lesion to be minimally FDG avid; it demonstrated internal calcifications and distal bronchiectasis, consistent with a carcinoid tumour. The patient underwent resection of the lesion. Post-operatively, GH and IGF-1 returned to within the reference range. The patient reported normalisation of menses, cessation of snoring, and 7 kg weight reduction. Histology confirmed grade 1 neuroendocrine tumour (NET). Immunostaining for GH was negative, and immunohistochemistry for growth hormone releasing hormone (GHRH) is awaited. MEN1 gene analysis is underway.

### Discussion

Acromegaly due to ectopic secretion of GHRH accounts for less than 1% of cases<sup>1</sup>. Literature is limited to case reports<sup>2</sup> and two case series<sup>3,4</sup>. The clinical phenotype varies in severity, and IGF-1 values range from 1.1 to 4.2 times the upper limit of normal. When available, GHRH is always elevated and is a helpful distinguishing tool<sup>3</sup>. Pituitary imaging reveals diffuse enlargement in the majority of cases, with the minority being normal or having a pituitary adenoma<sup>2,3</sup>. Bronchial and pancreatic NETs are most often causative and, when performed, GHRH immunostaining is positive<sup>2,3</sup>.

### Conclusion

Acromegaly secondary to ectopic GHRH secretion from a NET is rare, but should be considered in cases where pituitary imaging is not strongly suggestive.

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## A case of multisystem Langerhans-Cell Histiocytosis: A rare but important cause of diabetes insipidus

Rebecca J Foskey<sup>1</sup>, Christopher J Yates<sup>1,2</sup>

1. Western Health, Sunshine, Victoria, Australia

2. Department of Medicine (RMH), The University of Melbourne, Parkville, Victoria, Australia

**Introduction** Diabetes insipidus may rarely be due to Langerhans-Cell Histiocytosis (LCH) and is the presenting symptom in 14-15% of patients with LCH.

**Clinical case** A 46-year-old male presented with typical symptoms of diabetes insipidus on a background of presumed alcoholic steatosis, anxiety and gastro-oesophageal reflux disease. Central diabetes insipidus was confirmed by a water deprivation test. Pituitary biochemistry was otherwise unremarkable, however an MRI demonstrated a thickened, homogeneously enhancing pituitary gland and stalk with loss of the pituitary bright spot. A CT demonstrated lytic lesions in the pelvis, [pulmonary micronodular infiltrate in bilateral upper lobes, and liver changes confirmed on MRCP to be a lesion infiltrating hepatic biliary ducts. Biopsy of the bone lesion was non-diagnostic, but lung biopsy eight months post-presentation was consistent with LCH.

The patient was managed with cytarabine chemotherapy for 12 months; an FDG PET scan after three months of treatment revealed no avid lesions. At completion of the course clinical remission was achieved, with the exception of ongoing diabetes insipidus. Seven months after completing chemotherapy, the patient developed hypogonadotropic hypogonadism requiring testosterone replacement. The remainder of the pituitary biochemistry was unremarkable. His liver function tests continued to be deranged. Gene testing was positive for the BRAF V600E mutation.

Two years following completion of chemotherapy, the patient developed progressive diabetes insipidus necessitating increased doses of desmopressin. He reported subjective cognitive slowing, headaches, nausea, bone pain and fatigue. Cognitive testing was unremarkable and pituitary MRI demonstrated probable size increase of both the pituitary gland and stalk, however repeat

PET imaging was normal. Without further therapy, the patient described resolution of all symptoms except diabetes insipidus within five months.

**Clinical lessons** Langerhans-Cell Histiocytosis should be considered as a differential diagnosis for diabetes insipidus with pituitary stalk thickening. Even with effective systemic therapy, pituitary hormone dysfunction persists indefinitely.

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## Lactation ketoacidosis: case study and literature review of this unusual entity

**Aakansha Zala**<sup>1, 2</sup>, **Henrik Falhammar**<sup>2, 3, 4</sup>

1. *University of Queensland, Brisbane, QLD, Australia*

2. *Royal Darwin hospital, Darwin, NT, Australia*

3. *Department of Molecular Medicine and Surgery, Karolinska Institute,, Stockholm, Sweden*

4. *Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden*

### Abstract:

Lactation ketoacidosis is becoming an increasingly recognised phenomenon. We present the case of a 32-year-old female, 5 months post-partum, who presented with lethargy, nausea and abdominal pain. She was a fitness instructor and still breastfeeding. 48 hours prior to admission, she had worked double training shifts involving high intensity exercise and due to time constraints had reduced her oral intake. Initial blood tests revealed a high anion gap metabolic acidosis with hypoglycaemia [pH 7.26 (normal 7.30–7.40), bicarbonate 12.3 mmol/L (normal 21.0–28.0), anion gap 14.7 mmol/L (normal 8–12), glucose 2.9 mmol/L (normal fasting 3.9–5.8) and ketones 4.8 mmol/L (normal <0.6)]. She had no known diabetes. She was treated with IV dextrose and other causes of hypoglycaemia were excluded. She had complete recovery within 24 hours with resolution of her ketoacidosis. She was discharged home with the diagnosis of lactation ketoacidosis and remained well on a balanced diet with advice on limiting high intensity exercise while breastfeeding. Her lactation ketoacidosis was believed to be triggered by reduced carbohydrate intake and increased high intensity exercise while breastfeeding. We explore the literature surrounding this increasingly identified phenomenon.

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## Metastatic paraganglioma in an SDHB mutation positive patient

**Jasmine J Zhu**<sup>1</sup>, **John R Burgess**<sup>1, 2</sup>

1. *Department of Endocrinology, Royal Hobart Hospital, Hobart, TAS*

2. *School of Medicine, University of Tasmania, Hobart, TAS*

Paragangliomas are rare neoplasms arising from pluripotent neural crest cells in the autonomic nervous system. They may secrete catecholamines, leading to flushing and hypertensive crises. Although resection is recommended as first-line treatment for localised disease, peptide receptor radioligand therapy (PRRT) is effective and well-tolerated for disseminated disease that expresses somatostatin receptors. A unique feature of paragangliomas is their slow and minimal response to PRRT, radiotherapy and somatostatin analogues. Even when successfully treated, they often demonstrate a residual mass, the presence of which does not necessarily indicate treatment failure. We report a case of metastatic paraganglioma in an SDHB mutation positive patient who was successfully treated with PRRT. A 12-year-old male experienced a hypertensive crisis during routine anaesthetic induction for an elective procedure. After  $\alpha$  followed by  $\beta$  blockade, an extra-adrenal secretory paraganglioma was resected. He was subsequently found to be SDHB-mutation positive. At the age of 15, he underwent re-resection of locally recurrent disease. Due to a progressively rising chromogranin A, an FDG-PET scan was performed at the age of 22. This showed retroperitoneal soft tissue disease and widespread skeletal metastases. These lesions were avid on a GATATE PET scan but not on a MIBG scan. Retrospective review of MRI images from the previous year revealed that one of the bony lesions had already been present on this scan and had not changed in size. In combination, these imaging findings suggested slowly progressive disease with high somatostatin receptor expression. The patient was treated with three cycles of PRRT combined with 5-FU as a radiosensitising agent, which was well tolerated. Serial imaging over a five-year period has demonstrated stable lesions. He is currently being managed with somatostatin analogues. This case illustrates the role of different imaging modalities in the evaluation of neuroendocrine tumours and the complexities of managing disseminated disease.

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## Analysis of Histone 3 Lysine 9 acetylation in the 2-cell mouse embryo

**Gulfam Ahmad**<sup>1</sup>, **Yan Li**<sup>1</sup>, **Chris O'Neill**<sup>1</sup>

1. *Human Reproduction Unit, Kolling Institute for Medical Research, Northern Clinical School, Sydney University and Royal North Shore Hospital,, St Leonards, NSW, Australia*

Histones package DNA into nucleosomes and lysines within their tail are common targets for acetylation. Acetylation neutralizes the negative charge on lysine allowing DNA to be less tightly wrapped around the nucleosome. This facilitates access by the transcriptional machinery. Acetylation is also a docking site for bromodomain proteins that serve as assembly sites for transcriptional complexes. The minor round of transcription that occurs in the 1-cell embryo requires the onset of global histone 3 lysine 9 acetylation (H3K9ace) and this was perturbed by culture of zygotes in vitro<sup>1</sup>. Here we report on the association of H3K9ace with the epigenetic reprogramming that occurs in the 2-cell embryo and the effects of embryo culture on this process.

Hybrid mouse embryos were collected at the 1-cell (18 h post hCG) or 2-cell (42 h post hCG) (fresh) stage. 1-cells were then cultured under standard conditions to an equivalent time of 2-cell development (cultured). H3K9ace was quantified in the resulting 2-cell embryos by indirect fluorescence immuno-staining intensity using Image Pro Plus software.

Mean nuclear H3K9ace staining per pixel and the total staining across the nuclei were significantly lower in cultured embryos compared to fresh ( $p < 0.0001$ ;  $0.02$ ), respectively. Average nuclear cross-sectional area was significantly larger in cultured embryos ( $p = 0.001$ ) as was the number of nucleoli precursor bodies per nuclei ( $p = 0.0004$ ). The distribution of heterochromatic staining and its association with H3K9ace was also disturbed in cultured embryos.

Contrary to the hyperacetylation observed in cultured zygotes<sup>1</sup>, culture to the 2-cell stage was associated with a relative global hypoacetylation of the genome. This was accompanied by changes in nuclear organisation, including nucleoli maturation. Our studies point to a complex pattern of alterations in the epigenetic landscape in the early embryo in response to the various stresses imposed upon it by culture *in vitro*.

1.Reproduction 2017:154:375-385

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## Diverse origins of the Fallopian tube epithelium

**Arnab Ghosh<sup>1</sup>, Pradeep S Tanwar<sup>1</sup>**

1. *University of Newcastle, Newcastle, NSW, Australia*

Publish consent withheld

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## The effects of IVF on the regulation of histone acetylation in the preimplantation embryo

**Wed Salah<sup>2,1</sup>, Chris O'Neill<sup>2</sup>, Yan Li<sup>2</sup>**

1. *Jeddah University, Jeddah, Saudi Arabia*

2. *University of Sydney, St Leonards, NSW, Australia*

The onset of gene expression from the new embryonic genome requires the conversion of chromatin from a transcriptionally repressive to permissive state. One component of this conversion is the extensive global acetylation of nuclear histones, including acetylation of lysine 9 within histone 3 (H3K9ace). H3K9ace is not detected in the newly fertilized embryo, with acetylation starting around the time of first DNA replication<sup>1</sup>. This reprogramming coincides with the period in which embryos are exposed to the culture environment during assisted reproduction technologies. We showed that embryo culture caused a marked perturbation in the onset of H3K9ace which in turn altered the earliest round of gene expression<sup>1</sup>. In this study we explored which aspects of culture are associated with this perturbation.

Quantitative indirect immunofluorescence was used to assay changes in global H3K9ace levels. We compared the H3K9ace levels in zygotes fertilized *in vitro* and then culture *in vitro* (IVF) with zygotes fertilized and matured within the reproductive tract (Fresh) and those fertilized *in situ* and then cultured *in vitro* (ISF). H3K9ace was significantly greater in IVF zygotes compared to either ISF or Fresh, while ISF was higher than Fresh. The effect of IVF was independent of media formulation. Superovulation had no impact compared to spontaneous ovulation.

Our results confirm that manipulation of the embryo *in vitro* causes a global disturbance in epigenetic reprogramming and shows that this effect is compounded by IVF. This appears to be a stress response to the conditions of culture *per se*, since another major putative stressor, superovulation, had no impact. These results confirm the utility of H3K9ace measurements as a biomarker of the effects of stress on the early embryo.

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## Analysis of Circulating MicroRNAs Expression in Heat stressed Dairy Cows

**Jihwan Lee<sup>1</sup>, Seunghwan Lee<sup>2</sup>, Jongho Kim<sup>2</sup>, Taiyoung Hur<sup>1</sup>, Hyun-Joo Lim<sup>1</sup>, Junkyu Son<sup>1</sup>, Jihoo Park<sup>1</sup>, Seungmin Ha<sup>1</sup>, Euntae Kim<sup>1</sup>, Donghyeon Kim<sup>1</sup>, Inchul Choi<sup>2</sup>**

1. *National Institute of Animal Science, Seonghwan-eup, Seobuk-go, Cheonan-si, Chungcheongnam-Do, South Korea*

2. *Department of Animal Biosystem Sciences, College of Agriculture and life sciences, Chungnam National University, Daejeon, Republic of Korea*

There has been a widespread and rapid rise in annual average temperature over the Korean peninsula, for example, 0.41°C from 1981 to 2010 and 0.5°C from 2001 and 2010. Particularly, subtropical weather such as humid and high temperature in Korea affects the productivity of Holstein dairy cows that are more sensitive to heat stress. miRNA profiles are widely used to investigate physiological changes in dairy cows exposed to heat stress (HS), however the profiles were limited to economic traits including milk production. In this study, we analyzed miRNA expression from whole blood and compared the expression between HS and non-HS cows to understand regulation of biological process in HS cows. The whole blood was collected from the jugular vein of the same cows (n=9) at summer (THI >86; average rectal temp., 39.6°C) and autumn (<63; 38.1°C), respectively. Further, decline in milk yield was recorded in the cows during HS condition. To analyze the profiles of miRNAs, RNA-seq was performed on an Illumina Hiseq 2500 sequencer. The data identified 79 differentially expressed miRNAs that

were significantly changed between HS and non-HS cows (fold change  $\geq 1.5$ ,  $P < 0.05$ ); 43 miRNAs were up-regulated (for example, bta-miR-15b, bta-miR-19a, bta-miR-19b, bta-miR-20a, bta-miR-29c, bta-miR-301a, bta-miR-301b, bta-miR-374b, bta-miR-199b) and 36 miRNAs down regulated in HS cows. Target- gene analysis using miRmap DAVID showed that the selected miRNAs might be associated with Leukocyte transendothelial migration, adherens junction, chemokine signaling pathways. Interestingly, splicesome, base excision repair, and TGF-beta and Glucagon signaling pathway were also detected in the analysis. In summary, our study provides expression profiles of miRNAs that are differently responded to HS and the profiles can be used to understand physiological changes and as potential markers for evaluation of HS cows.

## Expression of TETs in epithelial cells across the menstrual cycle

**Vishakha Mahajan**<sup>1</sup>, **Cindy Farquhar**<sup>2</sup>, **Anna P. Ponnampalam**<sup>1,2,3</sup>

1. *The Liggins Institute, The University of Auckland, Grafton, Auckland, New Zealand*

2. *Obstetrics and Gynaecology, The University of Auckland, Grafton, Auckland, New Zealand*

3. *Department of Physiology, The University of Auckland, Grafton, Auckland, New Zealand*

**Background:** Ten Eleven Translocation Proteins (TET) mediate DNA hydroxymethylation, a biologically critical epigenetic mechanism that is known to activate gene expression. Previously, our data demonstrated significant and dynamic changes in the mRNA expression of TETs across the menstrual cycle. The aim of this study is to localise TET proteins and determine the hormonal regulation of TETs in the endometrium.

**Methods:** Endometrial tissues were obtained after informed written consent from women with normal cycles. TETs were localised using colorimetric immunohistochemistry. To determine the hormonal regulation of TETs, HES cells (endometrial epithelial cell-line) were first treated with estradiol for 24 hours and subsequently treated with a combination of estrogen and progesterone for 24, 48 and 72 hours, *in vitro*. RNA was extracted and TET gene expression was determined using real-time PCR.

**Results:** TET proteins were localized in the nucleus of epithelial and stromal cells, throughout the menstrual cycle. Strong immunostaining of TET1 and 2 were observed in the glandular epithelium, during proliferative, early and mid-secretory phases which reduced during the late-secretory phase. Conversely, TET3 immunostaining was the highest during proliferative phase, then reduced during the mid to late-secretory phases. Strong universal staining was seen throughout the glandular and luminal epithelium. In contrast, staining in the stroma was not universal with immunostaining present in some cells and absent in others throughout the stroma. TET 1 and TET 3 transcriptions in HES cells were up-regulated in response to combined treatment of estrogen and progesterone for 72 hours compared to control. While, TET2 transcription was the highest with the combined treatment for 48 hours.

**Conclusion:** Our data imply that TETs are expressed in a cell-specific and dynamic manner in the endometrium and they are responsive to varying levels of estrogen and progesterone. Further investigations are underway to elucidate their role and interaction with other epigenetic machineries in the endometrium.

## Expression quantitative trait loci in endometrial stromal cells isolated from women with and without endometriosis

**Brett McKinnon**<sup>1,2</sup>, **Sally Mortlock**<sup>1</sup>, **Joanna Crawford**<sup>1</sup>, **Michael Mueller**<sup>2</sup>, **Grant Montgomery**<sup>1</sup>

1. *Institute for Molecular Bioscience, St Lucia, QLD, Australia*

2. *Department of Gynaecology, Frauenklinik, Inselspital, Bern, Switzerland*

Endometriosis is a chronic inflammatory disease characterised by the growth of endometrial epithelial and stromal cells outside the uterine cavity. It is associated with pelvic pain and puts a significant strain on the individual and health care system. Genome wide association studies have identified 27 regions associated with increased susceptibility to endometriosis. The critical SNPs in each region are intergenic, or intronic. The next critical step is identifying the target genes and we are analysing the genetic effects on gene expression. Expression quantitative trait loci (eQTL) are genetic regions that influence the expression of multiple genes. An overlap between endometriosis associated genetic variants and eQTLs could identify genes related to endometriosis. We previously identified eQTL in endometrial tissue using microarray data identifying eQTLs for 453 unique genes using the Bonferroni genome wide significant threshold. Endometrial tissue however consists of both epithelial and stromal cells and eQTL are tissue and cell specific. It is likely distinct eQTLs may exist in both the epithelial and stromal cells.

Pure populations of endometrial stromal cells were isolated from 59 endometrial biopsies and genome wide genotyping performed and additional single nucleotide polymorphisms imputed using the 1000 genomes project. Genome wide gene expression was determined using RNA-seq and genome-wide genetic influence on gene expression was determined.

The result identify 14,307 genes expressed in 90% of samples with 6,424 cis-eQTL passing the Bonferroni test ( $p, 3.3 \times 10^{-9}$ ) for 111 unique genes. Of these 111 genes, cis-eQTL for 29 genes were not previously detected in whole endometrial tissue. The 29 unique genes are not located within the 27 regions associated with endometriosis. However, the genetic regulation of gene expression in individual cell types from the endometrial tissue should be considered to understand gene regulation in the endometrium and their potential role in complex genetic reproductive disorders.

## RNA-seq analysis of the developing Müllerian duct in the Chicken embryo

**Zahida Yesmin Roly<sup>1</sup>, Andrew Major<sup>1</sup>, Craig Smith<sup>1</sup>**

1. Monash Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia

Müllerian ducts are embryonic structures of the female reproductive tract (Fallopian tubes, uterus and upper vagina in mammals, the oviducts in birds). In most vertebrates, embryonic Müllerian ducts initially develop in both sexes, but later they regress in males under the influence of testis-derived Anti-Müllerian Hormone (AMH). While a number of genes or regulatory factors have been implicated in proper duct development and differentiation, many of the molecular details remain obscure. We used RNA sequencing to profile gene expression in the Müllerian duct at key embryonic time points in the chicken embryo; specification (day 4.5/stage 25), invagination (day 5.5/stage 28) and elongation (day 6.5/stage 30). Compared to posterior mesonephros negative control, 234 genes were differentially expressed in the day 4.5 Mullerian duct, 447 at day 5.5 and 610 genes by day E6.5. Gene ontology and pathway analysis indicated that many of these genes encoded proteins involved in extracellular matrix receptor function, Calcium signaling pathway, cell differentiation and migration. Expression of ten novel genes was validated by RT-PCR and whole mount *in situ* hybridization. These genes were *POSTN*, *SMARCA2*, *TSHZ3*, *TGFBI*, *COL1A2*, *HTRA3*, *PRICKLE*, *LOXL2*, *FOXE1* and *RUNX1*. Functional Analysis of some of these genes is being conducted using *in ovo* electroporation (CRISPR/Cas9 editing). This research will provide new insights into Müllerian duct development and reveal potential new players in Müllerian duct disorders.

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### Differential effects of activin A on the responses to inflammatory activation in cultures of bone marrow-derived and cell line macrophages

**Julia Bender<sup>1</sup>, Yalong Yang<sup>1</sup>, Monika Fijak<sup>1</sup>, Kate Loveland<sup>3,2</sup>, Sudhanshu Bhushan<sup>1</sup>, Mark Hedger<sup>3,2</sup>, Andreas Meinhardt<sup>1,3,2</sup>**

1. Institute of Anatomy and Cell Biology, Justus-Liebig-University of Giessen, Giessen, Hesse, Germany

2. Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, Victoria, Australia

3. Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia

Testicular macrophages contribute to the immune privilege of the testis through their immunoregulatory properties. The mechanisms of regulation of the essentially anti-inflammatory testicular macrophage phenotype are not well understood, but attention has been drawn to the role of the immunoregulatory cytokine, activin A. Within the immune system, activins exert both pro- and anti-inflammatory actions, depending on the immunological context. Activin A is produced by a number of testicular cell types, including the Sertoli cells, which have been shown to possess graft-protecting activities in co-transplantation studies. In order to investigate macrophage responses to activin, two murine macrophage models were compared: CSF1-matured bone marrow-derived macrophages (BMDM) and the RAW246.7 (RAW) macrophage cell line, which differ significantly in their activation status and downstream inflammatory signalling pathways. In BMDM cultures, activin A (50 ng/ml) significantly induced the secretion of the pro-inflammatory cytokine TNF upon stimulation (6 and 24h) with bacterial lipopolysaccharide (10-100 ng/ml). The secretion of the anti-inflammatory cytokine, IL-10, upon LPS challenge was significantly decreased by activin A. Additionally, the mRNA expression of pro-inflammatory markers (*Tnf*, *Gpr18*) were significantly increased by activin A in both resting and LPS-activated BMDM, while the expression levels of anti-inflammatory markers (*Chil3*, *Mrc1*) were decreased. In contrast, production of TNF was significantly decreased by activin A in RAW macrophages. A reduction in production of the pro-inflammatory mediator, NO, was observed in both macrophage types, while its anti-inflammatory counterpart, *Arg1*, was elevated. In summary, these studies indicate that activin A exerts predominantly pro-inflammatory effects in BMDM, whereas preliminary data in RAW macrophages suggest an anti-inflammatory response towards activin A treatment. Further investigation of the differences between the responses of macrophages under different activation states will help to elucidate the role that activin A plays in regulating macrophage activity in the testis and other tissues.

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### DLA Class II alleles and haplotype analysis for DLA-identical immunomodulatory effect in canine

**Hayeon Wi<sup>1</sup>, Seunghoon Lee<sup>1</sup>, Keonbong Oh<sup>1</sup>, Ran Lee<sup>1</sup>, Youngyim Kim<sup>1</sup>, Eung Woo Park<sup>1</sup>, Sun A Ock<sup>1</sup>**

1. Animal Biotechnology, National Institute of Animal Science, Rural Development Administration, 1500, Kongjwipatjwi-ro, Iseo-meyon, Wanju-gun, Republic of Korea

Recently, number of dogs have been treated with cell therapy showing variable effect among the treated dogs. Dog Leukocyte Antigen (DLA) have many genes associated with immune system. Therefore, matching of DLA gene is considered an important factor for cell therapy. The aim of the this study was to investigate dog leukocyte antigen(DLA) class II alleles and haplotypes. Sequence-based genotyping of the polymorphism region exon2 from DQA,DQB,DRB class II loci were performed the normal 5 dog(Beagle) in 2 pedigree(parent2,sibling3). The allele names determined according to the universal nomenclature found in IPD-MHC sequence data base. DLA- DQA : 014012, DQB : 04201, DRB : 02002 were used as a reference gene and alignment was performed using seqman software. We identified haplotype (DLA-DQA\*00401/DQB\*02301/DRB\*04801) in first pedigree and (DLA-DQA\*00101/DQB00201/DRB\*00101) in second pedigree. Many Single nucleotide polymorphism(SNPs) to tag haplotype compared to the reference gene. In these SNPs DLA-DRB has the highest SNP(1 pedigree:22(60%), 2 pedigree :28(67%)). These results suggest that DRB is the most highly polymorphic in classII loci. This study serve as a reference for donor and recipient matching in the canine model for investigated immunomodulatory function of DLA-identical cell therapy.

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## Region-specific inflammatory responses in the male reproductive tract: studies on a murine autoimmune epididymitis model

**Rukmali Wijayarathna<sup>1,2</sup>, Alen Pasalic<sup>1,2</sup>, Rama Ravinthiran<sup>1,2</sup>, Nour Nicolas<sup>3</sup>, Kate Loveland<sup>1,2</sup>, Andreas Meinhardt<sup>4</sup>, Monika Fijak<sup>4</sup>, Mark P Hedger<sup>1,2</sup>**

1. Hudson Institute of Medical Research, Clayton, VIC, Australia
2. Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Clayton, VIC, Australia
3. Laboratory of Development of the Gonads, Unit of Genetic Stability, INSERM, Paris, France
4. Department of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany

Epididymitis is a leading cause of male reproductive disease and infertility. Our previous studies in a bacterial epididymitis model revealed that the cauda epididymis was severely damaged by fibrosis, while the caput region remained relatively intact. The caput epididymis, where spermatozoa are first exposed to the immune system outside the immune-privileged testis, contains numerous immunoregulatory cells (dendritic cells, macrophages) and genes, such as activin A (*Inhba*), activin B (*Inhbb*), and indolamine 2,3-deoxygenase-1 (*Ido1*). These immunological parameters were considerably reduced in the cauda, where spermatozoa are stored prior to ejaculation.

A murine model of experimental autoimmune epididymo-orchitis was used to investigate the regional differences in the immune response. Adult C57/Bl6 mice were immunised with syngeneic testicular homogenates in adjuvant (s.c. 3x, fortnightly). Controls received adjuvant only, or were untreated. Tissues were collected 50 days following the first immunisation.

All animals that developed orchitis also showed epididymitis, but epididymitis alone was observed in some animals. Epididymitis was characterized by epithelial damage, immune cell infiltrates and fibrosis in the cauda. In extreme cases, sperm were observed in the interstitium. The caput appeared relatively normal in all animals. Based on histopathology and cytokine expression, a damage score ranging from 0-5 was established (0 = normal, 5 = severe epididymitis). The severity of orchitis and epididymitis were positively correlated. The expression of genes important in epididymal immunoregulation and inflammation, including *Ido1*, *Foxp3*, *Tnf*, *Il1b*, *Il10*, *Tgfb1* and *Ccl2*, correlated with the damage score in the cauda. Only a minor upregulation of genes was seen in the caput.

These data indicate that the caput and cauda have very different immunological environments. We postulate that the caput is more tolerogenic/anti-inflammatory to protect immunologically 'foreign' sperm from the body's immune system, while the cauda is primed to combat ascending infections, and is therefore more susceptible to inflammation.

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## Comparisons of Male Reproductive Parameters between Type 1 and 2 Diabetes Mice

**Supatcharee Arun<sup>3,1,2</sup>, Apichakan Sampanang<sup>2</sup>, Sitthichai Iamsaard<sup>3,1,2</sup>**

1. Performance & Health Promotion (HHP&HP) Research Institute, Khon Kaen University, Khon Kaen, Thailand
2. Mekong Health Science Research Institute, Khon Kaen University, Khon Kaen, Thailand
3. Anatomy, Khon Kaen University, Ampoe Muang Khon Kaen, KHON KAEN, Thailand

Diabetes mellitus (DM) is a cause of male infertility, but the differences of adverse reproductive parameters have never been elucidated. This study aimed to distinguish between type 1 and type 2 diabetes (T1DM and T2DM) on reproductive parameters using male mice treated with multiple-low doses of streptozotocin (MLD-STZ, T1DM) and high-fat diet with streptozotocin (HFD-STZ, T2DM) for 36 and 72 days. In T1DM, mice were intraperitoneally injected with STZ (40 mg/kg body weight) for 5 days. For T2DM, mice received HFD for 14 days and induced by STZ (85 mg/kg body weight) with fed by HFD continuously. Male reproductive parameters, indicating sperm quality and histology were evaluated. The results showed that reproductive organ weights were significantly decreased in DM mice, but seminal vesicle plus prostate gland of T1DM was lower than that of T2DM. The increases of blood glucose levels, sperm abnormality, and incomplete sperm DNA packaging were observed in T1DM and T2DM groups. Sperm concentration and normal sperm morphology were significantly decreased in T1DM compared to those of T2DM. Seminiferous histopathology of DM animals was classified into 7 types. Morphometry of seminiferous tubule and penis was decreased whereas tunica albuginea thickness and penile collagen fibers were increased in DM mice. Round cells were abundantly found in the epididymal lumens of DM mice. In conclusion, the adverse reproductive parameters of T1DM were more present than T2DM.

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## Enumerating macrophages in the mouse testis using classical histological and immunohistochemical techniques

**Sneha Biniwale<sup>1</sup>, Rukmali Wijayarathna<sup>1</sup>, Sivanjah Indumathy<sup>1</sup>, Samira Hosseini<sup>1</sup>, Sudhanshu Bhushan<sup>2</sup>, Andreas Meinhardt<sup>2</sup>, Kate Loveland<sup>1</sup>, Mark Hedger<sup>1</sup>**

1. Hudson Institute of Medical Research, Clayton, VIC, Australia
2. Institute of Anatomy and Cell Biology, Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany

Immunohistochemistry is widely used to identify immune cells, but reliable detection of antigens is not always compatible with good-quality fixation. This is particularly problematic for rodent testes, where preservation of morphology is crucial. Testicular cells can be differentiated in well-fixed, paraffin-embedded sections, but morphometric analysis using morphological criteria is time-consuming. Furthermore, testicular interstitial macrophages may be easily discriminated, but the peritubular macrophages are difficult to visualise. The aim of this study was to optimise and validate methods for detection and quantification of



macrophages using several histological stains and immunohistochemical protocols in Bouin's-fixed, paraffin-embedded testes from mice with altered levels of the immunoregulatory cytokine, activin and its binding protein, follistatin: (a) *Inhba*<sup>-/-</sup> (reduced activin), (b) *Inha*<sup>-/-</sup> (activin over-production), and c) TghFST315 transgenics (reduced follistatin). May-Grunwald-Giemsma stain provided the best discrimination between macrophages and other interstitial cells, based on differences in nuclear morphology and chromatin patterns, but identification of peritubular macrophages was less reliable using this approach. Optimisation of antigen retrieval, serum blocking, washing and primary to secondary antibody concentrations for F4/80 immunohistochemistry to detect macrophages was established. Peritubular macrophages were readily discriminated using this optimised method and there was a good correlation between the numbers of interstitial macrophages identified by histology and immunohistochemistry. However, preliminary morphometric analysis indicated no significant differences in total macrophage numbers between the various activin/follistatin mouse models. This was also verified by F4/80 and CD45 gene expression measured by qRT-PCR. These studies represent a significant methodological advancement in the discrimination and quantification of macrophages in well-preserved mouse testis tissue. These preliminary results suggest that alterations in endogenous activin or follistatin have minimal effects on the total number of peritubular and interstitial macrophages in the adult mouse testis. Optimisation of these techniques to quantify macrophage functional subsets and other immune cells in the testis is in progress.

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### Effect of artificial insemination with frozen-thawed cauda epididymal spermatozoa in hanwoo Bull

**Sangrae Cho<sup>1</sup>, Sung-sik kang<sup>1</sup>, Ui-hyung Kim<sup>1</sup>, Myung-suk Lee<sup>1</sup>, Seok-dong Lee<sup>1</sup>, Man-hee Han<sup>1</sup>, Min-Hyeong Jeon Jeon<sup>1</sup>**

1. RDA, National Institute of Animal Science, Pyeongchang-gun, Gangwon-Do, South Korea

**Objective:** Deep uterine artificial insemination (DUAI) was examined to improve pregnancy rate with epididymal spermatozoa in Hanwoo cattle.

**Methods:** The estrus cycles of 100 Hanwoo cows were synchronized, and the cows were used for insemination. Seventeen cows were inseminated by DUAI with epididymal spermatozoa, and 32 cows were inseminated by body of uterus AI (BUAI). As a control, 51 cows were inseminated by BUAI with frozen-thawed ejaculated spermatozoa from one bull. After 70 to 80 days of AI, pregnancy was evaluated by rectal palpation and ultrasound. Motility of epididymal spermatozoa were examined in DUAI and BUAI immediately after thawing, as well as at 3 and 6 h of incubation.

**Results:** The pregnancy rate of DUAI with epididymal spermatozoa tended to be greater than that of BUAI with epididymal spermatozoa (DUAI and BUAI vs. 58.8% and 31.1%,  $p=0.075$ ). The control spermatozoa group showed a 56.9% pregnancy rate. The fast, progressive motility of the control group was significantly higher than in epididymal spermatozoa in DUAI and BUAI groups immediately upon thawing and after 3 and 6 h of incubation ( $p<0.05$ ). VSL and VAP of epididymal spermatozoa in DUAI and BUAI at 6 h of incubation periods were both significantly lower than those in the control spermatozoa group ( $p<0.05$ ). LIN and ALH of epididymal spermatozoa were low compared those at 6 h ( $p<0.05$ ). BCF and hyperactivation of epididymal spermatozoa were lower than in the control spermatozoa group immediately after thawing and at 3 h ( $p<0.05$ ). These motility parameters suggest that epididymal spermatozoa have low motility and fertilizability compared to control spermatozoa.

**Conclusion:** The DUAI method can overcome the low pregnancy rate of epididymal spermatozoa, even though epididymal spermatozoa have low motility and fertilizability. This method will contribute to improvement of pregnancy rate for genetically valuable and post-mortem bulls.

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### Melatonin attenuates rat testicular damage induced with methotrexate via decrease of caspase-3 expression and changes of tyrosine phosphorylation

**Sitthichai Iamsaard<sup>2,1</sup>, Jariya Umka Welbat<sup>2</sup>, Wannisa Sukhorum<sup>2</sup>, Suchada Krutsri<sup>2</sup>**

1. Performance & Health Promotion (HHP&HP) Research Institute, Khon Kaen University, Khon Kaen, Thailand

2. Khon Kaen University, Khon Kaen, THAILAND, Thailand

Methotrexate (MTX), a chemotherapeutic agent, was shown to adversely affect testis especially seminiferous epithelium. Since melatonin, an endocrine hormone, can normalize testicular functions, its antioxidant property on prevention of MTX-induced testicular damages has never been demonstrated. This study aimed to investigate the protective effect of melatonin on such damage. Sixty adult male rats were divided into 5 groups ( $n=12$ /each). Control rats were injected with vehicle whereas MTX, rats were intravenous injected with MTX (75 mg/kg) at days 8 and 15. Melatonin animals were injected with melatonin (8 mg/kg, i.p.) for 15 consecutive days. Rats in preventive or throughout groups were co-treated with melatonin and MTX for 15 or 30 consecutive days. The reproductive parameters including expressions of testicular tyrosine phosphorylation, steroidogenic acute regulatory (StAR), and caspase-3 proteins and malondialdehyde (MDA) level were examined. The results showed that melatonin significantly improved sperm concentration and seminiferous epithelium with decrease of caspase-3 expression. Additionally, the intensity of tyrosine phosphorylated proteins of 32 kDa was decreased while 47 kDa was increased in melatonin treated groups compared to MTX group. However, StAR protein expression was not altered compared among groups but testicular MDA levels of melatonin-MTX groups were significantly increased as compared to MTX rats. In conclusion, melatonin improved the MTX-induced testicular damage via antiapoptotic pathway of caspase-3 and increase the tyrosine phosphorylated proteins.

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## The difference in contractility between prostatic ducts and glands and how oxytocin is involved

**Beatrix Stadler<sup>1</sup>, Marian Kampschulte<sup>2</sup>, Florian Wagenlehner<sup>3</sup>, Betty Exintaris<sup>4</sup>, Ralf Middendorff<sup>5</sup>**

1. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia

2. Department of Radiology, Justus-Liebig-University, Giessen, Hessen, Germany

3. Department of Urology, Pediatric Urology, and Andrology, Justus-Liebig-University, Giessen, Hessen, Germany

4. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia

5. Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Hessen, Germany

Benign prostatic hyperplasia (BPH) affects up to 80% of eighty-year-old men, is often accompanied by lower urinary tract symptoms and can therefore significantly diminish quality of life. Medical treatment predominantly consists of relaxing the smooth muscle cells in the prostate by using alpha-1-blockers which bears the risk of side effects such as erectile dysfunction. In search of alternative effectors of prostatic contractility we investigated oxytocin which has been found to be involved not only in female but also in male reproductive processes. We analysed the contractile pattern of prostatic glands and ducts separately, allowing to predict effects and local side effects of drugs used as potential treatment options for BPH. Most of the experiments were performed in rodent tissue since in the human prostate data on structure and functional regulation of excretory ducts as well as the potential of targeting other receptors instead of adrenergic ones are missing.

The duct system of the human prostate was visualized using corrosion cast models, smooth muscle staining and Micro-CT-imaging. The effects of oxytocin were investigated using video microscopy.

We were able to clarify the human prostatic ductal system and also got information about the organisation of the surrounding smooth muscle cells. Oxytocin increased the frequency of spontaneous prostatic contractions. There was a visible difference between oxytocin- and noradrenaline-induced contractions, which also differed between prostatic ducts and glands.

Revealing differences between ducts and glands in the prostate not only extends our basic anatomical and physiological knowledge but might prove valuable for evaluating local side effects in pursuit of creating even more targeted medications. These insights in combination with the oxytocin data we obtained could open up new strategies in BPH treatment especially by comparing our novel oxytocin antagonists in the near future.

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## Rapid response to oestrogen in a human testis-derived cell line blocks SOX9

**Melanie K Stewart<sup>1</sup>, Andrew J Pask<sup>1</sup>, Deidre M Mattiske<sup>1</sup>**

1. University of Melbourne, Parkville, VIC, Australia

The nuclear localisation of SOX9 is essential for Sertoli cell differentiation and subsequent development of the testis. Exogenous oestrogen exposure of Sertoli cells can cause cytoplasmic retention of SOX9, leading to upregulation of key ovarian markers. The MAPK pathway can promote or inhibit SOX9 or b-catenin to tilt the balance between testis and ovarian genes in somatic cells. Thus, the MAPK pathway presents as a potential target of oestrogen to impact SOX9. Furthermore, the MAPK pathway and a key MAPK, ERK1/2, is known to affect the microtubule network. Cytoplasmic SOX9 requires a stabilised microtubule network, therefore we hypothesised that oestrogen could stabilise microtubules via ERK1/2 to promote cytoplasmic retention of SOX9. We treated the human testis cell line NT2/D1 with oestrogen for 30 minutes in the presence or absence of the ERK1/2 inhibitor U0126 and examined SOX9, tubulin and phosphorylated ERK1/2 by immunofluorescence. Oestrogen rapidly blocked the nuclear translocation of SOX9 in NT2/D1 cells and the microtubule network appeared stabilised. Phosphorylated ERK1/2 was more abundant and localised in the nucleus following oestrogen treatment. This effect was reduced upon pre-treatment with the ERK1/2 inhibitor U0126. Overall, these data suggest that oestrogen can rapidly activate ERK1/2 to stabilise microtubules and cause cytoplasmic retention of SOX9. We have revealed a previously unknown mechanism for oestrogen in impacting the function and differentiation of Sertoli cells.

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## Genetic characteristics and variation of coat color in Jeju Black Cattle

**Byoung-Chul Yang<sup>1</sup>, Jae-Young Choi<sup>1</sup>, Nam-Young Kim<sup>1</sup>, Sang-Min Shin<sup>1</sup>, Jae-Hoon Woo<sup>1</sup>, Moon-Cheol Shin<sup>1</sup>, Ji-Hyun Yoo<sup>1</sup>, In-Cheol Cho<sup>1</sup>, Nam-Geon Park<sup>1</sup>, Su-Yeon Kim<sup>1</sup>, Yong-Jun Kang<sup>1</sup>**

1. Subtropical Livestock Research Institute, National Institute of Animal Science, RDA, Jeju, Korea

This study was conducted to analyze the coat color characteristics in Jeju Black Cattle by comparative the coat color patterns. Black and others coat color skin tissues were collected from 6 Jeju Black Cattle. RNA-seq analysis was conducted to identify coat color related genes and expression levels of the genes were identified using qPCR. Three different staining methods were used to examine the distribution of melanin in skin tissues. Results of RNA-seq analysis between black and others coat color skin tissues in Jeju Black Cattle. We found a total of 271 DEGs, with 42 genes up-regulated and 229 genes down-regulated. Hierarchical clustering analysis showed that the expression patterns of DEGs were different between black group and others color group. We conducted GO Term analysis and KEGG pathway analysis on the found genes to select candidate genes associated with coat color expression. Expression levels were identified using the qPCR for 8 (DCT, KIT, MC1R, OCA2, PAX3, PMEL, SLC45A2 and TRPV2) of the selected candidate genes. The expression level of the candidate genes in others coat color skin were identified to be at least 10 times to 33 times lower than that of the black coat color skin. Expression of genes identified by experiments showed the same expression pattern as that of bioinformatics analysis. Tissue staining was also conducted to determine the distribution of melanin and the like in the black and others skin. In the analysis, melanin component was identified in the black coat color skin. In this study, the difference of gene expression was identified by comparison between black skin and other skin in Jeju Black Cattle, and higher expressions were observed in black skin. The black and others skin transcript profile will help further research to understand gene expression networks that regulate skin physiology and melanogenesis in cattle.

## Structural changes to the pouch of male Pot-bellied Seahorses (*Hippocampus abdominalis*) facilitate gas exchange during pregnancy

**Jessica Dudley<sup>1</sup>, Polly Hannaford<sup>2</sup>, Mike Thompson<sup>2</sup>, Camilla Whittington<sup>2</sup>**

1. *Anatomy and Histology, School of Medicine, The University of Sydney, Sydney, NSW, Australia*

2. *Biological Sciences, School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia*

Developing embryos need to exchange respiratory gases to grow and survive. In egg laying species, gas exchange occurs directly with the surrounding environment, across moist membranes inside the egg. However, in live-bearing female amniotes, gas exchange occurs across the placenta, between the developing embryo and the bloodstream of the parent. The development of the placenta is facilitated by rapid remodelling of the uterine epithelium and surrounding tissues during pregnancy. This remodelling includes an increase in vascular growth to facilitate the increasing oxygen needs of the embryo.

The members of the family Syngnathidae have male pregnancy and are an ideal opportunity to examine the biology and evolution of pregnancy independent of the female reproductive tract. In the seahorse *Hippocampus abdominalis*, the female transfers yolk-rich oocytes to the brooding pouch of the male, where they are internally fertilised and kept within the sealed pouch during development. Seahorse young are released as independent and free-swimming fry. The lining of the father's brood pouch shares the same function as an amniote placenta, in that it likely functions in waste removal, gas exchange, osmoregulation, and nutrient provision. *H. abdominalis* shares homologous genes for pregnancy with mammals, suggesting that the genetic pathways that regulate pregnancy could be shared. However, there is little known about the structural and cellular changes to the brood pouch of *H. abdominalis* during pregnancy, including the mechanisms by which embryonic gas exchange is achieved in the sealed pouch. Our study characterised the structural changes to the inner tissue layers of the brood pouch throughout pregnancy. We found an increase in the epithelial surface area and pouch vascularity during pregnancy, which likely facilitates respiratory gas exchange during male pregnancy in this species. These changes mirror the changes to the vascular bed of gestational tissue in viviparous amniotes.

## Differentiation of the gonads of the embryo and hatchling echidna

**Jane C Fenelon<sup>1</sup>, Stephen Johnston<sup>2</sup>, Marilyn Renfree<sup>1</sup>**

1. *University of Melbourne, Parkville, VIC, Australia*

2. *School of Agriculture and Food Science, University of Queensland, Gatton, Queensland, Australia*

The short-beaked echidna (*Tachyglossus aculeatus*) is one of only four extant species of egg-laying mammals (Monotremata: three echidnas and one platypus), but despite its common and ubiquitous distribution throughout Australia, information on its reproductive biology is limited. What is known is that the reproductive biology of the echidna is anatomically and behaviourally distinct in comparison with both marsupial and eutherian mammals. After a short gestation, the egg is incubated in the pouch and once hatched, the young puggle continues its development while residing in the pouch, sucking milk from the mammary patches.

In conjunction with Currumbin Wildlife Sanctuary (Queensland) we have begun to investigate echidna reproduction and development. The newly hatched puggle is developmentally remarkably similar to a marsupial neonate, but nothing at all is known of the genes and hormones that influence the growth and development of the embryo *in utero* or *in ovo*. Together with the platypus, the echidna has a unique sex chromosome make up (echidna 5Xs:4Ys; platypus 5Xs and 5Ys), but as yet there is no evidence of *Sry*. We have begun to examine the developmental anatomy of the echidna embryo and pouch young and to look at the expression of genes involved in sex determination pathways in the gonads of other mammals. The phallus already appears as a distinct structure, while the gonads have a typically mammalian embryonic appearance in that the ovaries remain elongated by day 3 post-hatching, whereas the testis has already begun to round up. Many of the common mammalian sex-determining genes are present in the gonads of the echidna at the RNA level and we have optimised antibodies for a number of these and will be examining their protein expression. Together, these results will provide the first insights into the evolution of sex-determining pathways between monotremes, marsupials and eutherians.

## Freezing the rainbow a pot of gold for fish conservation in Australia

**Nicola M Rivers<sup>2,1</sup>, Jonathan P Daly<sup>1,3</sup>, Peter C Currie<sup>2,4</sup>, Robert L Jones<sup>5,6</sup>, Peter D Temple-Smith<sup>2,1</sup>**

1. *Australian Frozen Zoo, Melbourne, VIC, Australia*

2. *Monash University, Prahran, VIC, Australia*

3. *Smithsonian Conservation Biology Institute, Front Royal, VA, USA*

4. *Australian Regenerative Medicine Institute, Melbourne, VIC, Australia*

5. *The Aquarium Vet, Melbourne, VIC, Australia*

6. *SeaLife Aquarium, Melbourne, VIC, Australia*

Declining fish populations highlight the importance of techniques that safeguard fish species against extinction. Biobanks or "frozen zoos" store cryopreserved cells from endangered species in the hope of generating new individuals in the future. However, cryopreservation of fish gametes and embryos has been challenging leaving endangered fish species without a backup plan in the event of extinction. Current research in fish now turns to germline progenitor cells: the spermatogonia and

oogonia located in the gonadal tissue. We present a cryopreservation protocol for testes from the rare Murray River Rainbowfish (*Melanotaenia fluviatilis*).

Testes were cryopreserved in one of the following permeating cryoprotectants- DMSO, EG, methanol or glycerol at 1.3M with 0.1M trehalose in 1.5% BSA and mixed salt solution (296mOsm, pH=7.4). Viability of cells in post-thaw testis suspensions was determined using SYBR14/Propidium Iodide (PI) staining and flow cytometry. To isolate and assess spermatogonia from cell suspensions, size-specific beads were used to gate spermatogonia-sized cells, as determined from histological assessment of the testes. Analysis using one-way ANOVA and Tukey's *post hoc* test showed 1.3M DMSO was the most effective permeating cryoprotectant, yielding the highest post-thaw viability at  $72.6\% \pm 10.48\%$ , significantly higher ( $p < 0.05$ ) than the next best-performing cryoprotectant, 1.3M EG ( $35.53\% \pm 2.37\%$ ), and cryoprotectant-free negative control ( $10.68\% \pm 7.09\%$ ). Changes to DMSO concentration (1.0M, 1.6M, and 2.0M) showed no significant difference in viability between DMSO cryopreserved tissue and fresh samples ( $78.76\% \pm 7.65$ ).

Recovery of viable cells from cryopreserved gonadal tissue is the first step to developing new management and conservation options for vulnerable fish species. Future studies aim to generate offspring from cryopreserved tissue via a surrogate using germ cell transplantation techniques.

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## Telomere elongation patterns during mouse pre-implantation embryogenesis

**Yasmyn E Gordon<sup>1,2</sup>, Macarena Gonzalez<sup>1,2</sup>, Ryan D Rose<sup>1,3,2</sup>, Darryl L Russell<sup>1,2</sup>, Rebecca L Robker<sup>1,2</sup>**

1. University of Adelaide, Adelaide, SA, Australia

2. Robinson Research Institute, Adelaide, SA, Australia

3. Fertility SA, Adelaide, SA, Australia

Telomeres are repetitive DNA sequences that cap the ends of eukaryotic linear chromosomes and play major roles in cell cycle control, cellular lifespan and maintaining genome integrity. Telomere length is an important determinant in health, longevity, and disease, with short telomeres being associated with adverse pathologies. Telomeres naturally shorten with each cell cycle, therefore their length must be regenerated at the outset of each new generation as it is essential for the health span of the offspring. Early embryogenesis is a time of exponential, rapid cell divisions to form a 'new' organism, which originates from a single cell. Importantly, telomeres lengthen rapidly during pre-implantation development, and this appears to be a major mechanism by which telomere length is reset across generations; however, there is little understanding of the mechanisms by which this occurs. Thus we conducted a detailed investigation of telomere elongation during mouse pre-implantation embryogenesis. Analysis of mRNA expression of key proteins that regulate telomere elongation (ALT- or telomerase-mediated) showed they were expressed in oocytes and embryos. We developed a highly quantitative qPCR assay which measures telomere length per cell in individual mouse oocytes and embryos. We then conducted an in-depth time course measuring telomere length in oocytes, sperm and across multiple stages of embryogenesis (2-cell, 4-cell, 8-cell, blastocyst), in 4 different crosses involving 2 strains of inbred and 1 strain of outbred mice. We found that telomere elongation occurred during embryogenesis but with subtly different kinetics in each cross. Further, telomere lengths were dynamic in both the Inner Cell Mass and Trophectoderm cell lineages. To investigate whether the capacity for elongation resides within the oocyte itself, we assessed elongation trends in parthenogenetically activated embryos and again found distinct patterns. These results demonstrate that telomere elongation occurs rapidly during embryogenesis, but exhibits distinct patterns dependent on mode of conception.

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## SIRT3 is dispensable for oocyte mitochondrial function and female fertility during ageing and obesity

**Juvita Iijas<sup>1</sup>, Hayden Homer<sup>1</sup>**

1. The Christopher Chen Oocyte Biology Research Laboratory, The University of Queensland-Centre for Clinical Research, Brisbane, Queensland, Australia

Publish consent withheld

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## Modulatory effects of TGF- $\beta$ and BMP6 on thecal angiogenesis and steroidogenesis in the bovine ovary

**Dareen Mattar<sup>1</sup>, Moafaq Samir<sup>1</sup>, Mhairi Laird<sup>1</sup>, Phil G Knight<sup>1</sup>**

1. University of Reading, Reading, United Kingdom

Angiogenesis plays an integral role in follicular and luteal development and is positively regulated by several intra-ovarian factors including vascular endothelial-derived growth factor A (VEGFA) and fibroblast growth factor 2 (FGF2). Various transforming growth factor-beta (TGF-beta) superfamily members function as intra-ovarian regulators of follicle and luteal function but their potential roles in modulating ovarian angiogenesis have received little attention. In this study, we used a bovine theca interna culture model to examine the effects of TGF-beta1 and bone morphogenetic protein 6 (BMP6) on angiogenesis and steroidogenesis. VEGFA/FGF2 treatment promoted endothelial cell network formation but had little or no effect on progesterone and androstenedione secretion or expression of key steroidogenesis-related genes. TGF-beta 1 suppressed basal and VEGFA/FGF2-induced endothelial cell network formation, progesterone secretion and expression of key steroidogenesis-related genes, actions that were reversed by an activin receptor-like kinase 5 (ALK5) inhibitor (SB-431542). The ALK5 inhibitor alone raised androstenedione secretion and *CYP17A1* expression. BMP6 also suppressed endothelial cell

network formation under VEGFA/FGF2-stimulated conditions and inhibited progesterone secretion and expression of several key steroidogenesis-related genes under basal and VEGFA/FGF2-stimulated conditions. These actions were reversed by an ALK1/2 inhibitor (K02288). Moreover, the ALK1/2 inhibitor alone augmented endothelial network formation, progesterone secretion, androstenedione secretion and expression of key steroidogenesis-related genes including *CYP17A1*. The results indicate dual suppressive actions of both TGF-beta 1 and BMP6 on follicular angiogenesis and steroidogenesis. Further experiments are needed to unravel the complex interactions between TGF-beta superfamily signalling and other regulatory factors controlling ovarian angiogenesis and steroidogenesis.

## The role of autophagy in the ageing oocyte

**Alexandra E Peters<sup>1,2</sup>, Bettina P Mihalas<sup>1,2</sup>, Elizabeth G Bromfield<sup>1,2,3</sup>, Shaun D Roman<sup>1,2,4</sup>, Brett Nixon<sup>1,2</sup>, Jessie M Sutherland<sup>1,2</sup>**

1. Priority Research Centre for Reproductive Science, Schools of Biomedical Science & Pharmacy and Environmental & Life Sciences, University of Newcastle, Callaghan, New South Wales, Australia

2. Hunter Medical Research Institute, New Lambton Heights, New South Wales, Australia

3. Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

4. Priority Research Centre for Drug Development, University of Newcastle, Callaghan, New South Wales, Australia

Accompanying the dramatic age-related decline in female fertility is a concomitant increase in the proportion of poor-quality oocytes that remain within the ovary. Based on the known proteomic changes that occur during oocyte ageing, and extrapolating from somatic cell literature, we hypothesised that a key contributor to the deterioration of oocyte quality is dysfunction of the essential protein degradation pathway known as autophagy. Thus, the aim of this study was to characterise autophagy in young and aged oocytes using a well-established mouse model of female reproductive ageing. Two primary autophagy pathway markers were assessed in this model; microtubule-associated protein 1 light chain 3B (LC3B), a constituent of the autophagosome membrane, and Beclin 1 (BECN1), an initiator of autophagosome formation. In addition, functional *in vitro* maturation studies were performed on pre-ovulatory germinal vesicle stage oocytes subjected to autophagy inhibition to assess oocyte maturation potential. This study revealed an increased number of large puncta (foci) containing LC3B and BECN1 in aged oocytes compared to young oocytes ( $n=3$ ,  $p=0.046$  and  $p=0.045$  respectively). This was accompanied by a change in the localisation of LC3B and BECN1 puncta to favour the periphery of aged oocytes ( $n=3$ ,  $p=0.029$  and  $p=0.081$  respectively). The use of the macroautophagy inhibitor, 3-methyladenine, led to a 27% reduction in the number of oocytes reaching the post-ovulatory metaphase II stage and extruding a polar body ( $n=3$ ,  $p=0.044$ ), indicative of meiotic failure. These findings shed light on the altered autophagy mechanisms occurring in aged oocytes and reveal an important role for autophagy in ensuring the competence of oocytes to mature to the post-ovulatory stage of development, a requisite for fertilization. This establishes the importance of autophagy in oocyte maturation and emphasises the need to further investigate the contribution of dysregulated autophagy pathways to age-related female infertility.

## Effect of nicotinamide mononucleotide on the *in vitro* maturation of porcine oocytes

**Charley-Lea Pollard<sup>1</sup>, Zamira Gibb<sup>2</sup>, Michael J Bertoldo<sup>3</sup>, Lindsay E Wu<sup>3</sup>, Christopher G Grupen<sup>1</sup>**

1. Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camden, NSW, Australia

2. Faculty of Science and IT, University of Newcastle, Callaghan, NSW, Australia

3. School of Women's and Children's Health, Fertility and Research Centre, University of New South Wales, Sydney, NSW, Australia

Sirtuins are a family of NAD<sup>+</sup>-dependent protein deacetylases that have been implicated in cellular aging and oocyte quality. Supplementing the diet of mice with the NAD<sup>+</sup> precursor nicotinamide mononucleotide (NMN) has been shown to ameliorate the detrimental impacts of generational obesity on female fertility [1], suggesting involvement of NMN in promoting oocyte maturation. Using small antral follicle-derived porcine oocytes, a well-established model of poor oocyte quality, the aim of this study was to examine the effect of NMN supplementation during *in vitro* maturation (IVM) on oocyte developmental competence. Oocytes were matured for 44 h in defined IVM medium without (control) or with increasing doses of NMN (0.1, 1, 10 and 100  $\mu$ M). Mature oocytes were artificially activated by sequential treatment with ionomycin and 6-dimethylaminopurine/cytochalasin B. Presumptive zygotes were cultured for 7 d in Porcine Zygote Medium-3. Cleavage and development to the blastocyst stages were assessed and total blastocyst cell numbers were determined. The experiment was replicated five times. Supplementing the IVM medium with NMN did not significantly affect the rates of cleavage (range: 62.8 $\pm$ 8.5% to 74.6 $\pm$ 5.7%) or blastocyst formation (range: 21.8 $\pm$ 3.7% to 28.6 $\pm$ 6.9%) compared with the control group (cleavage: 67.0 $\pm$ 8.8%; blastocyst: 19.6 $\pm$ 9.1%). The proportion of blastocysts that had partially or fully hatched from their zona pellucida by 7 d of culture was markedly greater in the 1 and 100  $\mu$ M NMN groups (29.5 $\pm$ 2.2% and 23.3 $\pm$ 3.8%, respectively) compared with the control group (12.5 $\pm$ 9.7%;  $P<0.05$ ). Surprisingly, the total blastocyst cell numbers did not differ among the groups ( $P>0.05$ ), suggesting the NMN treatment had resulted in changes to the function and/or development of blastocyst cells. Further studies are needed to determine the processes by which NMN influences the acquisition of developmental competence during oocyte IVM.

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## Effect of GMCSF on mouse *in vitro* oocyte maturation

**Anmol Saini<sup>1</sup>, Michelle Lane<sup>1</sup>, Mark Nottle<sup>1</sup>**

1. School Of Medicine, Robinson Research Institute, The University of Adelaide, Adelaide, South Australia, Australia

The addition of GM-CSF to embryo culture media has been shown to improve embryo development in a range of species including humans, however, its effects on in vitro oocyte maturation have not been widely examined. The present study was undertaken to determine whether the addition of GM-CSF to mouse oocyte maturation media could increase preimplantation embryo development. C57Bl6 x CBA F1 female mice age 21-23 days were injected IP with dose 0.1ml of eCG and cumulus oocyte complexes (COCs) aspirated from large antral follicles 46-48 h post injection. Ten COCs were cultured per 50µL drop in bicarbonate-buffered  $\alpha$ -MEM containing 3 mg/ml BSA and 1 mg/ml Fetuin and 5 mIU/mL FSH plus 0, 2 or 10ng/ml of GM-CSF. Following fertilization presumptive zygotes were cultured in Cleave Media (Cook Medical) and cleavage rate and Day 4 and 5 blastocyst rates determined. Blastocysts were differentially stained on day 5 to determine inner cell mass, trophectoderm, and total cell number. The experiment was replicated six times with 50-60 oocytes per group in each. Data was analysed using one-way ANOVA and Turkey post hoc test. The addition of GM-CSF during oocyte maturation had no effect on cumulus expansion and cleavage rate. The number of blastocysts present on Day 4 was not different between the groups. The number of blastocysts present on Day 5 and the proportion of these that were expanded and hatched were not different between the groups. However, the addition of 10 ng/ml of GM-CSF significantly increased blastocyst inner cell mass and total cell numbers compared to control by 52.1% (18.5 vs 12.2%) and 32.9% (55.2 vs 41%) respectively ( $P < 0.01$ ). Further studies are warranted to confirm our initial findings as well as to determine whether these increases implantation and live birth rates.

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## Possible involvement of thyroglobulin type-1 domain of IGF-BPs on the development and maturation of ovarian follicles

**Motoyuki Shimonaka<sup>1</sup>, Kana Kobayashi<sup>1</sup>, Asahi Tsukahara<sup>1</sup>, Marie Tamaki<sup>1</sup>, Moyuru Hayashi<sup>1</sup>**

1. Tokyo University of Science, Shinjuku-ku, Tokyo, Japan

It is well understood that several hormones, growth factors and proteases regulate the development of ovarian follicles, however, the mechanisms of the selection of dominant follicles has not been elucidated. Insulin-like growth factor (IGF)-binding proteins (IGF-BPs) are also involved in the regulation of follicular growth by controlling the availability of IGF. One of the structural features IGF-BPs possess is thyroglobulin type-1 domain (Tg-1) on their C-terminal region. Although Tg-1 has been shown to function as a protease inhibitor, the role of it in gonadal tissues is unknown. In this study, we examined the possibility that Tg-1 acts as a local modulator of folliculogenesis. Recombinant rat IGF-BP 1 and 5 as well as Tg-1 of rat thyroglobulin were expressed in *E. coli*. To evaluate the effect of Tg-1, chimeric proteins of IGF-BP 1 and 5 were constructed and also expressed. Primary follicles were enzymatically isolated from ovaries obtained from immature 14-day-old rats. One primary follicle was plated onto each well and cultured for several days with recombinant proteins. Diameter of follicles were measured through the culture period. Without recombinant proteins, the follicles gradually increased their size to formed antral-like structure, which was characteristic of the secondary follicular stage. IGF-BP 1 slightly inhibited the FSH-induced growth of primary follicles, whereas IGF-BP 5 showed the LH-like activity which induced a follicle to the stage of ovulation. The effects of Tg-1 on the follicles were similar to that of IGF-BP-1. However, the phosphorylation of Akt in the follicles was stimulated by Tg-1 but not by IGF-BP 1. When two primary follicles were co-cultured in the same well with recombinant proteins, communication between those two follicles in the course of growth was observed. In conclusion, we found a novel function of thyroglobulin type-1 domain of IGF-BPs on the regulation of folliculogenesis.

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## Determining the impact of environmental toxins on the germline epigenome and offspring development

**Elyse OK Swindells<sup>1</sup>, Jessica M Stringer<sup>1</sup>, Karla J Hutt<sup>1</sup>**

1. Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

Atrazine is one of the most widely used pesticides worldwide, with annual use exceeding 35,000 tonnes in the USA (1) and 3,000 tonnes in Australia (2). Atrazine is a common ground and surface water contaminant, with up to 1.65ng/ml detected in southeast Australia(3), and up to 7.6ng/ml in Queensland (4). Accumulating evidence suggests atrazine may act as an endocrine disruptor, interfering with reproductive health and function (5-7). Additionally, atrazine is shown to alter male germline epigenetic programming (8, 9). These studies, however, utilise short term, high dose exposures, focusing on male health and reproductive outcomes. Comprehensive analyses of environmentally-relevant doses and of chronic, multi-generational exposure on ovarian function and female fertility are lacking.

We aim to characterise the impacts of atrazine on female germline development, epigenetic programming, DNA damage, and fertility, using low (0.02ng/ml), medium (0.02ug/ml), and high (0.02mg/ml) exposure levels. To characterise embryonic exposure, pregnant dams will receive atrazine via drinking water from E9.5-17.5, encompassing sex-specific epigenetic reprogramming of the embryonic germline. Additionally, to assess direct insult to the ovaries, we will supplement ovary cultures with atrazine. To characterise adult exposure, female mice will receive atrazine for three weeks, a period encompassing follicle growth from activation to pre-ovulatory stage. Finally, to assess long term atrazine exposure, we will supplement drinking water of female mice for at least 3 generations.

This study will generate data to guide atrazine legislation and regulation. Elucidating the impacts of exposure may aid fertility management not only in humans, but also in agricultural cattle and native wildlife breeding programs.

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## The reduced locomotor activity in male and female Kiss1r KO mice is dependent on gonadal sex steroid status

**Raj Patel<sup>1</sup>, Shane Maloney<sup>1</sup>, Jeremy Smith<sup>1</sup>**

*1. School of Human Sciences, The University of Western Australia, Perth, WA, Australia*

Kisspeptin regulates reproduction by activating gonadotrophin-releasing hormone (GnRH) neurons through its receptor, Kiss1r. Kiss1r knockout (KO) mice develop an obese and diabetic phenotype compared to wild type (WT) littermates. We used Kiss1rKO and WT male and female mice to investigate the relationship between absent kisspeptin signalling and locomotor behaviour by allowing mice free access to running wheels (Lafayette Mouse Activity Wheel Chambers). These studies were also performed following gonadectomy (GDX), to control for gonadal steroids. We examined the real-time characteristics of wheel running over a 3-week period and its flow-on effects on body weight. In intact males, there was a 90% reduction in total distance travelled per 24h in KO mice compared to WT (WT, 6363±643m; KO, 652±219m; P<0.0001). Moreover, the circadian pattern of wheel running activity (dark phase activity) clearly present in WT mice was severely diminished in KOs. However, in GDX males there was no difference between WT and KOs, (WT, 1652±474m; KO, 998±219m). In intact females, there was a 77% reduction in total distance travelled per 24h in KO mice, compared to WT (WT, 6030±747m; KO, 1379±364m; P<0.004). In OVX females there was no difference between KO mice and WT (WT, 4150±1367m; KO, 3117±830m). Body weight analysis showed that wheel running prevented the weight gain normally attributed to the Kiss1rKO mouse. In fact, in GDX males and females (at days 21 and 22 of wheel running) KOs were significantly lighter than WT (at day 22: Males, WT 28.67g; KO, 23.70g; P<0.05; Females, WT, 27.38g; KO, 23.30g; P<0.05). We show the reduced locomotor activity in male and female Kiss1r KO mice is dependent on gonadal sex steroid status. Whether absent kisspeptin signalling acts as a regulator of voluntary activity is debatable but patterns of locomotion behaviour could be disrupted, potentially involving circadian rhythm, this is under further investigation.

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## Involvement of Bromodomain and Extra-Terminal (BET) Proteins in Inflammatory Gene Regulation in Human Decidual Stromal Cells (hDSCs)

**Sandeep S Aijaonkar<sup>1</sup>**

*1. University of Newcastle, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia*

Recent evidence suggests that immune cell activity in the decidua is controlled by decidual stromal cells, which promote immune tolerance and shelter the fetus from the maternal immune system while maintaining maternal immune responses. The protective function of the decidua wanes at term leading to decidual activation triggering labour. However, the mechanisms remain unknown. We hypothesised that labour-promoting inflammatory genes in the decidual cells are regulated epigenetically by the BET family of epigenetic reader proteins which specifically recognize lysine-acetylated histones at gene regulatory regions of the chromatin via bromodomains (BRDs).

Decidual stromal cells were purified by Percoll density centrifugation followed by magnetic immune-selection and cultured under appropriate conditions. Purity was confirmed by flow-cytometry and immunocytochemistry. Cultures were treated with the selective BET-BRD inhibitors (+)-JQ1, I-BET-762 and the inactive control compound (-)-JQ1 at 0.5-1 mM as per Structural Genomics Consortium (SGC) guidelines for 48h and subsequently stimulated with lipopolysaccharide (LPS, 1 mg/ml) for 24h. The expression of the prostaglandin synthetic enzymes (*PTGS1*, *PTGS2*, *PGES*), inflammatory factors (*IL6*, *IL8*, *TNF-α*) and anti-inflammatory factors (*IL10* and *IDO-1*) was determined by qRT-PCR.

LPS stimulated robustly the expression of *PTGS2*, *IL6*, *IL8*, *IL10* and *IDO-1*, moderately the expression of *PGES* and *TNF-α* and had no effect on *PTGS1*. The BET-BRD inhibitors reduced LPS-induced expression of *PTGS2*, *PTGES*, *IL6*, *IL8*, *IL10*, *TNF-α* and *IDO-1* while *PTGS1* expression was unaffected. The control probe ((-)-JQ1) was ineffective. We have also found that decidual stromal cells express high levels of the BET-BRD family members *BRD2* and *BRD4*, but low levels of *BRD3*. These results indicate that the acetyl-histone binding epigenetic reader proteins, especially *BRD2* and *BRD4*, participate in the regulation of key labour-associated genes in term decidua. We anticipate that histone acetylation is an epigenetic mechanism that controls decidual stromal cell function during pregnancy and labour.



### Effect of pentoxifylline on straightness rate of cryopreserved boar spermatozoa

**SunYoung Baek<sup>1</sup>, Hak-Jae Chung<sup>1</sup>, Soojin Sa<sup>1</sup>, HongWan Anh<sup>1</sup>, HakSoo Kim<sup>1</sup>, BongKi Kim<sup>2</sup>, KyuHo Cho<sup>1</sup>**

1. National Institute of Animal Science, RDA, Cheonan

2. Kongju National University, Yesan

The objective of this study was to investigate the influence of the addition of extenders after semen cryopreservation-thawing on boar sperm quality. To evaluate the effect of pentoxifylline and investigate optimum concentrations in extender freezing medium on motion characteristics, motility, viability and mitochondrial activity of spermatozoa at post thawing of cryopreservation. Semen were collected from five boars and frozen on the same day. Qualified semen samples (motility >80%) from each boar were subdivided into four groups, 0 (control), 5 mM (T1), 10 mM (T2) and 20 mM (T3) to evaluate the effects of pentoxifylline. Motility was assessed for % motile cell characteristics using computer-assisted semen analysis (CASA; SAIS SI-100, Medical supply, Korea). Viability was assessed using the LIVE/DEAD kit (Molecular Probes, USA) and mitochondrial activity was examined by MitoTracker (Invitrogen, USA). Frozen sperms were thawed in Beltsville Thawing Solution (BTS) then incubated at 38°C for 30 minutes. The addition of 10 mM/L pentoxifylline to cryopreserved semen after thawing significantly increased progressive, total motility and mitochondrial activity compared to controls. Progressive motility, Linearity, Straightness ratio VSL/VAP were higher ( $P < 0.05$ ) in group T2 compared to control group. Nevertheless, pentoxifylline concentration treatment did not affect the viability of the spermatozoa as the concentration increased. The data showed that pentoxifylline as an additive cryoprotectant was able to improve sperm motility and also quality in cryopreservation.

### Markers of placental ageing may play a role in gestational disorders

**Lucy Bartho<sup>1</sup>, Joshua Fisher<sup>1</sup>, James Cuffe<sup>2</sup>, Tony Perkins<sup>1</sup>**

1. Griffith University, Southport, QLD, Australia

2. School of Biomedical Science, University of Queensland, Brisbane, QLD, Australia

Placental ageing has been associated with the pathogenesis of several complications of pregnancy including preterm birth, post-date pregnancies and stillbirth. However, the molecular mechanisms behind this are not fully understood. Alterations in the placenta associated with telomere attrition, DNA binding, genomic instability, epigenetic alterations, proteostasis, inflammation, mitochondrial dysfunction and cellular senescence have been linked with pregnancy pathologies. The aim of this study was to investigate the multifaceted role of aging in pre-term, term and post-term delivered placentae to identify specific genetic markers of ageing.

Human placenta samples (n=32) were collected from Gold Coast University Hospital, Queensland, Australia. Placental RNA was extracted and reverse transcribed using established methods. A total of 24 genes were analysed by qPCR covering 8 areas of interest including markers of telomere attrition and DNA binding (*POT1*, *TERF1*, *TERF2*, *EP300*), genomic instability and epigenetic alterations (*BUB1B*, *POLMRT*, *TFAM*, *TFB1M*, *TFB2M*, *ARID1A*), mitochondrial dysfunction and cellular senescence (*SIRT1*, *SIRT3*, *CDKN1C*, *VWA5A*, *WRN*), as well as proteostasis and inflammation (*FOXO1*, *HSF1*, *AIF*, *HSP70*, *TOLLIP*).

Gene expression of *SIRT1*, *TERF1*, *EP300*, *CDKN1C*, *FOXO1*, *TOLLIP* and *TFAM* was significantly reduced in post-term placentae when compared with control tissue ( $p < 0.05$ ). In addition, an increase in *VWA5A* gene expression was identified within post-term placenta ( $p < 0.05$ ).

The changes in gene expression within the 8 areas of interest identifies that genes associated with mitochondrial dysfunction, cellular senescence and genomic stability were differentially expressed in aged placenta. These outcomes may help to understand the link between placental ageing and the role it plays in pregnancy pathologies.

### Toll-like receptor-4 antagonist (+)-naloxone attenuates LPS-induced fetal programming of offspring adiposity in a fetal sex-specific manner in mice

**Peck Y Chin<sup>1</sup>, Camilla L Dorian<sup>1</sup>, David J Sharkey<sup>1</sup>, Mark R Hutchinson<sup>2</sup>, Kenner C Rice<sup>3</sup>, Lachlan M Moldenhauer<sup>1</sup>, Sarah A Robertson<sup>1</sup>**

1. Robinson Research Institute and Adelaide Medical School, University of Adelaide, Adelaide, SA 5005, Australia

2. Adelaide Medical School, University of Adelaide, Adelaide, SA 5005, Australia

3. Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20892, USA

Toll-like receptor 4 (TLR4) activation during infection or inflammatory insult can induce pro-inflammatory cytokines that adversely impact fetal development and growth, and increase susceptibility to metabolic diseases in later life. We utilized a mouse model to investigate the utility of a small molecule TLR4 antagonist (+)-naloxone, the non-opioid isomer of the opioid receptor antagonist (-)-naloxone, in protecting the offspring from altered fetal programming induced by a modest systemic inflammatory challenge. Pregnant C57B/6 females (n=8/group) were administered low dose (20 µg/kg) intraperitoneal lipopolysaccharide (LPS) on gestation day (GD)16.5, with or without (+)-naloxone and the females were killed 4 hours later to collect fetal brain, fetal membrane, placenta, decidua and uterus tissues (2 fetal sites/female) for qPCR analysis of inflammatory cytokines. In adult progeny exposed to LPS challenge in utero, male but not female offspring exhibited elevated adipose tissue, reduced muscle mass and elevated plasma leptin at 20 weeks of age. (+)-naloxone attenuated the effects of in

utero LPS exposure on offspring allometry and leptin. (+)-naloxone treatment alone elicited accelerated post-weaning growth and elevated muscle, fat and bone mass in adult male offspring, and elevated bone mass in females. qPCR showed that LPS induce expression of inflammatory cytokines *Il1a*, *Il1b*, *Il6*, *Tnf* and *Il10* in fetal, placental and uterine tissues of pregnant mice, and treatment with (+)-naloxone was found to suppress induction of the inflammatory cytokines caused by LPS. Fetal sex-specific effects were observed, with higher expression levels of *Il1A*, *Il1B*, *Il6* and *Il10* induced by LPS in implantation sites of male fetuses. Greater suppression of *Il6* levels was observed in implantation sites of female fetuses following co-treatment with (+)-naloxone. These data demonstrate that suppression of TLR4 signaling with (+)-naloxone provides protection from inflammatory injury in utero, to attenuate long-term developmental effects of excessive TLR4 activation, in a fetal sex-specific manner.

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## Growth Differentiation Factor 15 expression is increased in preeclampsia

**Tess Cruickshank<sup>1</sup>, Teresa MacDonald<sup>1</sup>, Susan Walker<sup>1</sup>, Stephen Tong<sup>1</sup>, Natasha Pritchard<sup>1</sup>, Natalie Hannan<sup>1</sup>, Ping Cannon<sup>1</sup>, Alesia Harper<sup>1</sup>, Elizabeth Murray<sup>1</sup>, Tu'uhevaha Kaitu'u-Lino<sup>1</sup>**

1. Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, VIC, Australia

Preeclampsia is a serious complication of pregnancy which is associated with poor placental invasion and significant maternal endothelial dysfunction. Growth Differentiation Factor-15 (GDF15) is a stress-response protein which has been considered as a biomarker for cardiovascular disease. We have shown that GDF15 is increased in the maternal circulation in women destined to develop preeclampsia.

This study aimed to assess GDF15 in the placentas of women with established preeclampsia and to undertake functional studies to assess its potential contribution to disease pathogenesis.

Immunofluorescence revealed that GDF15 is localised to the syncytiotrophoblast, or surface of the placenta. In a cohort of placentas collected from women who developed early onset preeclampsia at <34 weeks' gestation (n=67), GDF15 mRNA expression was significantly increased relative to preterm control placentas (n=18). Given GDF15 is elevated in the circulation of women preceding preeclampsia diagnosis and also increased in cardiovascular disease, we wondered whether placental secretion of GDF15 might contribute to endothelial dysfunction. In our preliminary studies (n=3 separate placental isolations), when we silenced trophoblast GDF15 using siRNA and then exposed endothelial cells to the media (or control media from trophoblast treated with scrambled siRNA), we saw no changes in markers of endothelial dysfunction, Endothelin-1 or Vascular Cell Adhesion Molecule 1. Drug treatments (esomeprazole, metformin or sulfasalazine) that we have previously shown reduce placental secretion of the anti-angiogenic factors of preeclampsia sFlt1 and soluble endoglin did not significantly alter GDF15 secretion.

In conclusion, GDF15 is elevated in preeclamptic placenta and elevated in the circulation of women preceding their diagnosis of preeclampsia. Our endothelial experiments suggest elevated GDF15 is unlikely to contribute to the endothelial dysfunction characteristic of preeclampsia and we have been unable to identify any potential therapeutics that alter its secretion. Further study is required to better understand how and why GDF15 is elevated in preeclampsia.

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## Association between iodine and selenium and oxidative stress in placenta

**Nahal Habibi<sup>1,2</sup>, Tanja Jankovic-Karasoulos<sup>2</sup>, Shalem Yiner-Lee Leemaqz<sup>2</sup>, Maxime Francois<sup>3,4</sup>, Shao Jia Zhou<sup>1</sup>, Anthony V Perkins<sup>5</sup>, Claire Roberts<sup>2</sup>, Tina Bianco-Miotto<sup>1,2</sup>**

1. School of Agriculture, Food and Wine, University of Adelaide, Adelaide, SA, Australia

2. Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

3. CSIRO Health and Biosecurity, Future Science Platforms Probing Biosystems, University of Adelaide, Adelaide, SA, Australia

4. School of Biological Science, University of Adelaide, Adelaide, SA, Australia

5. School of Medical Science, Griffith University, Gold Coast Campus, Parklands Drive, Southport, QLD, Australia

**Background** A balanced maternal diet during pregnancy is essential for child growth, development, and lifelong health. Micronutrients obtained from mother's diet are important for several processes in pregnancy including placentation and antioxidant defence system. Too much oxidative stress can adversely affect placental development and functions. Poor placentation and oxidative stress are associated with pregnancy adverse outcomes such as preeclampsia and preterm birth. Epidemiological studies have shown that lower maternal levels of selenium and iodine are associated with a higher incidence of a complicated pregnancy. These two essential micronutrients may be involved in neutralizing oxidative stress; thus higher placentation quality and healthy pregnancy outcomes. **Aim** To determine how selenium, iodine, and their combination may impact oxidative stress in HTR-8/SVneo trophoblast cells. **Method** HTR8 cells were supplemented with mineral or organic selenium compounds, iodine, or their combination for 24 hours. Cells were then treated with menadione or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 24 hours to simulate endogenous or exogenous oxidative stress, respectively. Cell viability and also lipid peroxidation as the biomarker of oxidative stress were assessed at the end of treatments. **Results** Both menadione and H<sub>2</sub>O<sub>2</sub> decreased cell viability and increased lipid peroxidation, significantly. Supplementation with selenomethionine or sodium selenite was associated with higher cell viability (P<0.05). Lipid peroxidation in cells-supplemented with selenium or iodine separately or together was significantly lower in comparison to no supplemented cells (P<0.05). **Conclusion** Selenium and iodine may protect placental cells against oxidative stress which is important for a healthy pregnancy. Currently we are expanding this research by performing similar experiments on first trimester placental tissue.

## Placental extracellular vesicles released from first-trimester, term, and pre-eclamptic placenta all carry intact fetal genes

**Matthew MK Kang<sup>1</sup>, Cherie CB Blenkiron<sup>1</sup>, Larry LWC Chamley<sup>1</sup>**

*1. The University of Auckland, Auckland, New Zealand*

The human placenta is lined by a single multinucleated cell, syncytiotrophoblast. The syncytiotrophoblast releases three sizes of extracellular vesicles (EV) that carry cell-free fetal DNA (cfDNA) into the maternal circulation; 1) macro-, 2) micro-, and 3) nano-EVs. We hypothesised that EVs released from first trimester, term, and pre-eclamptic placenta have varying cargos of fetal DNA including intact fetal genes.

Placental explant cultures followed by differential centrifugation were used to isolate macro-, micro-, and nano-EVs from first-trimester, term, and pre-eclamptic placentae (n=3-5). Following DNA extraction from the EVs, long-range PCR for *Csh1* (7150 bp) and *Vegfa* (18008 bp) were conducted to confirm the presence of intact genes, and TapeStation analysis was used to determine prominent DNA fragment sizes.

Long-range PCR indicated all three EV types from first-trimester, term, and pre-eclamptic placentae contained intact *Csh1* and *Vegfa* genes and their 5' regulatory regions. While accurate quantification is not possible using this method, the amplicons from first trimester macro-EVs were markedly stronger than those from term macro-EVs. In contrast, the amplicons from macro-EVs from preeclamptic placentae were comparable to those from first trimester.

TapeStation analysis confirmed that all EVs sizes contained abundant long DNA fragments, but the long fragments in term macro-EVs were on average shorter (9 - 13 kb) than those from either first-trimester (13 - 30 kb) or pre-eclamptic macro-EVs (9 - 25 kb). There were no obvious differences in DNA fragment size in micro- or nano-EVs from first trimester, term or preeclamptic placentae.

All placental EVs we examined, regardless of EV size or placenta of origin contained full-length genes encoding the model genes *Csh1* and *Vegfa*. We have previously hypothesised that placental EVs have the capacity to transfect maternal cells with fetal genes and this demonstration that all sizes of placental EVs carry intact fetal genes supports that hypothesis.

## Maternal hypothyroidism during pregnancy in the rat induces glucose intolerance, reduces fetal growth and alters placental morphology

**Nykola Kent<sup>1</sup>, Karen Moritz<sup>1</sup>, James Cuffe<sup>1</sup>**

*1. The University of Queensland, St Lucia, QLD, Australia*

**Introduction:** Hypothyroidism is estimated to affect 3% of pregnant women and has been linked to gestational diabetes mellitus (GDM) and fetal growth restriction. However, the role of the placenta in mediating this process is poorly understood. This study aimed to investigate placental alterations caused by maternal hypothyroidism that may contribute to perturbed glucose homeostasis in pregnancy.

**Methods:** Hypothyroidism was induced in nulliparous female Sprague-Dawley rats by exposure to 0.02% methimazole in their drinking water for seven days prior to mating and throughout pregnancy. On embryonic day (E) 16, pregnant dams underwent an intraperitoneal glucose tolerance test. Maternal blood was collected three days prior to mating and on E10, E16 and E20 for determination of plasma thyroxine (T4). Animals were culled on E20 for collection of placental tissue. Placentas were separated into the junctional zone (JZ) and labyrinth zone (LZ) and RNA extracted for qPCR analysis. Gene expression of placental factors implicated in GDM will be assessed by qPCR.

**Results:** Four days into the methimazole treatment, dams had a significant reduction in plasma T4 concentration relative to control animals and this remained significantly reduced throughout pregnancy. The glucose tolerance test indicated that dams were glucose intolerant by E16 and, on E20, there was a significant reduction in fetal weight. While absolute placental weight was unaffected, JZ weight was reduced and the placenta to body weight ratio increased.

**Discussion:** This study highlights the importance of thyroid hormone homeostasis for maternal glucose control. Given that the placenta secretes several hormones implicated in GDM, this study investigated how thyroid deficiency impacts placental function. Further analysis will investigate thyroid responsive pathways within the JZ and how these relate to production of placental hormones implicated in GDM. Given that fetal growth is impaired in this model, future studies will also characterise long term outcomes in offspring.

## Long-term trophoblast monolayer cultures may be useful to study placental extracellular vesicles

**Yohanes N. S. Nursalim<sup>1</sup>, Julie Wang<sup>1</sup>, Cherie Blenkiron<sup>1</sup>, Katie Groom<sup>1</sup>, Larry Chamley<sup>1</sup>**

*1. University of Auckland, Auckland, New Zealand*

The multinucleated syncytiotrophoblast covers the human placenta and releases large quantities of extracellular vesicles (EVs) into the maternal blood. These EVs are lipid enclosed packages of cellular contents that are implicated in the fetal regulation of maternal adaptation or mal-adaptation to pregnancy. We pioneered the use of placental explants to generate EVs but are not amenable to long-term manipulations. Here we investigate an alternative model capable of stable generation of placental EVs.

Trophoblast cells were obtained from term placentae following sequential Dispase digestion. Concentrations and sizes of EVs were determined by Nanoparticle tracking analysis (NTA). Purity and viability were determined by FACs analysis (w6/32 and propidium iodide negative, respectively). Results were expressed as mean  $\pm$  SEM.

There were approximately  $2.51 \times 10^6 \pm 0.23$  cells isolated/gram of placenta ( $n = 4$ ). Of these,  $91.3\% \pm 2.23$  were viable but  $3.7\% \pm 1.45$  ( $n = 3$ ) were not cytotrophoblasts (w6/32<sup>+</sup>). By day three of culture, syncytialisation of the trophoblasts was obvious with loss of adjoining plasma membranes visualized by PKH67 staining.

NTA analysis of media, sampled every second day, indicated there was no significant change in the rate of micro-EVs ( $p = 0.45$ ,  $n = 4$ ) or nano-EVs ( $p = 0.15$ ,  $n = 4$ ) produced from days 3-12. Cultured trophoblasts produced equivalent quantities of nano-EVs but significantly less micro-EVs ( $p < 0.0001$ ,  $n = 4$ ) per  $\text{cm}^2$  of syncytiotrophoblast than placental explants.

It has been long known that mononuclear trophoblasts spontaneously form syncytial structures during in vitro culture. Here we show preliminary evidence that the EVs produced by such cultures are similar in quantity to those produced by placental explants. Unlike explants, we have maintained some of these cultures with minimal contamination for at least 35 days and therefore they may be a useful tool for the study of placental EVs.

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## Placental syndecan-1 is dysregulated in fetal growth restriction and regulated by matrix metalloproteinases

**Damanpreet DS Singh<sup>1,2</sup>, Teresa TM MacDonald<sup>1</sup>, Susan SW Walker<sup>1</sup>, Stephen ST Tong<sup>1</sup>, Natalie NH Hannan<sup>1</sup>, Natasha NP Pritchard<sup>1</sup>, Ping PC Cannon<sup>1</sup>, Alesia AH Harper<sup>1</sup>, Elizabeth LM Murray<sup>1</sup>, Tu'Uhevaha TL Joy Lino<sup>1</sup>**

1. *Obstetrics and Gynaecology, University of Melbourne, Melbourne, Victoria, Australia*

2. *Obstetrics and Gynaecology, University of Melbourne, Melbourne, Victoria, Australia*

Fetal growth restriction (FGR) or small for gestational age (SGA), when a baby fails to reach its predestined genetic growth capacity, is a leading cause of stillbirth. The molecular pathogenesis of SGA is poorly understood and there are no accurate biomarkers.

We have recently identified Syndecan-1, a cell surface proteoglycan, as significantly reduced in the circulation of women preceding their diagnosis of SGA. In addition, we've shown that placental Syndecan-1 is reduced by hypoxia and its knockdown impairs trophoblast proliferation.

The aim of this study was to characterise Syndecan-1 in the placentas and blood of women with established FGR. In addition, we sought to determine how syndecan-1 is released from placenta and whether silencing syndecan-1 altered the expression of Placental Growth Factor, a molecule reduced in SGA and important in normal placental function.

In a cohort of placentas collected from women who delivered a FGR baby at  $<34$  weeks' gestation ( $n = 18$ ), Syndecan-1 mRNA expression was significantly increased relative to preterm control placentas ( $n = 15$ ), whilst circulating Syndecan-1 protein was significantly reduced in the FGR cohort ( $n = 9$ ) relative to controls ( $n = 17$ ). Treatment of isolated primary human trophoblast ( $n = 4$  separate isolations) with broad spectrum matrix metalloproteinase inhibitor batimistat potently reduced the secretion of Syndecan-1 in a dose-dependent manner without altering mRNA expression. siRNA knockdown of Syndecan-1 in primary trophoblast however did not alter the mRNA expression of Placental Growth Factor.

In conclusion, Syndecan-1 is reduced in the circulation of women with both established FGR and preceding diagnosis of FGR/SGA whilst placental mRNA expression is increased in FGR. We provide new evidence that members of the matrix metalloproteinase family are likely regulators of placental Syndecan-1 secretion.

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## The effects of periconceptional ethanol exposure on markers of oxidative stress in maternal liver and late-gestation placental tissue

**Sarah E Steane<sup>1</sup>, Tulika Das<sup>1</sup>, Jacinta I Kalisch-Smith<sup>1</sup>, Lisa K Akison<sup>2</sup>, Karen M Mortiz<sup>2</sup>, James SM Cuffe<sup>1</sup>**

1. *The University of Queensland, St Lucia, QLD, Australia*

2. *Child Health Research Centre, The University of Queensland, St Lucia, QLD, Australia*

Alcohol consumption during pregnancy can cause oxidative stress (OS) by impairing mitochondrial function in maternal and fetal tissues, potentially increasing the risk for chronic disease in offspring. The placenta is known to mediate fetal vulnerability to OS. However, it is unknown how alcohol impacts maternal and placental OS when exposure only occurs around conception. This study aimed to measure markers of OS in maternal and placental tissues following periconceptional alcohol exposure (PC:EtOH).

Sprague-Dawley dams were fed liquid diets containing 12.5% v/v EtOH (PC:EtOH) or 0% EtOH (control) from 4 days prior to mating until embryonic day (E)4. Maternal livers were collected at E5 and E20 and placentas at E15 and E20. Placentas were separated into junctional (JZ) and labyrinth (lab) zones and fetal sex determined. Mitochondrial content and markers of OS (*Sod1/2*, *Cat*, *Gpx1/3*, *Txn1*, *Nos3/4*) were measured using qPCR and 5 major oxidative phosphorylation (OXPHOS) protein complexes were measured by Western Blot. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) production and advanced glycation end-products (AGEs) were also measured.

Mitochondrial content was lower in maternal liver immediately following PC:EtOH at E5 (4-fold reduction compared to controls,  $P < 0.0001$ ), and remained low at E20 ( $P = 0.011$ ). Mitochondrial OXPHOS complexes were disrupted at E5 but there were no

## Detection of reduced oxygenation in 3D placental volumes by magnetic resonance imaging (MRI) in a rat model of fetal growth restriction (FGR)

**Yutthapong Tongpob<sup>1,2</sup>, Alexander Joos<sup>3</sup>, Caitlin Wyrwoll<sup>1</sup>, Kirk Feindel<sup>3,4</sup>**

1. School of Human Sciences, The University of Western Australia, Perth, WA, Australia

2. Department of Anatomy, Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand

3. Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Perth, WA, Australia

4. School of Biomedical Sciences, The University of Western Australia, Perth, WA, Australia

A key driver of FGR is placental insufficiency, including inadequate development of placental vasculature. However, there are currently limitations in assessing placental function *in-vivo*. MRI can measure relaxation time (T2\*), which correlates with oxygenation via alterations in blood haemoglobin saturation. Results of previous placental T2\* studies have been conflicting, due to technical and analytical challenges, including consideration of only 2D-regions of interest (ROI).

This study utilised a 3D-MRI approach in a rat model of FGR.

Time-mated rats were treated with either vehicle (Veh) or dexamethasone (Dex; 0.5µg/ml) in drinking water from embryonic day (E)13 onwards. Serial scans at E15, E18, and E21 were conducted using a 9.4T MRI *in-vivo*: a 3D-multi-gradient-echo sequence with oxygen challenge (oxygen vs medical-air) to obtain 3D-maps of the T2\* signal. E21 dams were euthanised, fetoplacental units dissected, and weighed. E21 T2\*-values were calculated for manually defined 3D-ROIs with custom-Matlab software using the SQEXP algorithm, which is better suited for low signal to noise data than standard algorithms. In a separate cohort of rats, fetoplacental vascular casts were generated, scanned using micro-CT and quantified with custom-Matlab software.

E21 fetal and placental weights decreased in Dex rats by 15.6% and 35.2% respectively in comparison to Veh ( $p < 0.0002$ ). MRI scans revealed that when shifting from oxygen to medical-air, mean whole-placental T2\* decreased in Veh and Dex by 35.2% and 20.9% respectively ( $p < 0.05$ ). Importantly, the relative shift in whole-placental T2\* differed significantly between Veh and Dex (3.1±0.4 vs 2.0±0.5 msec;  $p < 0.05$ ), indicating reduced blood oxygenation in Dex placentas. Furthermore, the MRI measures aligned with a marked decline in fetoplacental vascular complexity in Dex.

Ongoing analyses are determining whether these changes are dynamic across gestation. The results of this proof-of-concept study demonstrate that T2\*-based measurements of the placental blood oxygenation can provide non-invasive assessments of *in-vivo* placental health.

## Elevated HtrA4 in the maternal circulation of preeclampsia may promote premature endothelial aging

**Yao Wang<sup>1,2</sup>, Guiying Nie<sup>1,2</sup>**

1. Hudson Institute of Medical Research, Clayton, Victoria, Australia

2. Molecular and Translational Science, Monash University, Clayton, VIC, Australia

**Objectives:** Preeclampsia (PE) is a serious complication of human pregnancy affecting 2-8% of pregnancy worldwide. Women who have had PE, especially early-onset PE (EPE, occurring  $\leq 34$  weeks of gestation), have an increased risk of cardiovascular disease (CVD) later in life. However, how PE is linked to CDV is not well understood. HtrA4 is expressed specifically by the placenta, and is significantly elevated in the EPE circulation [1]. We have also reported that elevated circulating HtrA4 can inhibit proliferation of endothelial cells as well as endothelial progenitor cells (EPCs), which play a critical role in endothelial regeneration [2]. We thus hypothesized that elevated circulating HtrA4 may promote endothelial aging, which is the biggest risk factor of CVD.

**Aim:** To examine whether HtrA4 alters endothelial expression of genes associated with senescence.

**Methods:** Human umbilical vein endothelial cells (HUVECs) and primary EPCs isolated from cord blood of healthy pregnancies were used as models. HUVECs were treated with HtrA4 for 48h and screened with a cell senescence PCR array. The results were then validated by RT-PCR and ELISA in HUVECs and EPCs treated with different doses of HtrA4 for 24 and 48h.

**Results:** The array revealed that HtrA4 altered HUVEC expression of 21 genes associated with senescence, 6 upregulated and 15 downregulated. All these genes were validated by RT-PCR. In particular, HtrA4 significantly up-regulated *IGFBP3*, *SERPINE1* and *SERPINE2*, which all promote senescence. IGFBP3 protein was also significantly elevated in HtrA4-treated HUVEC media. Conversely, a number of genes including *CDKN2C* and *CHEK2* that regulate cell cycle, were downregulated by HtrA4. Many of these genes also showed a similar trend of change in EPCs following HtrA4 treatment, although the majority did not reach a statistical significance.

**Conclusions:** High levels of placenta-derived HtrA4 detected in EPE circulation may promote premature endothelial aging to contribute to CVD development.

1. Singh, H., et al., Human HtrA4 Expression Is Restricted to the Placenta, Is Significantly Up-Regulated in Early-Onset Preeclampsia, and High Levels of HtrA4 Cause Endothelial Dysfunction. *J Clin Endocrinol Metab*, 2015. 100(7): p. E936-45.
2. Wang, Y., R. Lim, and G. Nie, HtrA4 may play a major role in inhibiting endothelial repair in pregnancy complication preeclampsia. *Sci Rep*, 2019. 9(1): p. 2728.

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### **Adrenomedullin promotes functions of extravillous trophoblast and endothelial cells in spiral artery remodeling which be beneficial effect in preeclampsia symptom.**

**Qing Qing Zhang<sup>2,1</sup>, Cheuk-Lun Lee<sup>2,1</sup>, yonggang Duan<sup>2</sup>, Philip CN Chiu<sup>2,1</sup>**

1. *Hong Kong University, Hong Kong*

2. *Obstetrics and gynaecology, The University of Hong Kong-Shenzhen Hospital, Shenzhen, GuangDong, China*

Background:

Preeclampsia is a pregnancy complication which affects 5% of all pregnancies and defined as gestational hypertension and proteinuria. Although the exact cause of preeclampsia remains unclear, there are many studies focus on the relationship between preeclampsia with spiral artery remodeling. Adrenomedullin (ADM) is a peptide belonging to the calcitonin/calcitonin-gene-related peptide (CGRP)/amylin peptide family. In human, plasma ADM level is elevated after implantation and peaks in early pregnancy. The role of ADM in pathology of preeclampsia remains to be demonstrated.

Methodology: The involvement of human subjects in this study was approved by the Institutional Review Board (IRB) of The University of Hong Kong/Hospital Authority Hong Kong West Cluster. To investigate the functions of trophoblast including differentiation, invasion, migration, extravillous trophoblast (EVCT) derived from first trimester villous (gestation 5-12 weeks), term placenta, and chorionic villus samples (CVS) collected from placenta at 10–12 weeks gestation were used.

Results: ADM expression was reduced in term placenta. In CVS sample, ADM expression was found in syncytiotrophoblast and cytotrophoblast. Particularly, the expression of ADM in CVS of preeclampsia patients was reduced. EVCT invasion, migration and integration capability were significantly ( $P < 0.05$ ) enhanced by ADM (10 nM or 100 nM) treatment for 16-24 hours. In tube formation assay, 100 nM ADM significantly enhanced the tube formation ability of HUVEC cells. In permeability assay, 10 or 100 nM ADM reduced permeability of HUVEC cells. Immunocytochemistry staining of HUVEC cells showed that there was increased expression of cell junction marker, ZO-1 and VE-cadherin, after 10 or 100 nM treatment. In conclusion, Expression of ADM was reduced in term placenta and CVS sample of preeclampsia patients. ADM regulates the functions of human EVCTs and endothelial cells. Further study on the mechanism that regulates the biological activities of ADM would enhance our understanding on the physiology of early pregnancy in humans.

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### **ROS production and localisation of mitochondrial glycerol 3-phosphate dehydrogenase in first trimester placentae**

**Rachel Zussman<sup>1,2</sup>, Qi Chen<sup>2</sup>, Larry W Chamley<sup>2</sup>, Anthony J R Hickey<sup>1</sup>**

1. *School of Biological Sciences, The University of Auckland, Auckland, New Zealand*

2. *Department of Obstetrics and Gynaecology, The University of Auckland, Auckland, New Zealand*

Placental mitochondria have high activity of the enzyme glycerol 3-phosphate dehydrogenase (mGPDH), which feeds into the electron transport system. The specific role of mGPDH in the placenta is unknown, but it is linked to glucose and lipid metabolism and is known to produce high levels of reactive oxygen species (ROS). There is evidence of placental oxidative stress in pregnancy diseases such as preeclampsia, so understanding the role of mGPDH in the placenta is important, particularly early in pregnancy when placental development is critical.

Therefore, we aimed to confirm the activity and ROS production of mGPDH in first trimester placentae relative to other sources of mitochondrial ROS. Additionally, we investigated whether mGPDH is differentially expressed in cytotrophoblast (CTB) and syncytiotrophoblast (STB) mitochondria as they are known to have different morphology and activities.

All experiments were performed on first trimester placentae collected with informed consent. Simultaneous mitochondrial respiration and ROS production rates were measured in high resolution respirometers coupled with fluorimeters, using multiple substrate protocols ( $n = 7-10$ ). Localisation of mGPDH was determined using immunohistochemistry on paraformaldehyde-fixed paraffin-embedded placental samples (5-12 weeks gestational age,  $n=5$  samples per gestational week).

First trimester placental explants demonstrated an ability to utilise mGPDH to respire and produce ROS as expected. The rate of ROS production by mGPDH was significantly higher than that of complexes I or II. Both STB and CTB stained positively for mGPDH and in 40% of images analysed, we observed more intense staining in CTB than STB. This staining pattern was significantly more frequent in the samples less than 10 weeks of gestational age.

First trimester placental mitochondria have the potential to produce high levels of ROS through mGPDH and it may be more active in CTB than STB in early gestation.

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### **Exogenous melatonin increases sperm production in the ram during the non-breeding season**

**Kelsey R Pool<sup>1</sup>, Jessica P Rickard<sup>1</sup>, Taylor Pini<sup>1</sup>, Simon P de Graaf<sup>1</sup>**

1. *Faculty of Science, The University of Sydney, Sydney, NSW, Australia*

The pineal neurohormone melatonin modulates ovine reproductive seasonality, with natural increases in melatonin denoting the breeding season and subsequent escalation of fertility. Despite the commercial use of melatonin implants to promote out-of-season fertility in the ewe, their impact on the reproductive physiology of the ram remains poorly described. To remedy this, mature rams were treated with (n=14) or without (n=17) slow release melatonin implants (3 x18mg implants/ram; Regulon, CEVA Animal Health, NSW Australia) during the early non-breeding season (September). Endocrinological and reproductive parameters such as seminal plasma and blood serum melatonin levels, serum testosterone levels, testicular circumference, sperm production and quality were recorded in the weeks immediately prior to melatonin implantation, post-implantation during the non-breeding season (Sept-Dec) and again in the following breeding season (March-April). Melatonin implantation resulted in a substantial elevation of both blood serum and seminal plasma melatonin concentrations in comparison to both pre-implantation and control ram levels ( $P<0.001$ ), seminal plasma melatonin remaining heightened throughout the non-breeding season ( $P<0.001$ ). During this same period, melatonin-treated rams also exhibited an increase in blood serum testosterone ( $P<0.001$ ). The testicular circumference of melatonin-treated rams was significantly increased throughout the non-breeding season, corresponding with an increase in total sperm production per ejaculate ( $P=0.028$ ). Control rams showed no significant change in either testes size or sperm production. No effect of treatment was observed upon sperm motility or morphology. Increased sperm production in melatonin treated rams during the non-breeding season did not negatively affect sperm production or quality in the subsequent natural breeding season. This study indicates that exogenous melatonin promotes an up-regulation of the ram reproductive axis, effectively advancing the male breeding season and improving ram sperm production in the non-breeding season.

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### High density lipoproteins enhance cholesterol efflux and stimulate hyperactivation during ram sperm capacitation

**Naomi Bernecic<sup>1,2</sup>, Simon de Graaf<sup>1</sup>, Bart Gadella<sup>3,4</sup>, Tamara Leahy<sup>1</sup>**

1. Faculty of Science, School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia

2. Department of Biological Sciences, Laboratory of Animal Reproduction, The University of Limerick, Limerick, Ireland

3. Department of Biochemistry & Cell Biology, Utrecht University, The Netherlands

4. Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Sheep serum is commonly supplemented to capacitating media for ram spermatozoa in order to support *in vitro* fertilisation (IVF). Its exact role in capacitation is uncertain but it is thought to support cholesterol efflux owing to the presence of cholesterol acceptors such as albumin and the various subtypes of high density lipoproteins (HDLs). During cholesterol efflux, HDLs remove this sterol via a facilitated pathway that involves interaction with cholesterol transporters located in the plasma membrane, including ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor class B, type I (SR-B1). With this knowledge, the objectives of the current study were to i) determine if sheep serum or the specific cholesterol acceptor, HDLs, were able to elicit cholesterol efflux in ram spermatozoa and ii) whether the cholesterol transporters, ABCA1 and SR-B1 were functional during cholesterol efflux as assessed by the use of antagonists. Both sheep serum and HDLs were able to elicit cholesterol efflux alone by up to 40-50% (as measured with the BODIPY-cholesterol assay) and caused additional efflux when combined with 0.3% BSA. Surprisingly, the addition of HDLs to ram spermatozoa also induced hyperactivation in at least 19.2% (95% CI: 12.3%-25%) of the population, which was in contrast to the addition of BSA alone (2.9%; CI: 1.6%-3.9%). Following the inhibition of ABCA1 and SR-B1 by glibenclamide (both transporters) and valsopodar (ABCA1 alone), sheep serum-mediated cholesterol efflux was reduced by 15% compared to when these inhibitors were absent. In contrast, only glibenclamide was able to reduce HDL-mediated cholesterol efflux. Together, these findings indicate that cholesterol efflux in ram spermatozoa may be regulated by a pathway involving the interaction between HDL subtypes and the cholesterol transporters, ABCA1 and SR-B1. Furthermore, the induction of hyperactivation under these conditions suggests a functional link with HDL-mediated cholesterol efflux.

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### Assessment of acrylamide generated DNA damage in sperm on fertilisation and embryo development

**Nathan D Burke<sup>1,2,3</sup>, Elizabeth G Bromfield<sup>1,2,4</sup>, Jacinta H Martin<sup>1,2</sup>, Shaun D Roman<sup>1,2,3</sup>**

1. The Priority Research Centre for Reproductive Biology, The University of Newcastle, Callaghan, New South Wales 2308, Australia

2. Discipline of Biology, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, New South Wales, Australia

3. The Priority Research Centre for Drug Development, The University of Newcastle, Callaghan, New South Wales 2308, Australia

4. Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Spermatozoa DNA damage is intimately linked to several reproductive pathologies including paternal factor infertility. Ingested chronically, acrylamide is metabolised by CYP2E1 to a known reproductive toxicant and DNA alkylating agent, glycidamide. Glycidamide has been implicated in inducing DNA damage in sperm and acute acrylamide exposure *in vivo* has induced embryonic dominant lethality and chromosomal translocations. To investigate the ramifications of DNA damage on preimplantation embryogenesis, sperm were exposed to glycidamide in conditioned *in vitro* fertilisation (IVF) media generated by culturing immortalised epididymal cells rich in CYP2E1 with acrylamide (10mM). This novel treatment emphasised the maintenance of sperm function and no aberrations were detected in capacitation ( $p<0.05$ ) or motility (~80%). Single cell gel electrophoresis revealed significant levels of DNA damage in exposed sperm compared to control sperm ( $p<0.001$ ), with a 50-fold increase in the minimum DNA damage reported in all treated cells. Glycidamide treated sperm were subsequently used for IVF. The fertilisation rate of sperm with DNA damage (~92%) was unchanged compared to control cells (~93%), and over 90%

of embryos from all treatments underwent cleavage, forming 2-cell embryos, by 24 hours. Preimplantation embryo development continued unimpeded in embryos fathered by sperm carrying DNA damage; culminating in over 80% of embryos from all treatments forming blastocysts at 96 hours. A subset of embryos derived from glycidamide treated sperm (~30%) exhibited delayed development at 48 and 96 hours and may reflect the impediment of embryonic genome activation in 4-cell embryos. Investigations of DNA damage in paternal pronuclei ( $\gamma$ -H2A.x ICC and TUNEL staining) and blastocyst stage embryos ( $\gamma$ -H2A.x ICC) revealed no aberrations in embryos fathered by glycidamide treated sperm. Despite extensive DNA damage, treated sperm were able to fertilise and produce developmentally competent embryos, emphasising the importance of understanding the ramifications of DNA damage on pregnancy outcomes and offspring health.

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## Exploring changes to protein homeostasis in male and female germ cells in response to ageing and oxidative stress

**Shenae L Cafe<sup>1</sup>, Shaun D Roman<sup>1,2</sup>, Jacinta H Martin<sup>1,2</sup>, Brett Nixon<sup>1</sup>, Matthew D Dun<sup>3</sup>, Elizabeth G Bromfield<sup>1,4</sup>**

1. The University of Newcastle, Callaghan, NSW, Australia

2. The Priority Research Centre for Drug Development, University of Newcastle, Callaghan, NSW, Australia

3. School of Biomedical Science and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW, Australia

4. Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Oxidative stress has been implicated in an extensive range of age-related pathologies and is a well-known cause of infertility. The negative impacts of oxidative stress on male and female germ cell viability are primarily underpinned by the peroxidation of fatty acids, resulting in the production of highly reactive lipid aldehydes, such as 4-hydroxynonenal (4HNE). In many cell types, the portion of the cell proteome that is targeted for 4HNE-modification often experiences severe protein misfolding that, in turn, leads to a disruption of protein homeostasis (proteostasis). This study was designed to explore a relationship between oxidative stress, ageing and protein aggregation in male and female germ cells with a key focus on uncovering mechanisms to prevent protein damage. Through the development of several robust strategies for the detection of protein aggregates, this study has revealed a causative role for oxidative stress in the induction of protein aggregation in both pachytene spermatocytes and round spermatids. Specifically, the exogenous application of 0.1mM 4HNE to these cells resulted in a significant increase in aggregation ( $P < 0.005$ ) detectable with the amyloid-specific fluorophores Proteostat and Thioflavin T, and the conformer specific antibodies anti-A11 and anti-OC. In this study, nucleocytoplasmic transport was examined as a potential mechanism for the subcellular compartmentalisation of aggregating proteins. The inhibition of transport proteins karyopherin beta 1/alpha 2 (KPNB1/A2) and exportin 1 (XPO1), resulted in a significant increase in cellular protein aggregates in male germ cells ( $P < 0.005$ ). In the female germline, both *in vivo* and *in vitro* oocyte ageing resulted in the development of cytoplasmic protein aggregates in germinal vesicle oocytes, with the subcellular compartmentalisation of these aggregates tightly control by XPO1. These results reveal the importance of functional nucleocytoplasmic transport systems for the management of misfolded proteins in male and female germ cells.

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## L-carnitine is a pro-survival factor for ambient temperature storage of bull spermatozoa

**Erin K Klein<sup>1</sup>, Allan J Gunn<sup>2</sup>, Cyril P Stephen<sup>2</sup>, Aleona Swegen<sup>1</sup>, Robert J Aitken<sup>1</sup>, Zamira Gibb<sup>1</sup>**

1. The University of Newcastle, Callaghan, NSW, Australia

2. Charles Sturt University, Wagga Wagga, NSW, Australia

Artificial Insemination (AI) is underutilized by northern Australian beef cattle producers due to the logistical complications associated with using chilled or cryopreserved sperm which requires oestrus synchronization and precise timing of insemination. Through the development of an ambient temperature sperm preservation medium, the lipid membrane phase changes that occur during cooling can be avoided so that spermatozoa will maintain greater longevity and fertility after insemination. The aim of this study was to develop an ambient temperature sperm storage medium to maintain sperm motility and viability for at least one week. As the regulation of intracellular sodium presents a major source of ATP loss during cell storage, the effect of replacing sodium chloride with L-carnitine as the primary osmolyte in a modified Biggers, Whitten, and Whittingham (BWV) medium was investigated. Bull semen was collected by electroejaculation (N=8) and viable spermatozoa were selected using a density gradient. When compared to spermatozoa stored at 5°C in a commercial semen extender (INRA96), the carnitine-BWV (at RT) had significantly higher total (TM) and progressive (PM) motilities at day 3 (TM: 71.63±3.85% vs. 58.75±6.50%; PM: 38.00±4.10% vs. 11.50±2.73%;  $P \leq 0.0015$  and  $< 0.0001$  respectively), 7 (TM: 65.88±4.76% vs. 51.25±6.17%; PM: 27.25±5.18% vs. 9.50±3.39%;  $P \leq 0.0005$  and  $< 0.0001$ ), and 10 (TM: 59.88±4.25% vs. 35.25±8.14%; PM: 19.00±4.18% vs. 5.50±2.41%;  $P \leq 0.0008$  and  $\leq 0.0032$ ) and significantly higher viability (53.88±3.44% vs. 25.18±3.14%;  $P < 0.0001$ ) and acrosome integrity (41.34±4.34% vs. 23.66±3.03%;  $P < 0.0001$ ) after 3 days. Spermatozoa is stored at low temperatures to reduce its metabolic rate. At higher temperatures, the uncurbed metabolic activity results in high levels of reactive oxygen species (ROS) and depletion of ATP. The results of this study show that L-carnitine is a potent pro-survival factor for bull spermatozoa, most likely through its actions as an osmolyte and a powerful antioxidant, along with its role in mitochondrial ATP synthesis.

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## Mass spectrometry analysis of density separated spermatozoa reveals that nucleoplasm retention plays a role in nuclear maturity



**Jacob K Netherton<sup>1</sup>, Rachel A Ogle<sup>1</sup>, Louise Hetherington<sup>1</sup>, Mark A Baker<sup>1</sup>**

*1. The University of Newcastle, Callaghan, NSW, Australia*

Spermatogenesis is an extremely specialised process that generates a cell capable of the protection and delivery of the paternal genome to the oocyte. During the development of a spermatozoon, the basic chromatin structure of DNA bound to histones is drastically altered, and nuclear volume is greatly reduced. Importantly poor chromatin compaction has been commonly associated with cases of male factor infertility.

To better understand the process of sperm nuclear condensation and maturation, we isolated sperm nuclei from cells with markers of good and poor compaction from an ejaculate using a density gradient. Comparative proteomics was performed on the nuclear proteins, using the quantitative SWATH platform on the Sciex 6600 Triple ToF. We confidently identified 342 proteins, and of these proteins 20 were found to be more abundant in the sperm possessing poor chromatin compaction, many of which are associated with nucleoplasm. Immunoblots using an antibody against TOP2A and PDIA3 confirmed the proteomic analysis. We have also demonstrated that the number of cells in which we are able to detect TOP2A by immunocytochemistry is higher in spermatozoa with poor chromatin compaction. Unexpectedly, no changes were observed in any of the identified histone peptides (H4, H3.3, H1T, H2A/B), nor for protamine 2. Our data suggests an alternate explanation for poor chromatin compaction. Rather than changes in histone or protamine content, it appears that retained or excess nucleoplasm is more prevalent in poorly compacted nuclei.

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### Comparison of two methods for sperm viability evaluation in Jeju cross-breed horse

**Sang Min Shin<sup>1</sup>, Sung Woo Kim<sup>2</sup>, Nam Young Kim<sup>1</sup>, Nam Geon Park<sup>1</sup>, In Cheol Cho<sup>1</sup>, Moon Cheol Shin<sup>1</sup>, Ji Hyun Yoo<sup>1</sup>, Jae Young Choi<sup>1</sup>, Jae Hoon Woo<sup>1</sup>, Byoung Chul Yang<sup>1</sup>**

*1. National Institute of Animal Science, Jeju-si, Jeju-Do, Republic of Korea*

*2. National Institute of Animal Science, Namwon-si, Jeollabuk-do, Republic of Korea*

Sperm viability is an important indicator of sperm function evaluation and is associated with plasma membrane integrity. One of plasma membrane main functions is the excretion towards outside and the selection of molecules to pass from outside to inside. A defect in the plasma membrane can easily lead to the death of the sperm. Sperm viability can be assessed by microscopy, image cytometer, flow cytometry using fluorescent dyes. CASA(AndroVision) and flow cytometer(FACS Calibur) used in this study are expensive and highly accurate equipment, but flow cytometer is known to be more expensive and more accurate than CASA.

The purpose of this study was to compare two methods of evaluating sperm survival rates in the equine to find its usefulness

CASA used Hoechst 33342(H33342)/PI dual staining and flow cytometry used CFDA/PI dual staining.

The stain Hoechst 33342 permeates cell membrane and binds specifically to the DNA. All sperm are marked blue. CFDA is permeable cell membrane. It is transformed into a fluorescent called carboxyfluorescein by the esterase of living cells, and show green fluorescence. PI stain only permeates damaged membrane, and show red fluorescence.

The viable rate of spermatozoa with CASA(H33342/PI) and flow cytometry(CFDA/PI) was 84.27±7.55% and 82.91±6.68%, respectively(mean±SD%, n=118).

There was no significant difference in viability of spermatozoa between CASA and flow cytometry(CFDA/PI)(P >0.05). Therefore, CASA is judged to be more cost effective than flow cytometry.

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### Effect of Pentoxifylline Concentration on Sperm Quality in Horses

**Junkyu Son<sup>1</sup>, Seolhwa Park<sup>1</sup>, Sangmin Shin<sup>1</sup>, Namyong Kim<sup>1</sup>, Jihwan Lee<sup>1</sup>, Jaehoon Woo<sup>1</sup>, Jihyun Yoo<sup>1</sup>**

*1. National Institute of Animal Science, Cheonan-si, Chungcheongnam-Do, South Korea*

This study was conducted to determine the effect of pentoxifylline levels on sperm motility, survival rate, sperm membrane integrity of frozen semen and fresh-extended equine semen in Jeju cross-bred horses. As a result of sperm characteristic comparison depending on pentoxifylline levels at 30 minutes post-thaw, the progressive motilities were 53.25±2.87(4mM pentoxifylline) and 50.28±2.14(8mM pentoxifylline) and significantly higher compared to the control group(40.09±5.15) and other treatment group(16mM pentoxifylline, 41.27±2.82). The progressive fast motility were 22.44±1.62(4mM pentoxifylline,) and 22.74±3.07(8mM pentoxifylline) and significantly higher compared to the control group(13.47±1.48) and other treatment group(16mM pentoxifylline, 14.66±3.68)(p<0.05). As a result of sperm characteristic comparison depending on pentoxifylline levels at 30 minutes post-thaw were 68.96±1.64(4mM pentoxifylline) and 67.90±6.72(8mM pentoxifylline) and significantly higher compared to the control group(53.48±4.84) and other treatment group(16mM pentoxifylline, 58.14±2.65).(p<0.05) In conclusion, these results suggest that treatment groups with 4mM and 8mM pentoxifylline were higher compared to equine sperm mobility and the control group and treatment groups with more than 16mM pentoxifylline has a negative effect on sperm characteristics. After thawing, the total motility in post-thawed equine sperm has increased by 10 percent for 1 hour. these results suggest that pentoxifylline contributes to the improvement of the equine sperm motility and characteristics in post-thawed semen.

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### Targeted inhibition of the lipoxigenase – lipid peroxidation pathway protects equine spermatozoa against oxidative stress

**Jessica LH Walters<sup>1</sup>, Geoffry N De Juliis<sup>1</sup>, Matthew Dun<sup>2</sup>, Eileen A McLaughlin<sup>3,4</sup>, Robert Aitken<sup>1</sup>, Jessie Sutherland<sup>1,5</sup>, Brett Nixon<sup>1</sup>, Elizabeth G Bromfield<sup>1,6</sup>**

1. School of Environmental and Life Sciences, Discipline of Biological Sciences, Priority Research Centre for Reproductive Science, University of Newcastle, Callaghan, NSW, Australia

2. Hunter Medical Research Institute, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, Priority Research Centre for Cancer Research, Innovation and Translation, The University of Newcastle, Callaghan, NSW, Australia

3. School of Biological Sciences, University of Auckland, Auckland, New Zealand

4. School of Science and Technology, University of Canberra, Bruce, ACT, Australia

5. School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW, Australia

6. Discipline of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

In the standardbred equine breeding industry, approximately 90% of foals are conceived through artificial insemination. Currently, this practice relies on frozen or chilled sperm storage techniques that lower metabolic activity and attendant reactive oxygen species (ROS) production. However, these protocols are not without their limitations, including the risk of accentuating cellular damage that compromises the fertilisation capacity of equine spermatozoa. Although the industry would benefit from the development of effective ambient temperature storage protocols, a major hurdle to the implementation of such strategies is the inherent sensitivity of equine spermatozoa to ROS generated as a by-product of their normal metabolism. Indeed, elevated ROS precipitate a cascade of lipid peroxidation reactions that promote the formation of cytotoxic lipid aldehydes. Our previous work on human spermatozoa has established arachidonate 15-lipoxygenase (ALOX15) as a key catalyst for lipid peroxidation in the male germ line. Hence, we hypothesised that pharmacological inhibition of ALOX15 could offer protection against ROS and associated loss of function during the ambient temperature storage of equine spermatozoa. Consistent with this hypothesis, we have confirmed the conservation of ALOX15 in high quality equine spermatozoa and demonstrated its localisation to the midpiece of the cell, a domain that houses the mitochondria, responsible for driving ROS production. By contrast, ALOX15 was exclusively detected in the centriole of poor quality equine spermatozoa, which are characterised by elevated ROS production and reduced functional competence. We have also identified arachidonic, linoleic and docosahexanoic acid as the key physiological substrates that fuel lipid peroxidation in equine spermatozoa and importantly, have shown that pharmacological inhibition of ALOX15 significantly reduced lipid peroxidation levels in a dose-dependent manner compared to untreated controls ( $p < 0.001$ ). We shall now capitalise on these findings to explore the functional implications of ALOX15 inhibition in terms of sustaining equine spermatozoa longevity during ambient temperature storage.

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## **SEPT14 mutations impact on male infertility due to sperm DNA damage and abnormal morphology**

**Ya-Yun Wang<sup>1</sup>, Ying-Hung Lin<sup>1</sup>**

1. Fu Jen Catholic University, New Taipei City, Xinzhuang District, Taiwan

### **Research question**

Dysfunction of septins, the fourth cytoskeleton protein, leads to male infertility because of sperm morphological abnormalities. SEPT14 is highly enriched in testis, but there are no reports about the role of SEPT14 in infertility. Here we aimed to clarify whether and how SEPT14 cause sperm defects.

### **Design**

254 infertile patients and 116 controls were recruited. After extraction of genomic DNA from sperm, we used polymerase chain reaction-based direct sequencing to detect genetic variants in the entire coding region of SEPT14. The DNA damage and ultrastructure of sperm was evaluated. We also identified the binding partner of SEPT14 and evaluated how SEPT14 mutations affect the binding partner in the sperm.

### **Results**

Two heterozygous mutations, p.Ala123Thr and p.Ile333Thr, were identified in the patients with teratozoospermia, but not in controls. Both positions were highly evolutionarily conserved among vertebrates. Results from fine morphological and chromatin structural analysis indicated severely malformed sperm heads with abnormal chromatin packaging. Compared with controls, prominent apoptosis and high DNA fragmentation was revealed in the sperm from the patients carrying SEPT14 mutations. ACTN4, a cytoskeleton actin-binding protein and participates in the organization of actin framework, interacts with SEPT14 in germ cell line. Furthermore, the localization of ACTN4 was disturbed in the sperm from patients carrying SEPT14 mutations.

### **Conclusions**

The identification of two missense mutations with potentially deteriorating effects on sperm head morphology and DNA damage provides strong evidence to support the notion that SEPT14 is critical for male fertility. SEPT14 probably mediates the coupling of ACTN4 to actin and further affects the actin organization during head shaping.

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## **The modified resazurin reduction assay: a predictor of thoroughbred stallion fertility**

**Stephanie J Waugh<sup>1</sup>, Sarah R Lambourne<sup>1</sup>, Robert J Aitken<sup>1</sup>, Zamira Gibb<sup>1</sup>**

1. Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW, Australia

Artificial insemination is prohibited for the breeding of Thoroughbred horses, and as such there is no way to assess the quality of a Thoroughbred stallion ejaculate prior to breeding. However, residual 'dismount' semen samples are routinely collected from the urethra immediately after coitus to confirm ejaculation and assess basic sperm parameters to determine the need to re-

breed ('cross-cover') the mare prior to ovulation. The resazurin reduction assay (RRA) has previously been shown to provide a basic assessment of fertility potential in both rams and bulls, and the aim of this study was to investigate whether a modified RRA protocol would be effective in identifying sub-fertile ejaculates from stallions. Initially, the relationship between the RRA signal and sperm assessments including motility (CASA), antioxidant capacity (aldehyde dehydrogenase) and reactive oxygen species production (MitoSox Red) was established, after which a field trial was conducted in which the RRA, motility and morphology assessments were run on commercial Thoroughbred dismount samples (N=315) to compare their ability to discriminate between ejaculates which did, or did not result in pregnancies. RRA signal was influenced by sperm concentration, various CASA motility parameters and antioxidant capacity ( $R^2 = 0.83$ ), and the field trial revealed that breedings which resulted in pregnancies had significantly higher RRA signals than those that did not result in pregnancies ( $383.34 \pm 29.32$  vs.  $292.74 \pm 23.41$  AFU;  $P=0.02$ ). Interestingly, total motility ( $54.02 \pm 1.36$  vs.  $51.01 \pm 1.72$ ;  $P=0.17$ ), progressive motility ( $40.62 \pm 1.33$  vs.  $37.81 \pm 1.76\%$ ;  $P=0.19$ ), and normal morphology ( $70.45 \pm 0.78$  vs.  $69.10 \pm 0.90\%$ ;  $P=0.37$ ) were not significantly different between these two populations. This study indicates that integration of the RRA into dismount sample analysis would aid in improving management outcomes and welfare of both stallions and mares by minimising unnecessary re-breedings, with the potential for automation of the RRA for an on-farm device to further improve the efficiency of the Thoroughbred breeding industry.

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## The impact of elevated activin A on the mammalian fetal testis

**Penny A. F. Whitley<sup>1</sup>, Michael C. M. Luu<sup>1</sup>, Kate L. Loveland<sup>1, 2</sup>**

1. Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, Vic, Australia

2. Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Clayton, VIC, Australia

Male infertility and testicular cancer are significant health issues, and these conditions may stem from disrupted communication between testicular somatic (especially Sertoli cells) and germ cells (gonocytes) during embryogenesis. In the fetal mouse testis, the absence of activin A (*Inhba* KO) results in reduced Sertoli cell and increased gonocyte numbers (1). In this study we sought to clarify whether increased activin A (*Inha* KO) levels regulate fetal testis development, since elevated activin A is associated with complications during human pregnancy. Using indirect immunofluorescence with cell-specific markers we quantified Sertoli cell (Sox9+) and gonocyte (Ddx4+) populations, and testis cord formation, in sections of *Inha* and *Inhba* embryonic (E; E13.5 and E15.5) and newborn (0 dpp) mouse testes, comparing KO and wild type (WT) for each strain. Three sections were selected from the middle of the testis, spaced > 50 mm, from 3 - 4 animals/age/genotype. Slides were scanned (Olympus VS120® Virtual Slide Microscope System), images calibrated prior to measurements using ImageJ (2), and data collected blind.

The measured outcomes were opposite for increased and decreased activin A levels. *Inha* KO testes (elevated activin A) had: 1) a higher ratio of Sertoli cells to gonocytes, 2) gonocytes in the interstitium (only at E15.5), and 3) an increase in multinucleated cells at E15.5 and 0 dpp. These findings indicate that elevated activin A can result in gonocytes escaping the testis cords, and drive the phenotype of multinucleated gonocytes which is observed following exposure to endocrine disruptor chemicals and potentially linked with cryptorchidism in boys (3). The discovery that systemic activin A elevation can cause dysgenic testicular development in mice between E13.5 and birth provides evidence that pregnancy conditions which feature elevated activin A may impact male reproductive health later in life.

1. Mendis et al (2011). Biol Reprod, 84:379
2. Schneider et al. (2012), Nature methods 9(7): 671-675.
3. Cortes et al (2003). Apmis, 111, 25-31.

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## Genes involved in vascular compliance show dysregulated expression in placental mesenchymal stem/stromal cells (pMSCs) from growth restricted pregnancies.

**Anna L Boss<sup>1</sup>, Larry W Chamley<sup>1</sup>, Jo L James<sup>1</sup>**

1. Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

Placentae from pregnancies with fetal growth restriction (FGR) exhibit poor oxygen and nutrient exchange, in part due to impaired placental vascular development. pMSCs reside in a perivascular niche, where they may influence blood vessel formation and function. However, the role of pMSCs in influencing vascular dysfunction in FGR is unknown. To elucidate the mechanisms by which pMSCs may impact placental vascularisation we compared the transcriptomes of pMSCs from normal and FGR pregnancies.

pMSCs were isolated from FGR (<5<sup>th</sup> centile) (n=9) and gestation-matched control placentae (n=9). Transcriptomes were compared using Affymetrix microarrays. Genes/proteins of interest were validated by qPCR and by immunohistochemistry (IHC).

Whilst at the transcriptome level there were no statistically significant differences between normal and FGR pMSCs, the data were used to generate hypotheses. Several genes linked to vascular function exhibited distinct fold changes, supporting reports that FGR placentae exhibit differences in vascular compliance and structure that may impair blood flow and exchange. qPCR, demonstrated that the expression of Tenascin-X, ADAMTS1 and Fibulin-2 were significantly upregulated, whilst hyaluronan synthase-2 was significantly downregulated, in pMSCs from FGR placentae relative to controls ( $p < 0.05$  for all, n= 9 FGR and 9 control placentae). IHC indicated all four proteins were expressed perivascularly in third-trimester placentae.

Tenascin-X and Fibulin-2 maintain vessel elasticity, and their increased expression in FGR pMSCs could help explain the increased distensibility of FGR blood vessels. ADAMTS1 and hyaluronan synthase-2 regulate angiogenesis, and their differential expression by pMSCs from FGR placentae may contribute to the impaired angiogenesis in these placentae. Future

work to relate expression of these proteins to vessel compliance in normal and FGR placentae will inform in silico models to better understand normal and abnormal placental haemodynamics and exchange function.

## Investigating the heterogeneity of endometriotic lesions

**Eliza M Colgrave<sup>1</sup>, Sophie Bittinger<sup>2</sup>, Leonie Cann<sup>1</sup>, Peter AW Rogers<sup>1</sup>, Janet R Keast<sup>3</sup>, Jane E Girling<sup>4</sup>, Sarah J Holdsworth-Carson<sup>1</sup>**

1. Department of Obstetrics and Gynaecology, RWH, The University of Melbourne, Melbourne, Victoria, Australia

2. Department of Pathology, The Royal Women's Hospital, Melbourne, Victoria, Australia

3. Department of Anatomy and Neuroscience, The University of Melbourne, Melbourne, Victoria, Australia

4. Department of Anatomy, The University of Otago, Dunedin, Otago, New Zealand

**Background:** Endometriosis is characterised by the presence of endometrial-like lesions outside of the uterus. The presence of endometrial-like glands and/or stroma is the only observation required for a pathologist to diagnose endometriosis. More extensive pathologic phenotyping of lesions may improve diagnosis and treatment decisions. This project aims to characterise a broad range of morphological features in endometriotic lesions in the context of patient medical and phenotypic data (e.g. menstrual cycle stage) to better illustrate the makeup and heterogeneity of endometriotic lesions.

**Methods:** Endometriotic lesion biopsies (n = 42 patients from across the menstrual cycle, 152 biopsies, 1,069 endometriotic glands) were analysed by brightfield microscopy of haematoxylin and eosin stained sections (independently, by EC (researcher) and SB (pathologist)). A mixed effects logistic regression analysis was utilised to determine if patient variables (e.g. menstrual cycle stage) had a significant effect on the characteristics of the endometriotic lesions.

**Results:** There was significant inter- and intra-patient variability in the epithelium, stroma and tissue surrounding endometriotic lesions. Some subtle menstrual cycle-associated changes were observed among lesions, including a significant increase in epithelial mitoses in endometriotic glands from proliferative phase patients (18% of glands) compared to menstrual and secretory phase patients (0% and 2% of glands, respectively; OR = 7.26, p<0.001, 95% CI 3.04-17.29). In contrast, there were no significant differences in the proportion of glands with haemosiderin-laden macrophages across the menstrual cycle, indicating signs of haemorrhage were present in lesions independent of menstrual stage (prob > chi2 = 0.13).

**Conclusion:** We observed some significant changes in endometriotic lesions associated with features of the menstrual cycle, but they were not prominent in all lesions as has previously been proposed. The results of this project provide a foundation for further targeted characterisation of the heterogeneity of molecular features in endometriotic lesions.

## The Effect of Ovarian Hyperstimulation on Microvillar Length in Uterine Epithelial Cells

**Samson N Dowland<sup>1</sup>, Chad L Moore<sup>1</sup>, Christopher R Murphy<sup>1</sup>, Laura A Lindsay<sup>1</sup>**

1. Discipline of Anatomy & Histology, The University of Sydney, Sydney, NSW, Australia

During early pregnancy the luminal uterine epithelial cells (UECs) are extensively remodelled, microvilli are lost from the apical plasma membrane which enables interaction with the blastocyst. These dynamic changes in microvilli are unique to UECs, however the underlying molecular mechanisms driving the growth and retraction of these microvilli are currently unknown.

During early pregnancy after ovarian hyperstimulation (OH) there is a significant reduction in blastocyst implantation rate and a corresponding disruption of UEC remodelling.

The present study utilised transmission electron microscopy and morphometry techniques to measure the length of microvilli at the time of fertilisation and implantation in normal and OH pregnancy. Two cytoskeletal proteins have also been examined in UECs; the actin nucleation factor "Cordon-Bleu" (COBL) as it regulates the length of microvilli and the intermediate filament protein cytokeratin 15 (CK15).

At the time of fertilisation in normal and OH pregnancy, UECs possess short, regular microvilli with COBL concentrated at their base. At the time of implantation during normal pregnancy, the UECs lose microvilli and COBL remains localised to the region of the terminal web, where it may sever actin microfilaments to reduce the length of microvilli.

At the time of implantation in OH pregnancy microvilli are retained on the surface of the UECs. Morphometric analysis demonstrates that these microvilli are significantly longer than those seen at the time of fertilisation and they have an irregular, branching appearance. At the same time COBL is lost from UECs and CK15 is concentrated in the apical region of the UECs.

This study demonstrates significant changes in microvillar length in OH pregnancy compared to normal pregnancy and shows that their cytoskeletal supporting structures are changed. The significantly longer microvilli present at the time of implantation in OH pregnancy may inhibit blastocyst implantation and explain the reduced implantation rate seen in this model.

## Identification of estrogen driven pathways and novel targets in endometriosis using high throughput drug screens

**Jacqueline F Donoghue<sup>1</sup>, Molly L Churchill<sup>1</sup>, Sarah J Holdsworth-Carson<sup>1</sup>, Peter A W Rogers<sup>1</sup>**

1. University of Melbourne, Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Victoria, Australia

**INTRODUCTION:** Endometriosis is an estrogen-dependent, chronic, pro-inflammatory disease that develops in 6-10% of women of reproductive age. Symptoms include chronic pelvic pain, dysmenorrhea and subfertility. Current therapeutic interventions include hormone modifiers, non-steroidal anti-inflammatories and surgery. Unfortunately, these interventions are often short term and non-curative highlighting a significant need for the discovery of new compounds that disrupt the estrogen-

driven pathways in endometriosis. To achieve this, a high-throughput screen (HTS) of 3,500 clinically approved drugs was performed using immortalized human endometriosis stromal cell lines. **METHODS:** Stromal cells were seeded into 384 well plates and screened with a single dose of library drug [10  $\mu$ M] in the presence or absence of estradiol-17 $\beta$ . Three days later, the cells were fixed, stained and imaged using the Cell-Insight CX7 High Content Screen platform where a nuclear count was performed using HCS Studio Cell Analysis Software. **RESULTS:** Drug hits were identified as those that caused a >70% inhibition during estrogen treatment compared to vehicle. This process identified 40 unique drug hits for each cell line. These drug hits included a diverse range of compound types including anti-cancer, anti-diabetic, anti-histamine and antibiotics. **CONCLUSION:** The HTS on endometriosis stromal cells identified 40 drugs that interfered with estrogen driven cell growth and targeted novel pathways down-stream of the estrogen receptors. These findings highlight novel mechanisms that may contribute to endometriosis lesion formation and demonstrate strong heterogeneity of cell signalling between cell lines. This study also demonstrates the value of HTS for endometriosis therapeutic discovery and the potential for repurposing clinically approved drugs for personalised endometriosis therapy.

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## Identifying the functional role of *VEZT* in endometriosis endometrial stromal cells

**Jacqueline F Donoghue<sup>1</sup>, Katie-Rose Campbell<sup>1</sup>, Sarah J Holdsworth-Carson<sup>1</sup>, Premila Paiva<sup>1</sup>, Peter AW Rogers<sup>1</sup>**

1. University of Melbourne, Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Victoria, Australia

**INTRODUCTION:** Endometriosis is an estrogen-dependent, chronic, pro-inflammatory disease that develops in 6-10% of women of reproductive age. Symptoms include chronic pelvic pain, dysmenorrhea and subfertility. Recently, *VEZT* was identified as an endometriosis risk gene that was upregulated during the secretory phase of the menstrual cycle<sup>1</sup>. *VEZT* encodes for an adherens junction protein vezatin and to date, the function of *VEZT* in human endometrium is unknown.

**METHODS:** Immunohistochemistry was used to determine *VEZT* protein (vezatin) expression and localisation in a tissue microarray of eutopic endometrium. Cellular localisation of vezatin was further evaluated using immunofluorescence double staining of endometriosis ectopic lesions and eutopic endometrium compared to non-endometriosis eutopic endometrium. To demonstrate *VEZT* regulation by secretory phase hormones, immortalised endometriosis endometrial stromal cells and a non-endometriosis endometrial stromal cell line were treated with estradiol-17 $\beta$ , medroxyprogesterone acetate and cAMP to induce decidualisation and quantify *VEZT* mRNA by qPCR. RNASeq was then used on immortalised endometriosis endometrial stromal cells and a non-endometriosis endometrial stromal cell line that were transfected to over express *VEZT*. Ingenuity Pathway Analysis (IPA) was then performed on the top 500 up and down regulated genes to determine pathways significantly influenced by *VEZT* overexpression. **RESULTS:** Vezatin was localised to glandular epithelium, decidualised stromal cells, endothelium and leucocytes in secretory phase eutopic endometrium as well as CD10<sup>+</sup>stromal cells, epithelium and CD45<sup>+</sup>leucocytes in ectopic lesions. Vezatin also colocalised with CD31, CD56 and CD68<sup>+</sup>cells. Following decidualisation, *VEZT* mRNA expression was increased 2-fold compared to non-decidualised stromal cells and the most upregulated pathway by the overexpression of *VEZT* in stromal cells was the interferon pathway. **CONCLUSION:** *VEZT*/vezatin expression in decidualised stromal cells, endothelium and leucocytes indicates a functional role in mesenchymal-epithelial transition, angiogenesis and inflammation. *VEZT* may therefore modulate these processes in conjunction with interferon signalling and significantly contribute to the pathogenesis of endometriosis.

1. Holdsworth-Carson et al., 2016

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## Similarities in uterine changes during pregnancy in eutherian and marsupial species

**Jessica Dudley<sup>1</sup>, Christopher Murphy<sup>1</sup>, Mike Thompson<sup>2</sup>, Laura Lindsay<sup>1</sup>, Bronwyn McAllan<sup>1</sup>**

1. School of Medical Sciences and Bosch Institute, The University of Sydney, Sydney, NSW, Australia

2. Biological Sciences, School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia

Reproduction is a complex phenomenon that is fundamental to all living things. The uterine epithelium of viviparous mammals undergoes vast remodelling to accommodate the developing embryo. There are several levels of placental invasion in mammals that develop a placenta, based on the number of cell layers that separate the developing fetus and maternal blood stream. We investigated whether there are common molecular changes to the uterine surface during pregnancy in Theria by comparing uterine remodelling across several phylogenetic groups with independent origins of endotheliochorial placentae to determine how this placenta type evolved. We tested the generality of uterine remodelling during pregnancy in the marsupial, *Sminthopsis crassicaudata* (Dasyuridae; the fat-tailed dunnart) and the eutherian, *Felis catus* (Felidae; the domestic cat) and *Dipodomys merriami* (Heteromyidae; Merriam's kangaroo rat). Transmission and Scanning Electron Microscopy were used to study the ultrastructural changes to the uterine epithelium during pregnancy and immunofluorescence microscopy and Western blotting showed that there are common changes to the distribution of key lateral adhesion molecules, desmoglein-2 and E-cadherin. We confirmed that the same ultrastructural and molecular changes to the uterine epithelium are seen in marsupial and eutherian species which represent separate lineages of endotheliochorial placentation. We also determined the effect of the reproductive hormones, progesterone and 17 $\beta$ -oestradiol on the reproductive tract of *S. crassicaudata*, concluding that the plasma membrane transformation is regulated by the same hormonal mechanisms among therian species. The conclusions from this study support the theory that uterine remodelling and the plasma membrane transformation are crucial for successful pregnancy in viviparous mammals with commonalities in molecular and morphological changes among species.

## Mass spectrometry imaging to identify the spatial distribution of metabolites within endometriosis tissue

**Brayden Fraser<sup>1</sup>, Sarah Holdsworth-Carson<sup>2</sup>, Jane Girling<sup>2,3</sup>, Peter Rogers<sup>2</sup>, Berin Boughton<sup>4,1</sup>**

1. School of Biosciences, The University of Melbourne, Melbourne, Victoria, Australia

2. University of Melbourne Department of Obstetrics and Gynaecology, Royal Women's Hospital, Melbourne, Victoria, Australia

3. Department of Anatomy, University of Otago, Dunedin, New Zealand

4. Metabolomics Australia, The University of Melbourne, Melbourne, Victoria, Australia

**Background:** Endometriosis, which affects up to one in ten women worldwide, is defined by the ectopic growth of endometrial-like tissue outside of the uterus. Endometriosis has no cure and is associated with menstrual pain and infertility. There is a clear need for fundamental research to increase our understanding of the underlying disease mechanisms. This project has employed mass spectrometry imaging (MSI) to examine the spatial distribution of tissue metabolites in the eutopic tissues, endometrium and myometrium and disease-specific ectopic endometriotic lesions. Identification of endometriosis-associated metabolites may contribute to improving our ability to diagnose and treat the condition.

**Methods:** This study has used a large bank of formalin fixed paraffin embedded (FFPE) tissues that have been collected from patients as part of the Royal Women's Hospital Endometriosis Project. Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI) was employed to discriminate spatial metabolite profiles within the tissues of interest. Mass spectrometry imaging (MSI) adds another level of complexity to other metabolomic techniques by determining the spatial distribution of metabolites and biomolecules within tissues, that can be used to associate specific metabolites with tissue and cell types to create an in-depth comparative analysis of how diseases change over time.

**Results:** We have developed new methods to measure the spatial distribution of small chemicals and metabolites in archived FFPE tissues, measuring 5553 individual  $m/z$  (ions) showing spatial distribution within archived FFPE uterine and endometrial curettes through five stages of the menstrual cycle, of these we were able to annotate 168 metabolites using the metabolite annotation tool Metaspace ([www.metaspace2020.eu](http://www.metaspace2020.eu)).

**Conclusion:** This investigation has provided the first metabolomic MSI data on archived FFPE uterine tissues and endometrial curettes. This information will be compared to MSI data from eutopic and ectopic endometrial lesions and employed to improve our understanding endometriosis of disease mechanisms and classification.

## Obesity does not impact on endometrial gene expression in women with endometriosis

**Sarah J Holdsworth-Carson<sup>1</sup>, Jessica Chung<sup>2</sup>, Martin Healey<sup>1</sup>, Jenny N Fung<sup>3</sup>, Sally Mortlock<sup>3</sup>, Grant Montgomery<sup>3</sup>, Peter Rogers<sup>1</sup>, Jane E Girling<sup>4</sup>**

1. Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, VIC, Australia

2. Melbourne Bioinformatics, University of Melbourne, Parkville, VIC, Australia

3. Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

4. Department of Anatomy, University of Otago, Dunedin, New Zealand

**INTRODUCTION:** Obese women have a reduced incidence of endometriosis. We recently determined that while the incidence was lower in obese women, they had more severe endometriosis compared to women with lower BMIs and were also diagnosed with significantly less stage I disease. Endometriosis is considered a disease of endometrial origin; therefore, it is of interest to study endometrial gene expression in studies pertaining to endometriosis.

Our aims were to determine:

- 1) in obese women with endometriosis (all stages), does the endometrium demonstrate differential gene expression relative to women with endometriosis and a lower BMI, and
- 2) in obese women with stage I endometriosis, does the endometrium demonstrate differential gene expression compared to women with lower BMIs who also have stage I disease?

**METHODS:** Endometriosis was diagnosed following surgical and histopathological confirmation. Women were grouped by BMI ( $\text{kg/m}^2$ ). Total endometrial RNA was extracted and whole-transcriptome sequencing (RNA-Seq) was performed (Illumina TruSeq Stranded Total RNA protocol). Following multiple testing correction (including for menstrual cycle stage), genes with an adjusted P value (false discovery rate) less than 0.05 were deemed differentially expressed.

**RESULTS:** Endometrial gene expression from obese women with endometriosis ( $n=14$ ) were compared to women with endometriosis and underweight ( $n=6$ ), normal ( $n=69$ ) and pre-obese ( $n=30$ ) BMIs; no significant differential gene expression between groups was observed. We limited the analysis to women with stage I endometriosis (underweight  $n=5$ , pre-obese  $n=18$ , normal  $n=41$  versus obese  $n=6$ ); no significant differences were observed when obese women were compared to the lower BMIs.

**CONCLUSIONS:** Obesity has negative consequences on endometrial pathologies (including endometrial cancer and infertility). However, with respect to endometriosis (all stages and stage I alone), we conclude that obesity does not impact on endometrial gene expression. Future research will need to look beyond the endometrium to determine the influence of obesity on endometriosis disease mechanisms.

## Expression and function of the endometriosis risk gene Long Intergenic Non-Coding RNA 339

**Sarah J Holdsworth-Carson<sup>1</sup>, Jacqueline Donoghue<sup>1</sup>, Katie-Rose Campbell<sup>1</sup>, Molly L Churchill<sup>1</sup>, Leonie Cann<sup>1</sup>, Jessica Chung<sup>2</sup>, Clare Sloggett<sup>2</sup>, Martin Healey<sup>1</sup>, Raden Kendarsari<sup>3</sup>, Jenny N Fung<sup>3</sup>, Sally Mortlock<sup>3</sup>, Grant Montgomery<sup>3</sup>, Jane E Girling<sup>4</sup>, Peter AW Rogers<sup>1</sup>**

1. Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, VIC, Australia

2. Melbourne Bioinformatics, University of Melbourne, Parkville, VIC, Australia

3. Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

4. Department of Anatomy, University of Otago, Dunedin, New Zealand

**INTRODUCTION:** Meta-analyses of endometriosis GWAS have identified significant associations for single nucleotide polymorphisms (SNP) on chromosome 1. A common mechanism by which SNPs influence disease is by regulating transcription or expression quantitative trait loci (eQTL). The strongest eQTL is for 'long intergenic non-coding RNA 339' (*LINC00339*) where the risk allele decreases *LINC00339* expression in blood and endometrium. The aim of this study was to examine the expression and function of *LINC00339* in ectopic and eutopic endometrium.

**METHODS:** The expression of *LINC00339* was examined in endometrium and stromal and epithelial cultures by RNA-seq (n=184) and qRT-PCR (n=6-13). *LINC00339* in situ hybridization was performed on n=8 cases (lesion and endometrium) and n=8 controls (endometrium). *LINC00339* was overexpressed in 3 endometrial stromal cell lines and differential gene expression and pathway analysis was performed following RNA-seq and Ingenuity Pathway Analysis.

**RESULTS:** The most abundant *LINC00339* transcript was ENST00000416769, associated with *LINC00339* variants 2, 5 and 6 and validated by RT-PCR in endometrial stromal lines (n=13). *LINC00339* variants were not differentially expressed between cultures of stromal versus epithelial cells (n=6). *LINC00339* in situ hybridization demonstrated nuclear localization. Interestingly, in lesions, *LINC00339* was only expressed in disease foci, not in surrounding tissues. Examination of gene expression following *LINC00339* overexpression identified 290 significantly differentially expressed genes (adj. p-values <0.05) (top 5 genes *IL33*, *FENDRR*, *PRKCA*, *NACC2* and *RHEBL1*); Ingenuity Pathway Analysis revealed that the top functional biological pathways included Interferon Signaling, Role of Pattern Recognition Receptors and Hepatic Fibrosis.

**CONCLUSIONS:** *LINC00339* was expressed equally in stroma and epithelium of eutopic and ectopic endometrium but was absent in the tissues surrounding foci of endometriosis. Functional characterisation of *LINC00339* suggests the endometriosis-risk gene has a role in immune function and inflammation; features well established in the aetiology of endometriosis.

## Modelling human endometrial diseases using patient-derived organoids

**Manish Kumar<sup>1</sup>, Shafiq M Syed<sup>1</sup>, Arnab Ghosh<sup>1</sup>, Pradeep Tanwar<sup>1</sup>**

1. School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW, Australia

The human endometrium (inner lining of the uterus) undergoes hormone-regulated cycles of growth and shedding. Aberrations in this process are known to contribute to many diseases, such as infertility, endometriosis, and cancer. Due to obesity and other lifestyle factors, there is a significant rise in the number of cases of endometrial diseases. Therefore, there is an urgent need to develop preclinical models mimicking normal and disease human endometrium to develop new targeted approaches for patients with endometrial diseases.

Recently, the three-dimensional culture of endometrial epithelial cells has been developed to generate organoids which resemble their organ of origin. We developed organoids from normal (n=3) and endometrial cancer tissues (n=7) collected from patients undergoing hysterectomies at the John Hunter Hospital. These organoids expressed markers of the human endometrial epithelium (Cytokeratin 8), glands (Foxa2), and ciliated cells (HFH4), confirming these organoids mimic human endometrium.

Abnormal canonical Wnt signaling is involved in the pathogenesis of endometriosis, adenomyosis, and endometrial cancer. Our previous work has established that overactivation of this pathway leads to the development of endometrial hyperplasia and cancer in mouse models (Goat et al Carcinogenesis 2019). In this study, we investigated if targeting Wnt/b-catenin signaling in human endometrial cancer is a viable strategy to reduce the growth of cancer cells. We cultured human endometrial organoids in the presence of two well-known Wnt inhibitors (IWR1 and IWP2) and found a significant reduction in colony formation efficiency (0.36-fold in normal and 0.55-fold in cancer) and cell proliferation (0.45-fold in normal and 0.37-fold in cancer). We showed that these two inhibitors specifically targeted canonical Wnt signaling by examining the expression of active b-catenin protein. We are now developing organoids from additional patients covering several different histotypes of endometrial cancer to understand which group patients are more likely to respond to therapies targeting Wnt/ b-catenin signaling.



### Role of vitamin D in spiral artery remodeling

**Gendie Lash<sup>1</sup>, Joy Yue Zhang<sup>1</sup>, Danyang Chen<sup>1</sup>, Peihuang Wu<sup>1</sup>**

*1. Guangzhou Women and Children's Medical Center, Guangzhou, Guangdong Province, China*

**Background:** Low levels of vitamin D and diminished remodeling of the uterine spiral arteries (SpA) are associated with pre-eclampsia and fetal growth restriction. It is unknown whether vitamin D plays a direct role in mediating SpA remodeling, or modulates the ability of other cell types (uterine natural killer (uNK) cells or extravillous trophoblast cells) to mediate this process.

**Methods:** uNK cells and placental explants (PE) were isolated from first trimester (6-8 weeks gestation) decidua and placenta, and cultured in 25OHD (0, 10, 100nM) or 1,25(OH)2D (0, 1, 10nM) for 24 hours and conditioned medium (CM) harvested. Chorionic plate arteries were dissected from term placenta, cut into small portions and cultured in different CM generated above or 25OHD (0, 10, 100nM) or 1,25(OH)2D (0, 1, 10nM) for 5 days, sections were H&E stained and vascular smooth muscle (VSMC) organization assessed using a 4 point scale; 1=fully organized and 4=fully disorganized. VSMC migration and invasion in response to different CM and vitamin D were assessed by ExCelligence RTCA assay. VSMC cytoskeleton rearrangement was determined by immunofluorescence.

**Results:** uNK cell-CM induced VSMC disorganization, but PE-CM did not. 25OHD had no effect on VSMC organization, and did not alter the ability of uNK cells or PE to induce VSMC disorganization. In contrast, active vitamin D (1,25(OH)2D) induced VSMC disorganization in a dose dependent manner. In addition, in the presence of active vitamin D PE-CM was also able to induce VSMC disorganization, although there was no enhancement of the effect of uNK cell-CM. 1,25(OH)2D also increased PE secretion of G-CSF, and G-CSF alone could also induce VSMC changes. Active vitamin D induced VSMC migration, invasion and cytoskeleton rearrangement.

**Conclusions:** Vitamin D plays an important role in early stages of SpA remodeling, in both a direct manner and by altering the activity of other cell types.

### Redistribution of desmosomes in uterine epithelial cells at the time of receptivity after ovarian hyperstimulation in the rat

**Laura Lindsay<sup>1</sup>, Sam Dowland<sup>1</sup>, Chris Murphy<sup>1</sup>**

*1. Cell and Reproductive Biology Laboratory, School of Medical Sciences (Anatomy and Histology), The University of Sydney, Sydney, NSW, Australia*

There is a decrease in pregnancy rates following fresh IVF transfers compared to transfers of frozen embryos in a natural cycle. It has been proposed that this decrease in uterine receptivity is due to alterations in the endometrium and we have evidence from a rat ovarian hyperstimulation (OH) model to support this.

At the time of receptivity during normal pregnancy there are distinct changes in uterine epithelial cells including modifications to the lateral plasma membrane. These changes include morphological and biochemical alterations in tight junctions, loss of adherens junctions and a decrease in the number of morphological desmosomes down the lateral plasma membrane.

At the time of receptivity during OH compared to normal pregnancy we found an increase in the number of morphological desmosomes and a corresponding increase in desmoglein-2 (a desmosomal marker) down the length of the lateral plasma membrane. We also observed similar changes in Rab 13, a protein involved in endosomal trafficking of proteins to the lateral plasma membrane, spreading down the entire length of the lateral plasma membrane at the time of receptivity during OH pregnancy.

These results add to the developing picture of the alterations in morphological and molecular markers of receptive uterine epithelial cells as a result of the effects of IVF medications on the endometrium, explaining the decrease in uterine receptivity during fresh stimulated IVF cycles.

### Expression of the renin angiotensin system in placental models of syncytialisation: a comparative study

**Saije K Morosin<sup>1</sup>, Sarah J Delforce<sup>1</sup>, Eugenie R Lumbers<sup>1</sup>, Kirsty G Pringle<sup>1</sup>**

*1. School of Biomedical Sciences and Pharmacy, Priority Research Centre for Reproductive Science, Hunter Medical Research Institute, University of Newcastle, Newcastle, New South Wales, Australia*

**Background:** The syncytiotrophoblast is a large multinucleated cell layer that is maintained by continuous fusion of underlying cytotrophoblast cells in a process known as syncytialisation. The syncytiotrophoblast is the major functional cell layer of the placenta, responsible for regulating feto-maternal nutrient, gas and waste exchange. The placental renin angiotensin system (RAS) is essential for placental development however, little is known about its role in syncytialisation. We therefore aimed to determine the effect of syncytialisation on RAS expression. To do this we used two models of syncytialisation; forskolin-induced syncytialisation of BeWo choriocarcinoma cells and spontaneously fusing human primary trophoblast cells.

**Methods:** BeWo choriocarcinoma cells were treated with 100mM forskolin 24h post plating and left to syncytialise for 48h before cells and supernatant were collected. Primary trophoblast cells were isolated from human placentae and left to spontaneously syncytialise for 72h, with collection of cells and supernatant every 24h. The mRNA expression of RAS genes

including: prorenin (*REN*), (pro)renin receptor (*ATP6AP2*), angiotensinogen (*AGT*), angiotensin converting enzyme 1/2 (*ACE/ACE2*) and angiotensin II receptor type 1 (*AGTR1*), were assessed by RT-qPCR.

**Results:** Forskolin-induced syncytialisation of BeWo cells significantly decreased *ATP6AP2* and *ACE* expression ( $P=0.002$  and  $<0.0001$ , respectively), and increased *AGT* and *ACE2* expression (both  $P<0.001$ ). There were low to undetectable levels of *REN* and *AGTR1* mRNA in BeWo cells and these did not change with forskolin treatment. Spontaneous syncytialisation of trophoblast cells significantly decreased *ACE2* expression ( $P<0.0001$ ) and increased *AGT* and *REN* expression (both  $P<0.05$ ). *ATP6AP2*, *AGTR1* and *ACE* mRNA expression were unchanged.

**Conclusion:** BeWo and primary trophoblast cells exhibit different RAS mRNA profiles both before and in response to syncytialisation, highlighting a significant difference between these two *in vitro* models. BeWo cells predominantly utilise RAS-independent pathways induced by the (P)RR, whereas primary trophoblasts rely more on classical RAS pathways.

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## The impact of repeat gynaecological surgery in patients with endometriosis: a retrospective cohort study using clinical reports and questionnaires.

Byung Kyu (Anthony) Jun<sup>2,1</sup>, Martin Healey<sup>2,1</sup>, Sarah Holdsworth-Carson<sup>2,1</sup>, Peter Rogers<sup>2,1</sup>

1. University of Melbourne, Melbourne, VIC, Australia

2. The Royal Women's Hospital, Melbourne, VIC, Australia

### Introduction

Endometriosis is a common gynaecological condition with a high burden on the patient's quality of life due to the chronic pain and recurrence of disease. By studying a Melbourne cohort of endometriosis patients, our goal was to determine the incidence of repeat surgery, and to identify what interventions are performed on these women.

### Methods

De-identified clinical questionnaires were obtained through the Royal Women's Hospital with consent. Questionnaires contained data including demographics, diagnosis, disease severity, and other medically relevant information. For women with surgical diagnosis of endometriosis (visually confirmed at surgery), we identified those who later returned for another surgery related to endometriosis, and further analyzed their data from surgical, medical, and pathological reports.

### Results

From 2011 to 2017, we had a cohort of 420 patients with surgically diagnosed endometriosis. The mean age at initial operation was 30.48 (SD6.96). Of those with endometriosis, 23.3% (n=98) had further surgeries for endometriosis. The majority of patients had two surgeries in total (n=73), with a mean time to repeat surgery of 136 weeks.

Of all patients with endometriosis, 6.4% (n=27) had hysterectomies at some point, with 62.9% (n=17) of those at the second surgery. In terms of other procedures, 41.0% (n=172) patients had adhesions surgically divided, 36.4% (n=153) had cystectomies including ablation of endometriomas, 5.5% (n=23) had oophorectomies, and 13.1% (n=55) had salpingectomies.

### Conclusion

Our study demonstrated that a significant number of patients with surgically diagnosed endometriosis return for repeat surgeries, with many requiring procedures such as hysterectomies and adhesiolysis. We determined a mean time to repeat surgery of 136 weeks. Further work is required to identify possible clinical risk factors for disease recurrence. Currently, the information generated from this cohort is beneficial to understanding the clinical indication(s) of repeat surgery for endometriosis and has implications for the surgical management of these patients.

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## The pattern of epigenetic reprogramming during the 8-cell to blastocyst transition in mouse embryo

Yan Li<sup>1</sup>, Chris O'Neill<sup>1</sup>

1. Centre for Developmental and Regenerative Medicine, Kolling Institute, Sydney Medical School, University of Sydney, St Leonards, NSW, Australia

The first differentiation event in the mammalian life-cycle occurs during the transition from the 8-cell to the blastocyst. This results in the formation of the trophectoderm and inner cell mass lineages (ICM). Differentiation requires global reprogramming of the cell's epigenetic landscape and a range of covalent modifications to histone proteins and DNA bases are the dominant mechanisms. The combinatorial arrangement of these modifications determined chromatin structure and transcriptional capacity. In this study we examined global changes in the major histone modifications H3K9 acetylation (H3K9Ace), H3K9 trimethylation (H3K9me3), H3K4me3, and H3K27me3 across the 8-cell to blastocyst transition. We also undertake preliminary analysis of the interaction between the histone modification and the levels of DNA methylation.

Immunolocalization of these modifications showed that H3K9ace, H3K9me3, H3K4me3 and H3K27me3 were uniformly present in each cell of the 8-cell but by the blastocyst stage each modification became restricted to the trophectoderm while the ICM showed a marked loss of these modifications. These differential levels of one activating (H3K9ace) and three repressive modifications mirrored the differential levels of DNA methylation present between these two lineages. Exposure of embryos to the broad-spectrum histone deacetylase inhibitor (trichostatin A, TSA, 30nM) demonstrated an interaction between acetylation and DNA methylation, by reducing the global nuclear level of methylated cytosine in the resulting blastocysts.

These results demonstrate extensive global epigenetic remodeling of multiple epigenetic modifications accompany the first differentiation event in the embryo. The similar profile of global changes for both activating and repressive modifications indicate a complexity of the process. This complexity is exacerbated by interactions between modifications, with changes in acetylation levels influencing DNA methylation profiles. The study provides a foundation for detailed analysis of the control of this remodeling with differentiation.

## Hormones and sedatives used during artificial breeding alter the core body temperature of the ewe

Jessica P Rickard<sup>1</sup>, Max J Lloyd<sup>1</sup>, Simon P de Graaf<sup>1</sup>, Gordon Refshauge<sup>2</sup>

1. Faculty of Science, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia

2. Cowra Sheep Station, Department of Primary Industries, Cowra, NSW, 2794

The impact of heat stress on the fertility of the Australian Merino is of great interest following the reported increase in global ambient temperatures and anecdotal decline in fertility rates following artificial reproductive technologies. Yet, the fluctuations in core body temp which occur during the ewes' oestrous cycle remain to be fully elucidated, as does the influence of exogenous hormones and sedatives which are necessary for artificial insemination (AI). As such, the core body temperature ( $T_b$ ) of Merino ewes was investigated using novel intravaginal temperature data loggers following oestrous synchronisation and sham AI. Experiment 1 compared the core body temperature of ewes in a natural oestrus with those that had been synchronised for oestrus (progesterone pessary inserted for 14 days + 400 IU pregnant mare serum gonadotrophin at pessary removal). Experiment 2 observed whether sedation (Xylazine, Acepromazine-Ketamine-Lignocaine or saline) prior to sham AI affected ewe core body temperature. In experiment 1, although the magnitude of difference was small, ewes which were synchronised recorded a higher mean  $T_b$  ( $39.28 \pm 0.05$ ) than those undergoing a natural oestrus ( $39.22 \pm 0.05$ ;  $p < 0.05$ ). In experiment 2, both sedatives increased  $T_b$  in comparison to the control ( $p < 0.05$ ). Xylazine produced the highest maximum temperature ( $39.97 \pm 0.15$ ) with its effect on  $T_b$  over time shown to be immediate albeit short lived. The temperature increase resulting from Acepromazine-Ketamine-Lignocaine ( $39.89 \pm 0.11$ ) regime was slow in onset but produced a prolonged elevation in  $T_b$  when compared to xylazine, suggesting lasting effects beyond the day of AI. Overall, these findings indicate that oestrus synchrony and sedation alter the ability of ewes to thermoregulate, however further studies are needed to determine the biological significance of this increase in core body temperature on fertility when ewes are exposed to heat stress inducing conditions.

## Ontogeny of polycystic ovary syndrome (PCOS) traits in a PCOS mouse model

Valentina Rodriguez Paris<sup>1</sup>, Melissa C Edwards<sup>2,1</sup>, Ali Aflatounian<sup>1</sup>, William L Ledger<sup>1</sup>, Robert B Gilchrist<sup>1</sup>, David J Handelsman<sup>2</sup>, Kirsty A Walters<sup>2,1</sup>

1. School of Women's & Children's Health, University of New South Wales, Sydney, NSW 2052, Australia

2. ANZAC Research Institute, University of Sydney, Sydney, NSW 2139, Australia

Polycystic ovary syndrome (PCOS) affects 5-10% of women worldwide and is a complex disorder characterized by reproductive, endocrine and metabolic abnormalities. The aetiology and ontogeny of PCOS are poorly understood and therefore current medical management relies solely on symptomatic treatment. Defining the ontogeny of PCOS traits is important for early PCOS detection and treatment of this disorder. Hyperandrogenism is a defining characteristic of PCOS and clinical and animal studies support a role for androgen driven actions in the development of PCOS. Therefore, to determine the temporal and spatial pattern of PCOS trait development, we implanted mice with either a blank or a dihydrotestosterone (DHT) pellet at 4 weeks of age and evaluated PCOS features after 2, 4 and 8 weeks of androgen exposure. Compared to control mice, all androgen-exposed mice exhibited complete estrous acyclicity at all time-points ( $P < 0.001$ ), accompanied by anovulation as none of their ovaries exhibited corpora lutea. Large antral follicle populations were significantly increased in the 4 and 8 week DHT-exposed ovaries compared to controls ( $P < 0.05$ ). Body weight was significantly increased in androgen-exposed mice compared to controls at all time-points ( $P < 0.001$ ), with a significant increase in parametrial and retroperitoneal fat pad weight observed in the 8 week androgen-exposed group ( $P < 0.001$ ). Glucose tolerance was not impaired in any DHT-exposed mice compared to controls. However, basal glucose in the 8 week DHT-exposed females was significantly increased compared to control females ( $P < 0.05$ ), indicating development of the initial stage of hyperglycaemia that precedes type-2 diabetes. These results indicate that in the development of hyperandrogenic PCOS features, disrupted reproductive function and increased body weight precede the occurrence of additional PCOS related metabolic disturbances. These findings infer that altered reproductive function and weight gain are early indicators of PCOS, and may be key initial treatment targets to prevent or reduce the severity of PCOS.

## Dose optimisation to mimic the *in utero* neurosteroid environment in preterm neonates

Julia C Shaw<sup>2,1</sup>, Rebecca M Dyson<sup>3,4</sup>, Hannah K Palliser<sup>2,1</sup>, Ryan Sixtus<sup>3,4</sup>, Heather Barnes<sup>3,4</sup>, Maureen Prowse<sup>3,4</sup>, Clint Gray<sup>3,4</sup>, Max J Berry<sup>3,4</sup>, Jonathan J Hirst<sup>2,1</sup>

1. Mothers and Babies Research Centre, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia

2. University of Newcastle, New Lambton Heights, NSW, Australia

3. Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand

4. Centre for Translational Physiology, University of Otago, Wellington, New Zealand

**Introduction:** Approximately 10% of births in Australia are preterm and it is now established that these vulnerable neonates have myelination deficits, which are exacerbated by early exposure to the *ex utero* environment and reduced inhibitory tone. We propose this is due to the early loss of the placentally-derived neurosteroid, allopregnanolone. We have previously shown that postnatally restoring inhibitory neurosteroid action following preterm birth using the allopregnanolone analogue

ganaxolone, can restore myelination. The aim of the current study is to determine the lowest effective dose of ganaxolone to ensure restoration of myelination, but minimisation of sedation-related side effects.

**Methods:** Dunkin Hartley guinea pigs had labour induced to deliver neonates prematurely at gestational age 62 (term=GA69). Between birth and term equivalence age, preterm neonates received either 45%  $\beta$ -cyclodextrin (vehicle), 0.5mg/kg/day (low-GNX), 1mg/kg/day (mid-GNX), or 2.5mg/kg/day ganaxolone (high-GNX) twice daily via subcutaneous injection. Physical characteristics, blood sugar levels and well-being scores (indicator of activity, respiration, and posture) were monitored throughout with tissue collection occurring at term equivalence age.

**Results:** There was no difference in weight gain between the neonates receiving vehicle and those receiving GNX. Ponderal index was decreased for high-GNX males on postnatal day 1 (PND1), compared to those receiving vehicle. Daily non-fasting blood sugar averages were reduced for high-GNX males and females across PND3-6 compared to their vehicle counterparts. Well-being scores were also reduced in both sexes receiving high-GNX across PND1-3 compared to the vehicle neonates, and this was more pronounced 2 hours post drug treatment.

**Conclusion:** Based on preliminary analysis, a high dose of ganaxolone has defined effects on sedation (indicated by well-being score) and metabolism (indicated by ponderal index and blood sugar levels), which may limit the feasibility of this particular dose, whereas lower doses were without these effects. Further analysis, particularly of myelination in the brain, is required.

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## Successful detection of ram mating behaviour with tri-axial accelerometers

**Emmah L Tumeth<sup>1</sup>, Jessica P Rickard<sup>1</sup>, Luciano A González<sup>1</sup>, Simon P de Graaf<sup>1</sup>**

1. *The University of Sydney, Sydney, NSW, Australia*

Reproductive events such as oestrus, ovulation, mating and pregnancy status in extensively managed animals are notoriously difficult to monitor and detect. Next generation on-animal sensors that utilise accelerometer, Bluetooth proximity and geo-location data stand to revolutionise our ability to detect such events for use in precision reproductive management of livestock.

The aim of this study was to establish an acceleration 'signature' of ram mating activity using tri-axial accelerometers and to determine the optimal attachment point of the sensor. Accelerometers (Digibale Pty Ltd, Sydney, Australia) were fitted to the necks and ears of Merino rams (n=14) prior to introduction to a ewe (replicated twice). Using decision tree analysis (SAS; v. 9.4., 2003; SAS Institute Inc. Cary, NC, USA), accelerometers attached to the necks of rams successfully predicted mating events with a positive predictive value (PPV) of 68% and a sensitivity of 80%. The PPV and sensitivity of ear-attached accelerometers were considerably poorer at 59% and 43%, respectively, likely due to the mobility of the ear compared with the neck. The specificity for both accelerometer types was high (96% and 91% for neck-attached and ear-attached, respectively) as was the accuracy (94% for the neck-attached accelerometers and 89% for the ear-attached accelerometers). Mating events were characterised by extreme peaks and troughs in acceleration across all three axes for both accelerometer positions. As a result, the standard deviation of the z- and x- axes were the variables of most importance to the model for the neck and ear accelerometer, respectively. Future studies will further refine this mating signature before its application to paddock mating systems, where its use as an investigative tool will unlock nuanced information on the differences in fertility, libido and hormonal fluctuations of individual sheep.

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## Response rate and predictive factors of intravenous glucocorticoid treatments in patients with moderate-to-severe and active Graves' ophthalmopathy

**Hwa Young Ahn<sup>1</sup>**

1. *Internal Medicine, Chung-Ang University College of Medicine, Seoul, South Korea*

Background

Graves' ophthalmopathy (GO) is an autoimmune disease of the orbit. Treatment for GO patients depends on the activity and severity of the eye changes. In patients with moderate-to-severe and active GO, intravenous (IV) glucocorticoid treatment is considered as a first-line treatment. However, response rates have been different depending on the dose of steroid or race. In addition, factors that predict the response of the treatment are not well known.

Method

This study was retrospective observational study. We included and analyzed 49 moderate-to-severe and active GO patients who treated 4.5g IV methylprednisolone during 12 weeks from November 2011 to November 2018. The response was defined when two or more of the five indicators (CAS, soft tissue involvement, exophthalmos, lid width, diplopia) were improved 3 months after the end of treatment. We examined predictive factors for the response using logistic regression analysis.

Results

Twenty-three (46.9%) patients classified response group 3 months after IV steroid treatment. In multivariate logistic regression analysis, age was negatively associated with response (OR 0.865, 95% CI 0.753-0.994), width of superior rectus muscle was positively associated with response (OR 3.899, 95% CI 1.305-11.649). In addition, suppressive TSH (OR 0.012, 95% CI 0.000-0.832) and higher TSH binding inhibitory immunoglobulin (TBII, OR 0.766, 95% CI 0.622-0.943) were associated with negative response of IV steroid treatment. On the other hand, the larger change of TBII after IV steroid treatment was related to the response (OR 1.460, 95% CI 1.009-2.113). In our study, CAS and soft tissue involvement improved mainly by steroid treatment.

Conclusion

In Korean active GO patients, it seems that IV steroid treatment is not as effective as previously reported. Younger age, superior rectus muscle enlargement, normal TSH, lower TBII and higher changes of TBII after treatment are predictive factors for good response of IV steroid treatment.

### A case of unilateral Graves' disease in the right lobe of thyroid gland

**Dong-Hyeok Cho<sup>1</sup>, Jin-Ook Chung<sup>1</sup>, Dong-Jin Chung<sup>1</sup>, Min-Young Chung<sup>1</sup>**

*1. Chonnam National University Hospital, Dongku, Gwangju, South Korea*

Although unilateral Graves' ophthalmopathy is frequently seen, Graves' disease usually presents as a diffuse hyperthyroid goiter, involving both lobes of the gland. Unilateral Graves' disease is a rare disease variant that can occur in either lobe of the thyroid gland. We report here the case of a 39-year old man who presented with unilateral goiter and clinical and laboratory evidence for hyperthyroidism. I-131 radioisotope uptake was elevated at 45.8%, and scintigraphy revealed that uptake of the radioisotope was unilateral increased in the right lobe of the thyroid gland, whereas the uptake in the left lobe did not differ from the uptake in normal control. Ultrasonography of the thyroid gland revealed an inflammatory change and diffuse enlargement in Rt lobe; Doppler investigation of the right lobe showed hypervascularity classically seen in Graves' disease. Serum antibodies to TSH-receptor and thyroid peroxidase were increased. Consequently, hyperthyroidism of Graves' disease with the involvement of only right lobe of thyroid gland was diagnosed. The patient was treated with methimazole. Although the unilateral Graves' disease has occasionally been described in the literature, clinician should be aware that Graves' disease can present unilaterally in either lobe of the thyroid gland.

### Antithyroid drugs resistant patient with Graves' disease : A case report

**nanny NM soetedjo<sup>1</sup>, hikmat permana<sup>1</sup>, Maya Kusumawati<sup>1</sup>**

*1. Division of Endocrinology, Metabolic & Diabetes, Internal Medicine Department, Hasan Sadikin Hospital, Padjadjaran University, Bandung, West Java, Indonesia*

Graves disease is the most common cause of thyrotoxicosis. It is an autoimmune disorder characterized by a constellation of clinical features including hyperthyroidism, diffuse goitre, ophthalmopathy, and dermopathy. Conventional principal management of thyrotoxicosis includes antithyroid drugs, radioactive iodine, and surgery. Adjunctive treatment in the form of beta-blockers, corticosteroids, inorganic iodide, and iopanoic acid may also be used for more prompt control of symptoms. However, a few cases may require additional treatment despite these conventional modalities to achieve euthyroid state.

A 22-year-old woman with severe Graves' disease was referred from a local clinic because of her refractory hyperthyroidism. She presented with exophthalmos, nodule goiter, and tachycardia. She was treated with a maximal dose of methimazole and switching to propylthiouracil (PTU), and administering maximum doses of beta blocker and steroid. However, her thyroid function test (TFT) did not improve. TFT showed T3 level > 600 ng/dL (normal : 58 - 156), free T4 level > 24 µg/dL (normal 4.87 - 11.72), thyroid stimulating hormone (TSH) level < 0.0083 mIU/L (normal : 0.36 - 4.94) and Thyrotropin (TSH) Receptor Antibodies (TRAb) > 40 IU/L (normal : <1.75). She was then administered for radioactive iodine (RAI) and effective to achieve treatment goals after the second RAI.

Hyperthyroidism resistant to antithyroid drug therapy is rare but potentially life-threatening. Radioactive iodine is one of the definitive approaches used in the treatment of thyrotoxicosis, especially in patients resistant to medical treatment.

However, it is necessary to consider why the patient was resistant to the antithyroid drugs. Possible reasons may include drug malabsorption, rapid drug metabolism, antidrug antibodies, impairment of intrathyroidal drug accumulation or action, and predominant elevation of T3 rather than T4 levels.

### Graves' disease recurrence after subtotal thyroidectomy presenting with out-of-hospital cardiac arrest

**Ting Wei Lee<sup>1,2</sup>, Chuan Liang Chou<sup>1</sup>, Ting I Lee<sup>1,3</sup>, Yu Mei Chien<sup>1</sup>, Han Wen Liu<sup>1</sup>, Yu Chun Hsueh<sup>1</sup>**

*1. Division of Endocrinology and Metabolism, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*

*2. Division of Endocrinology and Metabolism, Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*

*3. Department of General Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*

**Background:** Graves' disease is the most common cause of hyperthyroidism. Untreated hyperthyroidism can lead to thyrotoxic cardiomyopathy and consequent acute heart failure, a life-threatening condition requiring support with extracorporeal membrane oxygenation (ECMO). Surgery is one of the treatment modality for Graves' disease. However, subtotal thyroidectomy is not the best surgical strategy because it is associated with higher recurrence rate.

**Case Presentation:** A 36-year-old woman presented to the emergency department with out-of-hospital cardiac arrest. The patient called 911 because of shortness of breath, but she was found to have loss of consciousness on the ambulance. Advanced cardiac life support protocol was initiated, and she regained spontaneous circulation. Electrocardiogram showed atrial fibrillation with rapid ventricular response (heart rate of 160 beats per minute). Chest X-ray revealed cardiomegaly with features of pulmonary edema. Two hours later, the patient was placed on mechanical circulatory support with ECMO because of recurrent pulseless electrical activity cardiac arrest. The surgical scar over anterior neck and her exophthalmos made us suspect that the patient might have recurrent Graves' disease, which was confirmed by subsequent laboratory findings. Tracing

back her past medical history, she had Graves' disease for more than ten years. She underwent subtotal thyroidectomy three years ago, but had no medical surveillance thereafter. Her cardiovascular function improved after five days of ECMO support with medical treatment for thyroid crisis. She was eventually discharged after 20 days of hospitalization.

**Conclusion:** Untreated hyperthyroidism is potentially fatal, and must be considered as part of the differential diagnosis in a patient presenting with acute heart failure. Patients with Graves' disease who underwent subtotal thyroidectomy remain at the risk of developing recurrent hyperthyroidism, thus regular follow-up is necessary.

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## Outcomes of medical therapy for Graves' Disease in Far North Queensland. Identifying factors predictive of treatment failure.

**Yu-Chin Lo<sup>1</sup>, Benjamin Sly<sup>1</sup>, Anna McLean<sup>1</sup>, Ashimm Sinha<sup>1</sup>**

1. *Department of Diabetes and Endocrinology, Cairns Hospital, Queensland Health, Cairns North, QLD, Australia*

Medical treatment for Graves' Disease (GD) results in only around 50% treated achieving sustained remission. <sup>1</sup> Anecdotally, a high proportion of patients with GD managed at Cairns Hospital fail to achieve sustained remission with anti-thyroid drug (ATD) therapy.

The clinical audit aimed to determine the rate of ATD failure in the GD cohort seen at Cairns Hospital. Treatment failure was defined as either relapse following remission after at least 12 months of ATD, on-going ATD requirement after more than 24 months or the requirement of definitive therapy after initial treatment with ATD. Pre-treatment factors which may be predictive of ATD failure were also reviewed.

We conducted a retrospective cohort study of patients with GD who were evaluated, managed initially with ATD and followed up at the Cairns Hospital Endocrinology clinic between 2006-2016.

118 patients fulfilled criteria for inclusion in our study. Of these patients 86 (73%) failed ATD. Baseline characteristics including age, ethnicity, gender, smoking status, baseline thyroid function tests and thyrotropin stimulating hormone receptor antibody (TSHRab) levels, ophthalmopathy, presence and size of goitre, were collected to determine whether these factors have significant association with ATD failure.

In our cohort, the high rate of ADT failure was confirmed and reported at 73%. Univariate analysis of baseline characteristics revealed Indigenous ethnicity and large goitre size have a significant association with ATD failure. Previous studies have reported an association with goitre size and ATD failure, however the link with Indigenous ethnicity has not previously been reported.<sup>1</sup>

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## Elephantiasic Extrathyroidal Graves' disease.

**Divya Namboodiri<sup>1</sup>, Helen Mackie<sup>2</sup>, Diana Learoyd<sup>3</sup>, Veronica Preda<sup>1</sup>, Bernard Champion<sup>1</sup>**

1. *Endocrinology, Macquarie University Hospital, Sydney, NSW, Australia*

2. *Rehabilitation Medicine, Macquarie University Hospital, Sydney, NSW, Australia*

3. *Endocrinology, Kolling Institute, Northern Clinical School, Royal North Shore Hospital, St Leonards, NSW, Australia*

We present, a 72-year-old lady, ex-smoker, with Graves' Disease (GD) and severe extrathyroidal manifestations of Graves' ophthalmopathy (GO), thyroid acropachy and elephantiasic form of pretibial myxoedema (PTM). At diagnosis, aged 58, she had mild GO with a TRAb of 6.4IU/L (< 1.8IU/L). Initial unsuccessful therapy with carbimazole was followed by radioactive iodine (RAI) ablation with subsequent hypothyroidism requiring thyroxine replacement. Post RAI therapy, her GO and dermopathy worsened significantly, with persistent massively elevated TRAb titres. Currently she is clinically euthyroid, but with inactive GO, marked thyroid acropachy in hands, wrists and feet and severe bilateral lower limb elephantiasic PTM, with a TRAb level of 883IU/L. Indocyanine green fluorescence lymphography, which has not previously been applied to this condition, confirmed dermopathy with normal lymphatic drainage.

Worsening of GO is well-reported following RAI and is thought to be a consequence of release of thyroid antigenic material and activation of autoimmune reaction directed to orbital tissues, from radiation induced thyrocyte injury<sup>1</sup>. Overexpression of IGF-1 receptors in orbital fibroblasts, which synergistically enhance actions of thyrotropin, has also been postulated. Post RAI worsening of GO has been associated with several risk factors including smoking, pre-existing GO, high TRAb titres and high serum free T3<sup>1</sup>, the first three of which were identified in our patient. The severe elephantiasic form of PTM, has been postulated to be secondary to stimulation of fibroblasts, increasing glycosaminoglycans and hyaluronic acid deposition in the dermis, as a result of TRAb immunoreactivity<sup>2</sup>. The finding of autoantibodies that activate IGF-1R signalling in GD, has led to development and trial of teprotumumab, a monoclonal antibody to block the IGF-1 receptor in GO, that has shown superiority to placebo<sup>3</sup>. Although not used in Australia, this severe case warrants consideration, as to whether this targeted therapy might prove beneficial for other severe extrathyroidal manifestations of GD.

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## Hyperthyroidism in a child treated with human growth hormone

**Sin-Ae Park<sup>1</sup>**

1. *Presbyterian Medical Center, Jeonju, Jeollabukdo, South Korea*

Hypothyroidism is occasionally developed during human growth hormone (hGH) treatment. However, there is little evidence for the development of hyperthyroidism associated with hGH treatment. The development of hyperthyroidism associated with hGH treatment has rarely been reported. Recently we experienced one case. Herein we describe the clinical course and outcome of hyperthyroidism in this patient. This case is familial short stature. This patient developed hyperthyroidism during 18 months treatment with hGH. He showed positive thyrotropin-binding inhibiting immunoglobulin (TBII). He did not show typical clinical symptoms of hyperthyroidism. After treatment with methimazole for 2 months, thyroid function returned to normal. Hyperthyroidism associated with hGH treatment may occur by chance. However, incidence of hyperthyroidism among patients with familial short stature and the absence of a family history of it indicate that hGH may have causative effect in the development of hyperthyroidism.

## Blocking and stimulating antibodies in autoimmune thyroid disease

**Ramesh Ranjan<sup>2,1</sup>, Elna Ellis<sup>2</sup>**

1. *General Medicine, Canberra Hospital and Health Services, Canberra, ACT, Australia*

2. *Endocrinology, Alice Springs Hospital, Alice Springs, Northern Territory, Australia*

We highlight the case of a 42 year old indigenous man from a remote town in Northern Territory with autoimmune thyroid disease, who had alternating hyperthyroid and hypothyroid states. He presented hypothyroid with sight-threatening thyroid orbitopathy, over ten years post an initial diagnosis of Graves' disease. He then achieved a euthyroid state within months without medication therapy.

Hashimoto's thyroiditis and Graves' disease are autoimmune thyroid conditions. They have previously been thought of as two different disease processes, however it is now thought that they are on two ends of the autoimmune thyroid spectrum.<sup>1</sup>

In patients with alternating thyroid function between hyperthyroidism and hypothyroidism, blocking and stimulating TSH receptor antibodies are thought to be the cause. Both these antibodies can occur in the same patient, explaining variable clinical presentations, with alternating dominance between the two antibodies reported in the literature. The change of antibody dominance, and thus change in thyroid state, are related to the differences in concentration, affinity and potency of these antibodies.<sup>1</sup> The most common scenario is the transition from Graves' disease to Hashimoto's thyroiditis.<sup>1,2</sup> The presence of both blocking and stimulating antibodies may also explain periods of euthyroidism in these patients.<sup>3</sup>

Improved bioassay technologies are currently available which allows for the detection and measurement of TSH blocking and stimulating antibodies. The ability to measure these antibodies can be helpful in the interpretation of thyroid dysfunction, and management of patients with autoimmune thyroid disease.<sup>3</sup> However, until such a time that these bioassays are readily accessible, the management of patients with autoimmune thyroid disease, and switching hyperthyroid and hypothyroid states can be challenging. In the absence of definitive management of their disease, regular blood tests and close monitoring is required.<sup>2</sup>

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## Diffusely increased thyroid radionuclide uptake is not always hyperthyroidism: case report

**Lijuan Sun<sup>1</sup>, Hui Jen Goh<sup>1</sup>, Priya Govindharajulu<sup>1</sup>, Melvin Khee-Shing Leow<sup>1</sup>**

1. *Singapore Institute for Clinical Sciences, A\*STAR, Singapore*

Nuclear imaging scans are especially useful in the diagnostic evaluation of thyroid disorders such as thyroid nodules and thyroiditis. The rationale lies in distinguishing hyperfunctioning thyroid tissue (eg. "hot" thyroid nodules and Graves' disease show accentuated focal and diffuse radionuclide uptake respectively) from "cold" thyroid nodules and thyroiditis associated with attenuated radionuclide uptake. The diagnosis of thyroiditis is often suspected based on negligible uptake of either technetium-99m (<sup>99m</sup>Tc) or radioiodine (<sup>123</sup>I or <sup>131</sup>I) on thyroid scintigraphy. Here, we wish to highlight a case that showed a high uptake in the thyroid gland on <sup>18</sup>F-fluorodeoxyglucose (<sup>18</sup>F-FDG) positron emission tomography (PET) scan which turned out to be due to autoimmune thyroiditis. A 25-year-old asymptomatic woman without any history of medical illnesses had volunteered for a brown fat research study. <sup>18</sup>F-FDG PET imaging revealed abnormally increased tracer uptake over the anterior neck. The images appeared strangely analogous to typical <sup>99m</sup>Tc or radioiodine uptake scans among patients with hyperthyroidism. Further investigative workup confirmed autoimmune thyroiditis. In conclusion, while many clinicians are aware that reduced

thyroid radionuclide uptake usually signifies thyroiditis, the inclusion of autoimmune thyroiditis into the list of differential diagnosis based on accentuated thyroid uptake of  $^{18}\text{F}$ -FDG tracer remains counterintuitive for most of them. As the incidence and prevalence of autoimmune thyroiditis is high coupled with an increasing use of  $^{18}\text{F}$ -FDG-PET scanning for various indications, physicians should recognize that diffusely increased  $^{18}\text{F}$ -FDG uptake by the thyroid can result from autoimmune thyroiditis which needs follow-up and treatment to avert the morbidity of hypothyroidism.

## Correlation between alteration of freeT4 and IL-12 level in Graves' disease patients receiving methimazole therapy

**Tania Tedjo<sup>1</sup>, Tjokorda Gde Dalem Pemayun<sup>1</sup>, Heri Nugroho Hario Seno<sup>2</sup>**

1. *Endocrinology Metabolism, DR. Kariadi General Hospital, Semarang, Central Java, Indonesia*

2. *Endocrinology Metabolism, Diponegoro University, Semarang, Central Java, Indonesia*

### Background

Graves' disease (GD) is an autoimmune thyroid disease which has sophisticated pathogenesis and therefore difficult to achieve complete remission. Interleukin-12 (IL-12) is the main cytokine produced by the Antigen Presenting Cell (APC) and plays an important role in the pathogenesis of GD. Treatment of GD using Methimazole (MMI) known to reduce freeT4 (fT4) level and give a suppression effect to the immune system. Even though, correlation between alteration of fT4 and IL-12 level during MMI therapy remains unclear.

### Method

This is a prospective cohort study. Thirty three patients aged 16-60 years with GD were evaluate for their fT4 (enzyme-immunoassay method) and IL-12 level (ELISA method) serially during 2 month therapy of MMI. Differentiation among and between serial level of fT4 and IL-12 were done using Friedman and Wilcoxon signed rank test. Correlation between delta of fT4 and IL-12 level done using Rank-Spearman correlation test. Statistical analysis using SPSS 16.0.

### Result

Respondents: 12 males, 21 females. Median of age 36(16-60) years old. All respondents were in thyrotoxic condition (mean score of Wayne index:  $21,4 \pm 7,4$ ). Serial median level of fT4 during treatment were  $2,77(1,60-7,77)$ ,  $1,34(0,61-7,77)$ ,  $1,04(0,40-5,00)$  ng/dL respectively;  $p=0,00;95\%CI$ . Serial median level of IL-12 during treatment were:  $167,8(56,1-556,6)$ ,  $200,9(54,5-521,8)$ ,  $176,0(55,3-480,3)$  pg/mL respectively;  $p=0,023;95\%CI$ . Alteration of fT4 level showed a weak correlation with the alteration of IL-12 level ( $p=0,007-0,048;rho=0,346-0,459;95\%CI$ ) in patients with GD receiving MMI therapy.

### Discussion

Two month period of MMI therapy normalized thyroid function significantly but weakly reduced the immunological state. Reduction of fT4 level occurred as soon as MMI administered, whereas reduction of IL-12 occurred slowly and unstable. Suppression of immunity in GD need much longer time since many factors might affect the synthesis of IL-12 from the APC.

### Conclusio

Thyroid function has a weak correlation with immunological state in GD patient receiving 2 month MMI therapy.

## Synergistic Anti-Cancer Effect of Histone Deacetylase Inhibition and Blockade of the Glycolytic Pathway

**Hojin Chang<sup>1</sup>, Heejun Kim<sup>1</sup>, Soo Young Kim<sup>1</sup>, Seokmo Kim<sup>1</sup>, Bup-Woo Kim<sup>1</sup>, Yong-Sang Lee<sup>1</sup>, Hang-Seok Chang<sup>1</sup>, Cheong Soo Park<sup>1</sup>**

1. *Gangnam Severance Hospital, Yonsei University, Seoul*

### Background

Advanced cancer has been shown to have a higher percentage of epigenetic changes are more often events than genetic mutations. Preclinical models have showed that combination of the HNHA (N-hydroxy-7-(2-naphthylthio) heptanamide) and 2DG (2-Deoxy-D-glucose) is a play crucial role in ATC (cancer stem-like cell, anaplastic thyroid cancer). The aim of this research is to study that caspase cleavage dependent apoptosis by combination therapy of HNHA and 2DG in ATC.

### Methods

ATC cell lines were exposed to HNHA and 2DG alone or combined, and cell viability was determined by MTT assay. Synergistic anti-cancer effects of the combination therapy on cell cycle and intracellular signaling pathways were estimated by flow cytometry and immuno blot analysis. The ATC cell lines xenograft model was used to examine the anti-tumor activity *in vivo*.

### Results

Consequently, our results are suggest that combination therapy of HNHA and 2DG is synergistically decreased cell viability in ATC cell, and also significantly induced apoptotic cell death in this cells, as showed by the cleavage of caspase-3. HNHA and 2DG combination was reduced anti-apoptotic factor in these cells. Thus, combination therapy with HNHA and 2DG most significantly reduced tumor volume in ATC cell xenografts.

### Discussion& Conclusions

The current study suggests that HNHA and 2DG combination treatment was more effective than treatment with the HNHA or 2DG alone. These findings may offer a new therapeutic approach to ATC include the cancer stem-like cells.



## Transoral Endoscopic Thyroidectomy by a Vestibular Approach with Endoscopic Retractor : Experience with The First 132 Patients

**Hojin Chang<sup>1</sup>, Seokmo Kim<sup>1</sup>, Heejun Kim<sup>1</sup>, Soo Young Kim<sup>1</sup>, Bup-Woo Kim<sup>1</sup>, Yong Sang Lee<sup>1</sup>, Hang-Seok Chang<sup>1</sup>, Cheong Soo Park<sup>1</sup>**

1. Gangnam Severance Hospital, Yonsei University, Seoul

### ABSTRACT

**Background :** Transoral endoscopic thyroidectomy by a vestibular approach (TOETVA) is a novel technique for thyroid cancer operation. The aim of this study was to report on our initial experiences with TOETVA with endoscopic retractor for the management of thyroid carcinoma.

**Methods :** From September 2016 to April 2018, 132 patients with thyroid cancer underwent TOETVA. We used a three-port technique through the oral vestibule with endoscopic retractor, and thyroidectomy with ipsilateral central compartment dissection was performed endoscopically using conventional laparoscopic instruments.

**Results:** All patients had papillary thyroid carcinoma. Less than total or total thyroidectomy with ipsilateral central compartment node dissection was performed (124 vs. 8). The mean operation time was 87.6 min (range, 56-213 min). The average number of lymph nodes resected was 2.6 (range, 1-12). Six patients experienced a transient hoarseness, which was resolved within 3 months. Most of the patients could return home within 3 days after surgery.

**Conclusions:** In this large series from a single center, we found that TOETVA with endoscopic retractor can be performed safely and radically in selected patients with thyroid

## Adverse Effects of Tyrosine Kinase Inhibitors - Real World Use

**Hojin Chang<sup>1</sup>, Seokmo Kim<sup>1</sup>, Soo Young Kim<sup>1</sup>, Heejun Kim<sup>1</sup>, Bup-Woo Kim<sup>1</sup>, Yong Sang Lee<sup>1</sup>, Hang-Seok Chang<sup>1</sup>, Cheong Soo Park<sup>1</sup>**

1. Gangnam Severance Hospital, Yonsei University, Seoul

### Background

Kinase inhibitors are recommended for treating radioactive iodine (RAI)-refractory differentiated thyroid cancer patients with metastatic, rapidly progressive, symptomatic, and/or imminently threatening disease that is not otherwise amenable to local control using alternative approaches. Although associated with a significant progression-free survival improvement, the benefit of TKIs needs to be proved in the context of associated moderate to severe toxicities that require frequent dose reduction and delays.

### Methods

Retrospective cohort study of medical records of 71 patients treated with tyrosine kinase inhibitors lenvatinib and sorafenib for thyroid cancer at Gangnam Severance Hospital from July 2016 to December 2017 was conducted. Baseline clinical parameters, dosage and adverse effects from initiation of treatment were collected.

### Results

Sorafenib (N=48) and lenvatinib (N=23) was used. Initial starting dose for lenvatinib was 20mg/per day in all of the 20 patients. Adverse effects occurred in 19 patients (82.6%), requiring dose reduction in 8 (34.8%) of patients and drug cessation in 1 patient (4.3%). For patients using sorafenib, the initial starting dose was £400mg daily in 12 patients (25.0%), 600mg in 16 patients (33.3%) and 800mg daily in 20 patients (41.7%). Dose reduction was needed for adverse effects in 27 patients (56.3%), and drug cessation was necessary in 4 patients (8.3%). When most common adverse effect was compared, hand-foot-syndrome was significantly more frequent in patient using sorafenib (42 (87.5%) vs 13 (56.2%), p=0.003).

### Conclusion

Both of the TKIs showed high rate of adverse effect. Adverse effect was more observed in sorafenib patients, which needed more often drug cessation.

## Bone mineral density and bone turnover markers after long-term suppressive levothyroxine therapy of differentiated thyroid carcinoma in premenopausal women

**Dong-Hyeok Cho<sup>1</sup>, Jin-Ook Chung<sup>1</sup>, Dong-Jin Chung<sup>1</sup>, Min-Young Chung<sup>1</sup>**

1. Chonnam National University Hospital, Dongku, Gwangju, South Korea

**Background:** The standard treatment strategy for differentiated thyroid cancer (DTC) includes surgery followed by radioactive iodine ablation and long-term thyrotropin (TSH) suppression therapy. It has been suggested that the long-term TSH suppression may be associated with undesired adverse effects of thyroxine on bone metabolism. However, there is no consensus about the effects of subclinical hyperthyroidism on BMD induced by TSH suppression therapy of DTC in

premenopausal women. In this study, we evaluated the impact on BMD and bone turnover markers of long-term suppressive levothyroxine therapy for DTC in premenopausal women.

**Methods:** The study enrolled 83 premenopausal women (mean age,  $42.2 \pm 8.3$  years) receiving levothyroxine after total/near-total thyroidectomy and radioactive iodine therapy for DTC (mean follow-up period,  $7.3 \pm 5.6$  years). The subjects were divided into three groups by TSH level [group 1 with TSH level  $\leq 0.10$   $\mu\text{IU/mL}$  ( $n=28$ ), group 2 with TSH level between 0.10 and 0.50  $\mu\text{IU/mL}$  ( $n=32$ ), group 3 with TSH level  $>0.50$   $\mu\text{IU/mL}$  ( $n=23$ )]. levothyroxine dosage, BMD (examined by dual-energy x-ray absorptiometry), and bone turnover markers were evaluated. Biochemical marker of bone resorption was measured by urine deoxypyridinoline and bone formation by serum osteocalcin.

**Results:** Mean duration of levothyroxine treatment was  $7.8 \pm 6.1$  years, and mean levothyroxine dose was  $135.3 \pm 41.7$   $\mu\text{g/day}$  (range, 75–225  $\mu\text{g/day}$ ). Mean BMD contents and T-scores for each group divided by TSH level did not differ significantly among groups. Also, bone turnover markers and prevalence of osteoporosis and osteopenia were not different among groups. The odds ratios for risk of osteoporosis and osteopenia in groups 2 and 3 were not significant when compared to the reference group (group 1).

**Conclusion:** This cross-sectional study suggests that Long-term levothyroxine suppressive therapy of DTC in premenopausal women did not affect BMD and bone turnover markers or increase the prevalence of osteoporosis.

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## Is the Internal Jugular Node Dissection without Level V Enough in Patients with Papillary Thyroid Carcinoma with Lateral Neck Node Metastasis?

**Hye Ryeon Choi<sup>1</sup>, Jin Kyong Kim<sup>1</sup>, Sun Hyung Choi<sup>1</sup>, Soon Min Choi<sup>1</sup>, Cho Rok Lee<sup>1</sup>, Sang-Wook Kang<sup>1</sup>, Jandee Lee<sup>1</sup>, Jong Ju Jeong<sup>1</sup>, Kee-Hyun Nam<sup>1</sup>, Woong Youn Chung<sup>1</sup>**

1. Yonsei University College of Medicine, Seodaemun-gu, Seoul, South Korea

Purpose : Papillary thyroid carcinoma(PTC) has a very high rate of lateral neck node metastases, and completeness of surgical resection is an important determinant of outcomes. The extent of therapeutic lateral neck dissection remains controversial. This study aims to access the impact of lateral neck node dissection of levels II to V in a large patient series.

A retrospective review of the clinical charts and hospital records of 778 consecutive patients who had metastatic PTC and who underwent unilateral cervical lymph node dissection at a single institution between 1999 January and 2009 December .

A total of 289 modified radical neck dissection(MRND) (levels II–V) and 489 internal jugular node dissection(IJND)(level II–IV) were performed in 781 patients. Among these initial dissections, 391(50.1%), 585(74.0%), 517(66.2%), and 98(12.5%) had positive lymph nodes in levels II, III, IV, and V, respectively. In multivariate analysis, female sex, tumor size, and multi-level simultaneous metastasis was an independent predictor for level V metastasis. A total of 84(10.8%) metastases occurred after initial operations and there were 5 patients of level V recurrence(one in SLND and four in MRND). Postoperative complications were similar in MRND and IJND group.

Conclusions : IJND achieves favorable postoperative results in PTC with lateral neck node metastasis patients and level V metastasis/recurrence incidence is low. Therefore, the extent of lateral neck node dissection whether IJND or MRND can be considered who showed multi-level simultaneous metastasis and large tumor size patients.

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## Case Report: Malignant struma ovarii with a robust response to radioactive iodine

**Matti Gild<sup>1,2,3</sup>, Lauren Heath<sup>2</sup>, Roderick Clifton-Bligh<sup>1,2,3</sup>, Bruce Robinson<sup>1,2,3</sup>**

1. Kolling Institute of Medical Research, St Leonards, NSW, Australia

2. Endocrinology Department, Royal North Shore Hospital, St Leonards, NSW, Australia

3. University of Sydney, Sydney

Struma ovarii is a rare ovarian tumour with malignancy occurring in <5% of cases. Metastases are uncommon and are usually visceral. Patients can present with hyperthyroidism together with ascites, pain and other classic effects of a pelvic mass. It is not well known how to best treat and follow patients with extensive disease. Case reports of radioiodine ablative (RAI) therapy following thyroidectomy can reduce recurrence.

We present the case of a 33 year old woman who presented with bone pain and was found to have lytic lesions on MRI. Numerous skeletal metastases were confirmed on biopsy which demonstrated metastatic follicular thyroid carcinoma (FTC). Subsequent thyroidectomy showed no remarkable pathology or evidence of malignancy. At 28, she had an ovarian cyst excised which was diagnosed as a mature cystic teratoma. Pathology confirmed a dermoid cyst at the time. In light of this metastatic FTC diagnosis, the cyst pathology was re-reviewed and the teratoma was determined to comprise thyroid tissue.

She underwent three cycles of RAI over 18 months. Following the RAI, her thyroglobulin dropped three orders of magnitude and is now undetectable. FDG-PET scan demonstrated no avidity and her last RAI uptake scan showed no iodine avid lesions. She remains on replacement thyroxine with a suppressed TSH.

This case demonstrates an unusual presentation of a rare disease and highlights how well malignant struma ovarii can respond to standard treatments for metastatic FTC.

## Thyroid lymphoma: multimodality imaging and pathologic correlation

**Ji-Young Hwang<sup>1</sup>, Jin Hee Moon<sup>1</sup>, Ji Young Woo<sup>1</sup>**

1. Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, South Korea

Purpose

We would describe multimodality imaging findings of primary and secondary thyroid lymphoma and also feasible pathologic correlation.

Content

1. To review of primary thyroid lymphoma manifesting as thyroid nodule
2. To review of primary thyroid lymphoma manifesting as diffuse thyroid lesion
3. To review of secondary lymphoma manifesting as lateral neck lymphadenopathy

Conclusion

Awareness of various ultrasonographic findings of thyroid lymphoma is helpful to differentiate from other malignancy. It would facilitate the direct application of core biopsy skipping over the inappropriate fine-needle aspiration and could establish the correct therapeutic guideline.

## Ectopic Thyroid Tissue mimicking Metastatic Thyroid Cancer

**Tjin Shing Jap<sup>2,1</sup>**

1. Division of Endocrinology and Metabolism, Weigong Memorial Hospital, Toufen, Miaoli county, Taiwan

2. Taipei Veterans General Hospital, Taipei, TAIWAN, Taiwan

Radioiodine whole body scintigraphy is a sensitive method for detection of thyroid cancer but false-positive scan results had been reported due to ectopic thyroid tissue in the mediastinum or thyroid tissue containing teratoma. Here we report two cases of ectopic thyroid tissue mimicking metastatic thyroid cancer. Case A was a 57-year housewife complained a lump over the right neck during the annual physical check-up. A sonography of thyroid gland showed a 1 cm hypoechoic nodule in the right lobe. Robotic total thyroidectomy was performed and disclosed a 0.7 cm single papillary thyroid cancer and local lymph node invasion with T1aN1aMx, staging. The follow up serum thyroglobulin level was 0.5 ng/mL, anti-thyroglobulin antibody 1.2 IU/mL (normal < 4.1 IU/mL) and 120 millicurie radioiodine whole body scintigraphy showed a nodular lesion with high tracer accumulation in the anterior mediastinum consistent with ectopic thyroid tissue. Case B was a 42-year housewife complained a lump over the right neck for six months without tenderness or swallowing disturbance. Neck sonography showed a 4 cm hypoechoic nodule in the right thyroid lobe. A neck exploration with total thyroidectomy showed a 4-cm papillary thyroid cancer without loco-regional invasion. Postoperatively the serum thyroglobulin was still as high as 39 ng/mL and 100 millicurie radioiodine treatment showed increased uptake over the right pelvic area and two intense spots over the neck. The patient underwent an abdominal exploration with right ovary resection, which showed teratoma containing thyroid tissue.

In conclusion, these two cases demonstrate the occurrence of false positive radioactive scintigraphy in patients with a history of papillary carcinoma of thyroid, and highlight the need to consider ectopic thyroid tissue as well as metastatic disease.

## Surgery alone for papillary microcarcinoma is more cost effective than long term active surveillance

**Jayani Jayasekara<sup>1</sup>, Jia Feng Lin<sup>1</sup>, Ahmad Aniss<sup>1</sup>, Diana Learoyd<sup>1</sup>, Roderick Clifton Bligh<sup>1</sup>, Schelto Kruijff<sup>1</sup>, Anthony Glover<sup>1</sup>, Stan Sidhu<sup>1</sup>, Mark Sywak<sup>1</sup>**

1. University of Sydney, Sydney, Australia

**Background:** Papillary thyroid microcarcinoma (PMC), is a subtype of thyroid cancer that may be approached with active surveillance (AS) rather than immediate surgery. AS reduces complication rates and may reduce health care cost. This study aims to analyze complication rates of thyroid surgery, PMC recurrence and survival rates. Additionally, the costs of surgery versus hypothetical AS for PMC are compared in an Australian cohort.

**Methods:** PMC patients were included from a prospectively collected surgical cohort of patients treated for papillary thyroid cancer between 1985 and 2017. Primary outcome was the complications of thyroid surgery, recurrence free survival, overall survival and the cost of surgical treatment and AS.

**Results:** In total 349 PMC patients with a median age of 48 years (range, 18-90 years). Permanent surgical complications rate was 3.7%. Postoperative RAI did not reduce recurrence free survival (P=0.3). Total costs of surgical treatment was A\$10,338,

whereas hypothetical AS was at a yearly cost of A\$722. We estimate that the cost of one surgical PMC treatment equals approximately 17.0 years of AS.

**Conclusion:** Surgery may have a long-term economic advantage for younger Australian PMC patients who are likely to require more than 17.0 years of follow-up in an AS scheme.

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## Changes of resting metabolic rate, body composition or muscle strength after thyroidectomy in female patients with differentiated thyroid cancer

**Kyong Yeun Jung<sup>1</sup>, Sun Wook Cho<sup>2</sup>, Jae Hoon Moon<sup>2</sup>, Hyo Jeong Kim<sup>1</sup>, Do Joon Park<sup>2</sup>, Ka Hee Yi<sup>2</sup>, Young Joo Park<sup>2</sup>**

1. Eulji University Eulji Medical Center, Nowon-gu, Seoul, South Korea

2. Seoul National University College of Medicine, Seoul

**Introduction:** Patients with differentiated thyroid cancer (DTC) often have complained of fatigue, weight changes or decreased quality of life (QOL) after thyroidectomy. The aim of this study was to identify whether there exist changes of resting metabolic rate, body composition, or muscle strength after thyroidectomy in female patients with DTC, and whether those are associated with QOL changes.

**Methods:** In this prospective study, we recruited 36 middle-aged female DTC patients scheduled for thyroidectomy. Among them, 18 subjects received radioactive iodine treatment (RAI), while 18 did not. Resting metabolic rate (RMR), muscle strength (knee extension), or body composition (DEXA) were measured at baseline, postoperative 7 and 12 months. Questionnaire for QOL (SF36), physical activity (IPAQ), and dietary records of 24 hour recall were also obtained.

**Results:** Baseline TSH values were  $1.74 \pm 1.34$   $\mu$ IU/mL and those of postoperative 7 and 12 months were  $0.23 \pm 0.41$   $\mu$ IU/mL and  $0.34 \pm 0.82$   $\mu$ IU/mL. The % body fat was decreased at postoperative 12 months compared to individual baseline ( $37.3 \pm 5.1$  vs.  $35.8 \pm 4.7$ ,  $p < 0.001$ ), although energy intake or physical activity was not changed in total 36 subjects. There were no significant changes in weight, BMI, RMR and total scores of SF-36. When we evaluated postoperative changes according to RAI treatment, the % body fat was significantly decreased at postoperative 7 and 12 months ( $37.4 \pm 5.5$  vs  $34.6 \pm 5.5$  vs.  $35.0 \pm 5.4$ ,  $p < 0.001$ ) only in no RAI group. On the other hands, muscle strength significantly increased at postoperative 12 months (Extension, peak torque:  $76.0 \pm 21.3$  vs.  $86.6 \pm 16.7$  nm,  $p = 0.024$ ) only in RAI group.

**Conclusions:** Our study demonstrated that body composition, muscle strength or QOL was not deteriorated after thyroidectomy in DTC patients.

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## Radiofrequency ablation of primary papillary thyroid carcinomas: evaluation of the treatment efficacy

**Solyung Jung<sup>1</sup>, Jeeyoung Kim<sup>1</sup>**

1. Radiology, Yeoudo St. Mary's Hospital, The Catholic University of Korea, Seoul, SEOUL, South Korea

### Objective:

The purpose of this study is to retrospectively evaluate the outcomes of radiofrequency ablation (RFA) of papillary thyroid carcinomas (PTCs) in patients who were unable to undergo the surgery due to severe disease or didn't want the surgery.

### Materials and Methods:

Between 2008 and 2017, pathologically proven 17 PTCs (mean diameter, 1.3 cm; range, 0.3 – 8cm; micropapillary carcinoma, 14) in 11 patients (9 women, 2 men; mean age, 59.4 years; range, 37-86 years) were treated with RFA by one radiologist with 10 year RFA experience. US-guided RFA was performed using a radiofrequency generator and an 18-gauge internally cooled electrode. We evaluated changes in tumor volume and local tumor regrowth or lymph node metastasis on follow-up ultrasonography.

### Results:

Among 17 PTCs, 14 underwent single session of RFA and three required two sessions. One patient was lost to follow up after RFA. During follow-up period (mean 27.2 months; range, 2–77 months), there was a significant volume reduction ( $92.8 \pm 1.5\%$ ) in 11 PTCs (11/16, 68.7%) and five (5/16, 31.2%) remained as hypoechoic nodules without volume reduction. 7 PTCs (7/16, 43.7%) were completely disappeared. No tumor regrowth were detected in any ablation zones at last follow up. No major complications were found.

### Conclusions:

In patient with PTC, RFA may be used as an effective tool for local tumor control, especially for patients who are unable to surgery or who do not want surgery.

## Tumor growth kinetic analyses might explain the excellent prognosis in childhood/adolescent papillary thyroid carcinoma

**Toshihiko Kasahara<sup>1</sup>, Akira Miyauchi<sup>1</sup>, Yasuhiro Ito<sup>1</sup>, Takumi Kudo<sup>1</sup>**

1. Kuma Hospital, Kobe, Hyogo, Japan

**Background:** Young patients with papillary thyroid carcinoma (PTC) have generally excellent prognoses despite their often-advanced disease status. To clarify the natural history of PTC in children and adolescents, we compared the tumor volume-doubling rate (TV-DR) under observation with hypothetical tumor volume-doubling rate (hTV-DR) before presentation in young PTC patients. DR is a reciprocal of doubling time and indicates the number of doublings that occur in a unit time. A negative value means the number of halvings per unit time.

**Methods:** We enrolled 20 patients with PTC diagnosed cytologically aged 12–19 years who were followed with periodical ultrasound examinations for  $\geq 3$  months before surgery due to various reasons. Seventeen of them later underwent surgery confirming the diagnosis. We calculated the TV-DRs using tumor diameters measured serially after presentation. We also calculated hTV-DRs using the tumor diameters and the patient's age at presentation, assuming that a single cancer cell was present at the patient's birth and that the tumor grew at a constant rate. These values indicate the least growth rates necessary for a single cancer cell to become the tumor size at the presentation.

**Results:** Thirteen patients had positive TV-DRs (/year) ranging from 0.09–1.89 indicating slow growth, and the remaining seven patients had negative values (-0.08 – -1.21) indicating regression. The median TV-DR was 0.29. The hTV-DRs (1.53–2.72, median 1.71) were significantly larger than the TV-DRs ( $p < 0.001$ ), indicating much faster growth before presentation.

**Conclusions:** These data suggest that deceleration of tumor growth has already occurred at presentation in the majority of the cases. This might explain why disease-specific survival is excellent, despite the frequent advanced disease in pediatric/adolescent patients with PTC.

## Robot-assisted Thyroid Lobectomy with SP System via Transaxillary Approach: Experience with the First 10 Papillary Thyroid Cancer Patients

**Jin Kyong Kim<sup>1</sup>, Sun Hyung Choi<sup>1</sup>, Soon Min Choi<sup>1</sup>, Hye Ryeon Choi<sup>1</sup>, Cho Rok Lee<sup>1</sup>, Sang-Wook Kang<sup>1</sup>, Jandee Lee<sup>1</sup>, Jong Ju Jeong<sup>1</sup>, Kee-Hyun Nam<sup>1</sup>, Woong Youn Chung<sup>1</sup>**

1. Yonsei University College of Medicine, Seodaemun-gu, Seoul, South Korea

**Introduction** Many studies have reported that robot-assisted thyroidectomy was proven to be a safe and feasible method and offer surgical outcomes similar to conventional open or endoscopic surgery. The aim of this study was to introduce our novel surgical technique using the da Vinci SP robot system through transaxillary approach.

**Methods** Ten patients underwent robotic-assisted thyroid lobectomy using the da Vinci SP robot system between December 2018 and April 2019 at Yonsei University Health System, Seoul, Korea. All procedures were performed successfully using the da Vinci SP robot system in two methods (4 gas method and 6 gasless method).

**Result** All ten patients were female who were diagnosed with papillary thyroid carcinoma (PTC). The mean operation time was  $148.7 \pm 26.8$  minutes. The mean operation time of gasless method was shorter than that of gas method ( $130.5 \pm 14.1$  vs.  $176.0 \pm 12.8$  minutes). The mean pain score on the operation day, 1<sup>st</sup> day, 2<sup>nd</sup> day and 3<sup>rd</sup> day was  $5.0 \pm 1.5$ ,  $2.2 \pm 1.0$ ,  $0.9 \pm 0.3$ , and  $0.6 \pm 0.5$ , respectively. All patients were discharged on the third day after operation without any complications.

**Conclusion** Robot-assisted thyroidectomy with SP system via transaxillary approach is a feasible and safe surgical method with outstanding cosmetic effects. This report is our successful initial surgical experiences. We need more surgical experiences to evaluate surgical outcomes and to verify technical feasibility.

## Clinical Usefulness of Preoperative Vitamin D Injection for Prevention of Postoperative Transient Hypocalcemia

**Kwangsoon Kim<sup>1</sup>, Jin Kyong Kim<sup>2</sup>, Cho Rok Lee<sup>2</sup>, Sang-Wook Kang<sup>2</sup>, Jandee Lee<sup>2</sup>, Jong Ju Jeong<sup>2</sup>, Woong Youn Chung<sup>2</sup>, Kee-Hyun Nam<sup>2</sup>**

1. Catholic university, Seoul

2. Yonsei university, Seoul

**Introduction** Transient hypocalcemia is a common complication after total thyroidectomy. The objective of this retrospective study was to evaluate the clinical usefulness of vitamin D injection before operation for the prevention of postoperative transient hypocalcemia.

**Methods** 2294 patients who underwent total thyroidectomy from January 2015 until October 2018 were analyzed retrospectively by complete chart review at Severance Hospital (Seoul, Korea). The patients were divided into two groups - vitamin D injection (VDI) group [n=342] and vitamin D non-injection (VDN) group [n=1952]. Transient hypocalcemia was defined as serum calcium  $< 8.2$  mg/dL and signs or symptoms of hypocalcemia.

**Results** The mean preoperative vitamin D level of VDI group was significantly lower than that of VDN group ( $16.5 \pm 6.9$  ng/mL vs.  $19.4 \pm 8.7$  ng/mL,  $p < 0.001$ ). Multivariate analysis indicated that the significant risk factors of postoperative transient

hypocalcemia include vitamin D non-injection (hazard ratio [HR]: 1.717, 95% confidence interval [CI]: 1.282-2.300,  $p < 0.001$ ), male gender (HR: 1.427, 95% CI: 1.117-1.822,  $p = 0.004$ ), and capsular extension (HR: 1.273, 95% CI: 1.011-1.603,  $p = 0.040$ ).

**Conclusion** Preoperative vitamin D injection effectively prevents postoperative transient hypocalcemia after total thyroidectomy. Further studies must be conducted to determine the effect of vitamin D injection.

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## Clinical and Pathologic Features for Predicting malignancy in Thyroid Follicular Neoplasms

**Kwangsoon Kim<sup>1</sup>, Ja Seong Bae<sup>1</sup>, Jeong Soo Kim<sup>1</sup>**

1. Catholic university, Seoul

**Background** Unfortunately, the cytology finding of follicular neoplasm does not distinguish between a thyroid adenoma and a carcinoma. The objective of this retrospective study was to identify the pathologic features that characterize patients with an increased risk of having a thyroid carcinoma.

**Methods** A total of 416 patients with follicular neoplasm who underwent thyroidectomy were reviewed at Seoul ST. Mary's Hospital (Seoul, Korea) from January 2010 to June 2018. Clinicopathologic features were analyzed retrospectively by complete medical chart review and pathologic slide review.

**Results** Thyroid malignancy was diagnosed in 209 patients (50.2%). 118 patients (28.4%) were diagnosed with follicular variant papillary thyroid carcinoma (fvPTC), 59 patients (14.2%) were diagnosed with follicular thyroid carcinoma (FTC), and 23 patients (5.5%) were diagnosed with conventional PTC. The number of patients with nuclear atypia was quite more in malignancy group than in benign group (16.4% vs. 1.9%,  $p < 0.001$ ). Multivariate analysis indicated that the significant risk factors of diagnosis of malignancy include cytological diagnosis with nuclear atypia (odds ratio [OR]: 10.762, 95% confidence interval [CI]: 3.002-38.575,  $p < 0.001$ ), NRAS mutation positive (OR: 2.483, 95% CI: 1.212-5.086,  $p = 0.013$ ), and male gender (OR: 2.027, 95% CI: 1.042-3.944,  $p = 0.037$ ).

**Conclusion** In our result, prevalence of carcinoma in patient with preoperative diagnosis of follicular neoplasm was much higher than previous reports. Cytological result with atypia is useful predictors for the presence of malignancy. Further studies must be conducted to support our results.

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## Initial experiences of transoral robotic thyroidectomy using 3 ports without axillary incision

**Wan Wook Kim<sup>1</sup>, Jin Hyang Jung<sup>1</sup>, Jeeyeon Lee<sup>1</sup>, Yang Soo Lim<sup>1</sup>, Ryu Kyung Lee<sup>1</sup>, Chansub Park<sup>1</sup>, Ku Sang Kim<sup>2</sup>, Young San Jeon<sup>3</sup>, Ho Yong Park<sup>1</sup>**

1. Surgery, Kyungpook National University, Daegu, South Korea

2. Surgery, Dongkang Medical Center, Ulsan, South Korea

3. Surgery, Koo hospital, Daegu, South Korea

**Background**

Transoral robotic thyroidectomy (TORT) has been popular due to new minimally invasive surgery and invisible scar. Conventional TORT with 4 ports allows fine dissection with counter-traction under optimal view and specimen can be easily removed. However, it needs axillary incision (it is not real scarless thyroidectomy) and wider flap dissection. The purpose of this study is to introduce the early experiences of TORT using 3 ports without axillary incision and compare the results from previous 4 ports.

**Material and Method**

A total of 80 patients were enrolled in consecutive order; 4 ports group ( $n = 47$ ) is done between December 2016 and July 2017, 3 ports ( $n = 34$ ) is from October 2018 to March 2019. Drain was put in all 4 ports through axillary incision and no drain in 3 ports. The parameters including clinicopathologic data, operation time, postoperative complications (recurrent laryngeal nerve palsy, hypocalcemia, bleeding), postoperative laboratory data (calcium, PTH), hospital stay, and postoperative pain (VAS score) were analyzed.

**Results**

Operation time (min) was shorter in 3 ports group than 4 ports group ( $142.0 \pm 38.6$  vs.  $166.3 \pm 35.4$ ,  $p = 0.006$ ) and hospital stay (day) was shorter in 3 ports group ( $2.1 \pm 0.3$  vs.  $3.8 \pm 0.4$ ,  $p = 0.001$ ) as well. Postoperative complications (hypocalcemia, nerve palsy, bleeding, seroma and infection), level of calcium (POD#1, 2, 10), PTH (POD#1, 10) and immediate postoperative pain (VAS score) were not different between the groups.

**Conclusions**

As the surgeon became accustomed to the operation, the operation time was continuously reduced even without the axillary port. Our study showed three ports TORT had a shorter operation time and hospital stay than conventional 4 ports. There were no significant differences between 3 ports and 4 ports TORT in almost postoperative surgical results. In conclusion, TORT using 3 ports is considered to be a comparable, safe and effective operation method as a real scarless operation.

## Should Total Thyroidectomy be Recommended for Patients with Familial Non-medullary Thyroid Cancer?

**Yon Seon Kim<sup>1</sup>**

1. Ulsan University Hospital, Ulsan, South Korea

**Background:** It is unknown if familial non-medullary thyroid cancer (FNMTC) has more aggressive clinical features and a worse prognosis than sporadic non-medullary thyroid cancer (SNMTC).

**Methods:** We retrospectively reviewed 2,894 patients with differentiated thyroid cancer who underwent primary thyroidectomy, identified 391 FNMTC cases, and compared the prevalence, surgical extension, and clinicopathologic features of FNMTC and SNMTC.

**Results:** A family history of thyroid cancer was noted in 391 patients (13.5%), with 85% having two affected relatives, and 15% with >3 affected relatives. A sibling was affected in 52.9% of cases and in 47.1%, both parent and child were affected. There were no significant between-group differences in sex, age, tumor size, extrathyroidal extension, or central lymph node metastases. Significantly more patients with FNMTC exhibited multifocal disease ( $p=0.020$ ) or benign nodules ( $p=0.015$ ). Lateral neck lymph node metastases were noted in 6.6% (SNMTC) and 9.7% (FNMTC;  $p=0.021$ ) of patients. In the FNMTC group, seven experienced disease recurrence, with no mortality noted during follow-up.

**Conclusions:** FNMTC is *not* more aggressive than SNMTC; however, FNMTC should be treated with total thyroidectomy because of the increased disease multifocality and presence of benign nodules. Lateral neck lymph node metastases were more likely in patients with FNMTC, although we could not estimate prognosis. All patients with thyroid cancer should be checked for family disease history and undergo preoperative ultrasonography to determine the extent of node dissection and the need for total thyroidectomy.

## The appropriate extent of surgery according to tumor size in thyroid cancer located in the isthmus

**Inhwa Lee<sup>1</sup>, Hyeung Kyoo Kim<sup>1</sup>, Jeonghun Lee<sup>1</sup>, Euy Young Soh<sup>1</sup>**

1. Ajou University Medical Center, Yeongtong-gu, Suwon-si, Gyeonggi-Do, South Korea

**Purposes:** The recent trend is towards reducing the extent of surgery considering the good prognosis of thyroid cancer and quality of life of patients. But there is still controversy about the appropriate extent of surgery for thyroid cancer located in the isthmus. In order to find the appropriate extent of surgery for thyroid cancer located in the isthmus, we compared clinical findings according to the extent of surgery and tumor size.

**Methods:** Between January 1997 and August 2017, a retrospective analysis was performed on all patients who underwent thyroid surgery for thyroid solitary cancer located in the isthmus. Finally 197 patients were included in this study.

**Results:** Median follow-up period was 54.4 months, recurrence rate was 0.5% (1 out of 197 patients). 41 patients (20.8%) underwent less than total thyroidectomy, 156 patients (79.2%) underwent total thyroidectomy, there was no statistically significant difference in recurrence rate. ( $p$ -value: 0.208) Groups with tumors larger than 1 cm had significantly increased microscopic extrathyroidal extension and gross extrathyroidal extension compared to groups smaller than 1 cm. ( $p$ -value: 0.032, 0.009, respectively)

**Conclusions:** There is no difference in recurrence rate according to the extent of surgery. Therefore, hemithyroidectomy should be actively considered in thyroid cancer located in the isthmus, especially in a solitary nodule smaller than 1 cm. Isthmusectomy is also an option.

## Occult Papillary Thyroid Carcinoma Presenting With Cervical Neck Lymph Node Metastasis

**Jong Eun Lee<sup>1</sup>, Sung Hoon Hong<sup>1</sup>, Sun Wook Han<sup>1</sup>, sung Yong Kim<sup>1</sup>**

1. Soonchunhyang University Hospital, Cheonan, Chungchungnam-Do, South Korea

### Occult papillary thyroid carcinoma presenting with cervical neck lymph node metastasis

Papillary thyroid cancer accounts for most cases of thyroid cancer. Neck lymph node metastases have been reported even in the absence of a primary tumor in the thyroid, and these cases are referred to as occult thyroid cancer (OTC). 74-year-old patient presented to the endocrinology department. Neck ultrasonography was conducted to assess the blood vessels. A 13 mm x 11 mm mass was incidentally found on the right thyroid that appeared benign. Additionally, a 22 mm x 16 mm mass was found at level III of the right neck lymph node, which appeared malignant. Another test was performed for thyroid, but no other abnormalities were observed in other areas. The right neck lymph nodes were confirmed to be a metastatic papillary carcinoma. Total thyroidectomy with right neck functional dissection was performed. The biopsy result confirmed that the right thyroid mass was a hyperplastic nodule, and also confirmed metastases to 7 of 21 neck lymph nodes. Although OTC has occasionally been reported in the past, its prevalence has decreased owing to improvements in ultrasonography and pathological tests. When a metastatic mass is found in neck lymph nodes but without a primary tumor in the thyroid, fine needle aspiration is the best diagnostic tool. If the biopsy result confirm metastatic thyroid cancer, total thyroidectomy and ipsilateral neck lymph nodes dissection should be performed.

Thank you for the opportunity to work on your manuscript. I have checked the complete document for language, grammar, style, clarity, and structure and have made changes as necessary, which I have edited while attempting to remain faithful to your intended meaning.

As requested and instructed, I have aimed to edit the text with a natural flow of native English for you.

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## Ultrasonographic Thyroid Nodule Classification Using a Deep Convolutional Neural Network

**Myung-Chul Lee<sup>1</sup>, Soon Woo Kwon<sup>1</sup>**

1. *Korea Cancer Center Hospital, Nowon-gu, Seoul, South Korea*

**Objective:** Ultrasonography with fine needle aspiration biopsy is commonly used to detect thyroid cancer. However, thyroid ultrasonography is prone to subjective interpretations and interobserver variabilities. The objective of this study was to develop a thyroid nodule classification system for ultrasonography using convolutional neural networks.

**Methods:** Transverse and longitudinal ultrasonographic thyroid images of 762 patients were used to create a deep learning model. After surgical biopsy, 325 cases were confirmed to be benign and 437 cases were confirmed to be papillary thyroid carcinoma. Image annotation marks were removed, and missing regions were recovered using neighboring parenchyme. To reduce overfitting of the deep learning model, we applied data augmentation, global average pooling, and 4-fold cross-validation. We employed a transfer learning method with the pretrained deep learning model VGG16.

**Results:** The average area under the curve of the model was 0.916, and its specificity and sensitivity were 0.70 and 0.92, respectively. Positive and negative predictive values were 0.90 and 0.75, respectively.

**Conclusions:** We introduced a new fine-tuned deep learning model for classifying thyroid nodules in ultrasonography. We expect that this model will help physicians diagnose thyroid nodules with ultrasonography.

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## High BRAF<sup>V600E</sup> Mutation Frequency in Chinese Patient with Papillary Thyroid Carcinoma Increases Diagnostic Efficacy in Cytologically Indeterminate Thyroid Nodules

**Shuhang Xu<sup>1</sup>, Xingjia Li<sup>1</sup>, Xiaodong Mao<sup>1</sup>, Chao Liu<sup>1</sup>**

1. *Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China*

**Objective:** To estimate the BRAF<sup>V600E</sup> mutation frequency in Chinese patient with papillary thyroid carcinoma (PTC), and the diagnostic value of BRAF<sup>V600E</sup> mutation status in thyroid nodules with indeterminate TBSRTC categories.

**Methods:** A total of 4875 consecutive samples for thyroid ultrasound-guided fine-needle aspiration cytology (FNAC) and BRAF<sup>V600E</sup> mutation analysis were collected. Among all the cases, 314 underwent thyroidectomy. FNAC was performed for a preoperative diagnosis. ROC of the subject was constructed to evaluate the diagnostic value of these two methods and their combination.

**Results:** BRAF<sup>V600E</sup> mutation in FNAC of thyroid nodules occurred in 2796 samples (57.35%). Of 353 nodule samples from 314 patients with thyroid operation, 333 were pathologically diagnosed as PTC. Of these PTC patients, 292 (87.69%) were found to have BRAF<sup>V600E</sup> mutation in their preoperative FNAC. In 175 cytologically indeterminate thyroid nodules, BRAF<sup>V600E</sup> mutation identified 88% of PTC. According to ROC data, BRAF<sup>V600E</sup> mutation testing had an obviously higher sensitivity (87.69%) and specificity (100.00%) than TBSRTC. Combining BRAF<sup>V600E</sup> mutation testing and TBSRTC achieved the largest AUC (0.954). For 41 PTC with a negative BRAF<sup>V600E</sup> mutation in preoperative evaluation, the repeated BRAF<sup>V600E</sup> mutation testing found out 12 samples with BRAF<sup>V600E</sup> mutation. The true BRAF<sup>V600E</sup> mutation rate of Chinese PTC patients was 91.29%.

**Conclusions:** Chinese patients with PTC have a higher frequency of BRAF<sup>V600E</sup> mutation. The BRAF<sup>V600E</sup> mutation testing affords a high diagnostic value in thyroid nodules with indeterminate cytology.

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## 6 cases of distant metastasis due to papillary microcarcinoma

**Yasushi Noguchi<sup>1</sup>**

1. *Department of Radiology & Nuclear Medicine, Noguchi Thyroid Clinic & Hospital Foundation, Beppu, Oita, Japan*

Papillary microcarcinoma (PMC) is generally considered as a very low risk disease. Therefore the possibility of distant metastasis is very low and the prognosis is excellent. But even with the very low risk, this do not mean that PMC will never have distant metastasis. We report 6 cases of distant metastasis due to PMC who underwent radioiodine therapy (RAI).



From January 2007 to December 2016, initial RAI were performed for distant metastasis in 143 cases. Among the 143 cases, we found that 6 cases of PMC. In the 6 cases, pathology showed that 3 had lymph node metastasis and 3 had multiple lesions in the thyroid, but only in 1 case lymph node metastasis were detected before surgery and none of the multiple lesions were detected on ultrasound. In 2 cases the original lesion could only diagnosed by pathology. 2 cases died due to thyroid cancer.

Because of the low risk of PMC, active surveillance is recommended. In active surveillance, the enlargement of the original lesion is said to be a sign of aggressiveness. Although in our group, the original lesions were still smaller than 10mm in diameter. Lymph node metastasis is also a sign of aggressiveness but 2 out of 3 cases could not be detected by ultrasound. The rate of distant metastasis of PMC is less than 1.0%, and some even claim that there are no incident of death. Despite this claim, 2 out of 6 cases died due to thyroid cancer in our group.

From our result, although the risk of PMC is very low, it could be a cause of death in some cases.

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## The diagnostic benefit of repeated FNA according to US patterns in thyroid nodules initially diagnosed as atypia/follicular lesion of undetermined significance

Wooyul Paik<sup>1</sup>, Ji Hoon Kim<sup>2</sup>, Dong Gyu Na<sup>1</sup>

1. Gangneung Asan Hospital, Gangneung-si, Gangwon-Do, South Korea

2. Radiology, Seoul National University Hospital, Seoul, South Korea

### ABSTRACT

**PURPOSE:** To determine the diagnostic benefit of repeated fine-needle aspiration (RFNA), according to the US patterns in thyroid nodules initially diagnosed as atypia/follicular lesion of undetermined significance (AUS/FLUS).

**MATERIALS AND METHODS:** This study included 273 consecutive nodules in which follow-up RFNA was performed among 502 thyroid nodules ( $\geq 1$  cm) initially diagnosed as AUS/FLUS from January 2010 to December 2014. The diagnostic benefit of obviating unnecessary diagnostic surgery was determined when the RFNA cytology result was benign. We assessed the rate of diagnostic benefit, surgery decision (RFNA result of category 4, 5, 6), and conclusive diagnostic result (RFNA result of category 2,4,5,6) on RFNA according to US patterns of nodules defined by Korean Thyroid Imaging Reporting and Data System (K-TIRADS).

**RESULT:** The diagnostic benefit of benign RFNA result was found in 49% in K-TIRADS 3, 37.8% in K-TIRADS 4, and 28% in K-TIRADS 5 nodules, and there was a decreasing trend of the diagnostic benefit rate on RFNA with increasing K-TIRADS score ( $P=0.034$ ). The surgery decision was made in 3.4% in K-TIRADS 3, 11.2% in K-TIRADS 4, and 28% in K-TIRADS 5 nodules ( $P<0.001$ ). There was no difference of conclusive RFNA results among K-TIRADS scores ( $p=0.773$ ). The AUS/FLUS subcategory and nodule size was not significantly associated with the diagnostic benefit of RFNA. The false negative rate of benign cytology result of the first RFNA was 1.7%–2.3% according to the criteria of final benign diagnosis.

**CONCLUSION:** The diagnostic benefit of RFNA to obviate unnecessary surgery was found at least 28% in initially diagnosed AUS/FLUS nodules. Therefore, repeated biopsy may be helpful to reduce the unnecessary diagnostic surgery even in AUS/FLUS nodules with high suspicion (K-TIRADS 5) US pattern.

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## Malignancy risk of thyroid nodules with isolated macrocalcification

Wooyul Paik<sup>1</sup>, Hye Yun Gwon<sup>1</sup>, So Jin Yoon<sup>1</sup>, Dong Gyu Na<sup>1</sup>

1. Gangneung Asan Hospital, Gangneung-si, Gangwon-Do, South Korea

**Purpose:** This study was performed to investigate the incidence and malignancy risk of thyroid nodules with isolated macrocalcification.

**Methods:** The isolated macrocalcification was defined as an entirely calcified nodule without any identified solid component on US, in which the assessment of other US characteristics was impossible owing to dense posterior shadowing. From January 2011 to June 2018, a total of 3061 consecutive patients with 3852 thyroid nodules ( $\geq 1$  cm) underwent ultrasonography (US)-guided fine-needle aspiration (FNA). We retrospectively reviewed all US images of those nodules to determine nodules with isolated macrocalcifications. We assessed the incidence, malignancy rate, and the size distribution of thyroid nodules with isolated macrocalcifications. The nodule size was categorized as 3 groups (group 1: 1~1.4 cm, group 2: 1.5~1.9 cm, group 3:  $\geq 2$  cm).

**Results:** Isolated macrocalcification was found in 38 (1.2%) of 3061 patients. Among 38 nodules with isolated macrocalcifications, the final diagnosis was achieved in 30 nodules and seven malignant tumors (6 conventional type and 1 follicular variant type papillary carcinomas) were diagnosed by surgery ( $n=6$ ) and FNA ( $n=1$ ). The malignancy rate of isolated macrocalcification was 23.3% in 30 nodules with final diagnoses and 18.4% in all nodules. The size of isolated macrocalcifications was group 1 ( $n=27$ , 71.1%), group 2 ( $n=8$ , 21%), and group 3 ( $n=3$ , 7.9%). The mean size of malignant tumors was  $1.5 \pm 0.4$  mm and the tumor size was group 1 ( $n=3$ , 43%), group 2 ( $n=3$ , 43%), and group 3 ( $n=1$ , 14%). Among 6 malignant tumors with surgical pathology diagnoses, the extrathyroidal extension was found in 4 (66.7%) and lymph node metastasis in 2 (33.3%) tumors.

**Conclusion:** Thyroid nodule with isolated macrocalcification ( $\geq 1$  cm) was found in 1.2% of our cohort patients and showed an intermediate malignancy risk (at least 18.4%).

## Impact of size threshold on diagnostic performance of US fine-needle aspiration for thyroid malignancy: comparison of three international guidelines

Wooyul Paik<sup>1</sup>, Dong Gyu Na<sup>1</sup>

1. Gangneung Asan Hospital, Gangneung-si, Gangwon-Do, South Korea

### Purpose

- To evaluate the impact of size thresholds on the diagnostic performance of US FNA criteria from KSThR/KTA, ATA, and ACR guidelines in the diagnosis of thyroid malignancy

### Patient

- Jan 2010 ~ May 2011 : 2000 nodules ( $\geq 1$  cm) : Benign (1546) Malignancy (454)

### Result

- Concordance rate and correlation of classified risk categories of nodules and comparison of simulated diagnostic performance with 4 size criteria thresholds for FNA in 3 guidelines

- Concordance rate and Correlation coefficients of risk categories**

KSThR/KTA vs. ATA 87.0% 0.937,  $p < 0.001$   
 KSThR/KTA vs. ACR 78.0% 0.889,  $p < 0.001$   
 ATA vs. ACR 84.0% 0.912,  $p < 0.001$

Size Threshold Criteria	SENS	SPEC	PPV	NPV	ACC	Unnecessary FNA Rate
<b>Criteria 1</b>						
KSThR/ATA	94.5	26.4	27.4	94.2	41.9	56.9**
ATA	93.8	27.9	27.7	93.9	42.9	55.7**
ACR	93.4	37.3	30.4	95.1	50.1	48.5**
<b>Criteria 2</b>						
KSThR/ATA	<b>90.5</b>	<b>43.3</b>	<b>31.9</b>	<b>94.0</b>	<b>54.1</b>	<b>43.8**</b>
ATA	<b>90.5</b>	<b>41.7</b>	<b>31.3</b>	<b>93.8</b>	<b>52.8</b>	<b>45.1**</b>
ACR	<b>90.3</b>	<b>48.4</b>	<b>34.0</b>	<b>94.5</b>	<b>58.0</b>	<b>39.9**</b>
<b>Criteria 3</b>						
KSThR/ATA	76.9*	55.2	33.5	89.0	60.1	34.7**
ATA	78.9*	53.2	33.1	89.5	59.0	36.2**
ACR	75.6*	61.4	36.5	89.5	64.7	29.8**
<b>Criteria 4</b>						
KSThR/ATA	75.6*	63.7	37.9	89.9	66.4	28.1**
ATA	77.5*	62.2	37.6	90.4	65.7	29.2**
ACR	74.7*	67.3	40.2	90.1	69.0	25.3**

- 

Size Threshold Criteria	SENS	SPEC	PPV	NPV	ACC	Unnecessary FNA Rate
Criteria 1	~1.1	~10.9	~3.0	~1.2	~8.2	~8.4
Criteria 2	~0.2	~6.7	~2.7	~0.7	~5.2	~5.2

Criteria 3	~3.3	~8.2	~3.4	~0.5	~5.7	~6.4
Criteria 4	~2.8	~5.1	~2.6	~0.5	~3.3	~1.1

- **Conclusion**
- The difference of diagnostic performance between KSThR/KTA or ATA and ACR guidelines is mainly related to the different size threshold of FNA.
- 2cm threshold for low suspicion nodules will decrease the unnecessary FNA rate more than 10% while maintaining the high sensitivity (> 90%) in both KTA/KSThR and ATA guidelines.

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## The learning curve of robotic thyroid surgery and the avoidance of temporary hypoparathyroidism after total thyroidectomy and concomitant central compartment node dissection: A single surgeon's experience

Jae Hyun Park<sup>1</sup>, Jong Ho Yoon<sup>1</sup>

1. Yonsei University Wonju College of Medicine, 20 Ilsan-ro, Wonju-si, Gangwon-Do, South Korea

**Background** The learning curve of robotic thyroid surgery has been evaluated solely in terms of operation time, and not with respect to surgical complications. The aim of this study was to evaluate the learning curve of robotic thyroid surgery with regard to both operation time and temporary hypoparathyroidism using quantitative statistical analysis.

**Methods** A total of 194 patients who underwent total thyroidectomy and concomitant central compartment node dissection for papillary thyroid carcinoma by a single surgeon between December 2008 and September 2017 were enrolled. The learning curve for operation time was assessed using the cumulative sum (CUSUM) technique, and the number of procedures required to reduce the incidence of temporary hypoparathyroidism to less than 30% was determined using the CUSUM and risk-adjusted CUSUM (RA-CUSUM) techniques. Age, gender, primary tumor size, multifocality, lymph node metastasis, and weight of thyroid gland removed were used as potential risk factors in the RA-CUSUM analysis.

**Results** The learning curve for operation time was divided into three phases: phase 1 (the initial learning period, 1st – 19th cases), phase 2 (the challenging period, 20th – 121st cases), and phase 3 (the competent phase, 122nd – 194th cases). To reduce the incidence of temporary hypoparathyroidism to <30% required 119 cases, and after adjustment for potential risk factors by RA-CUSUM analysis this extended to 173 cases. Parameters associated with surgical completeness, that is, number of lymph nodes retrieved and stimulated thyroglobulin levels, were maintained without variation during the study period.

**Conclusions** Technical proficiency for robotic thyroid surgery with respect to the avoidance of surgical complications probably requires a longer learning period than that required for operation time.

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## The appropriateness of ultrasound imaging for thyroid pathology, the standard of radiology reporting on thyroid nodules and the detection rates of thyroid malignancy: A tertiary centre retrospective audit.

Flavian Joseph<sup>1</sup>, Denis Rubtsov<sup>2</sup>, Peter Davoren<sup>1</sup>

1. Endocrinology, Gold Coast University Hospital, Gold Coast

2. General Medicine, Gold Coast University Hospital, Gold Coast

**OBJECTIVE:** We assessed the number of inappropriate thyroid ultrasound(US) scans performed, the quality of radiology reporting for thyroid nodules and the resultant number of thyroid cancers identified.

**MATERIALS AND METHODS:** Data was collected retrospectively for the period of July 2014 and July 2017. Data for 251 patients who had thyroid US scans and the final 201 patients with thyroid nodules were evaluated using descriptive statistics. Indications for thyroid US imaging amongst referring clinicians were assessed. We also compared radiology reporting practices of thyroid nodules to the published 2009 and 2015 American Thyroid Association(ATA) guidelines.

**RESULTS:** There were 50.2% of patients with initial thyroid US imaging deemed outside of expert recommendations including 46% of cases for hypothyroidism, 39.7% for hyperthyroidism and 14.3% for neck pain. Definite recommendation whether to further evaluate thyroid nodules were provided in 44.8% of radiology reports. There were no radiology reports that described thyroid nodules findings based on patterns as recommended by the 2015 ATA guidelines. Two cases of thyroid cancer were detected including one patient with a previous history of thyroid cancer and one patient with hypothyroidism.

**CONCLUSION:** Routine use of US thyroid imaging outside expert recommendation is common. There is lack of standardised reporting when assessing thyroid nodules on US. The appropriate utilisation of US imaging when investigating thyroid pathology and systematic reporting according to the 2017 guidelines published by the American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS) may reduce unnecessary investigations for thyroid nodules in the future.

## Celastrol inhibits the proliferation of thyroid cancer cells

**Shibiao Sang<sup>1</sup>, Jiandong Bao<sup>2</sup>, Huixin Yu<sup>2</sup>, Shengming Deng<sup>1</sup>**

1. *The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China*

2. *Jiangsu Institute of Nuclear Medicine, Key Laboratory of Nuclear Medicine, Ministry of Health, Wuxi, Jiangsu, China*

Celastrol (*Tripterygium wilfordii*) has been reported to play an important role in anti-cancer tumorigenesis such as colon cancer. However, the mechanism of its role in thyroid cancer remains unclear. The aim of this study was to investigate the killing effect and underlying mechanisms of celastrol in different types of thyroid cancer cell lines. After the treatment of celastrol at different concentrations (1, 5 and 10  $\mu$ M) for 24 h, the cell shape of papillary carcinoma cell BCPAP and anaplastic carcinoma cell 8505C cells turned round at a low concentration of 1  $\mu$ M, while follicular carcinoma cell FTC-133 showed morphological changes at 5 and 10  $\mu$ M of celastrol treatment for 24 h. Then  $IC_{50}$  values of BCPAP, FTC-133 and 8505C cells were determined by MTT assays to further determine the growth-inhibition effect of celastrol. The  $IC_{50}$  values of BCPAP, FTC-133 and 8505C cells were 4.7, 8.7 and 5.9  $\mu$ M, respectively, indicating that celastrol had a good killing effect on different kinds of thyroid cancer cells. We selected BCPAP cells to further explore the mechanism of celastrol in thyroid carcinoma. The proportion of JC-1 monomer, a sensitive marker of mitochondrial membrane potential, increased from 10.3% to 27.1% and 36.2% after celastrol (2.5 and 5 M) treatment for 24 h in BCPAP cells. PDHA2 is a member of the Pyruvate dehydrogenase complex (PDHc), an important rate-limiting enzyme family involved in mitochondrial metabolic glycolysis to the tricarboxylic acid (TCA) cycle. After 24 h of treatment with celastrol (1.25, 2.5 and 5 M) on BCPAP cells, the protein expression of PDHA2 decreased significantly, suggesting that celastrol may kill thyroid cancer cells through targeting PDHA2. Altogether, these findings elucidated the potential anti-tumor mechanisms of celastrol and shed a light on a prospective therapeutic target for thyroid cancer treatment.

## Association between TSH level after total thyroidectomy and hypercholesterolemia in patients with differentiated thyroid cancer

**Dong Yeob Shin<sup>1</sup>, Eun Jig Lee<sup>1</sup>**

1. *Yonsei University College of Medicine, Seoul, South Korea*

**Background:** TSH suppression below the reference range is not currently recommended for postoperative patients with differentiated thyroid cancer (DTC) at very low risk for recurrence. However, higher TSH levels may be associated with insufficient levothyroxine replacement and subsequent metabolic derangements such as dyslipidemia.

**Methods:** We reviewed 1092 women with DTC, who underwent total thyroidectomy at ages 19-79 years and were followed up with lipoprotein profiles at 1-4 years after the surgery. Postoperative changes in cholesterol levels were investigated according to the postoperative TSH levels. Multinomial multivariable logistic regression analyses were performed to assess the risks for dyslipidemia according to the TSH levels with adjustment for potential confounders including age, follow-up period, preoperative total cholesterol (TC), and body mass index.

**Results:** Preoperative to follow-up changes of TC were  $-3.69$  mg/dL ( $P=0.006$ ),  $+0.13$  mg/dL ( $P=0.926$ ),  $+12.46$  mg/dL ( $P<0.001$ ), and  $+16.46$  mg/dL ( $P<0.001$ ) in TSH  $<0.03$ ,  $0.03-0.3$ ,  $0.3-2.0$ , and  $2.0-5.0$  mIU/L groups. Compared with TSH  $0.03-0.3$  mIU/L, TSH  $0.3-2.0$  mIU/L was associated with hypercholesterolemia (Adjusted odds ratios [AOR] = 1.86 and 5.08 for TC  $200-240$  and  $\geq 240$  vs.  $<200$  mg/dL, both  $P<0.01$ ) and hyper-LDL-cholesterolemia (AOR = 2.76 for LDL cholesterol  $\geq 160$  vs.  $<130$  mg/dL,  $P = 0.012$ ). Moreover, TSH  $2.0-5.0$  mIU/dL was associated with higher risks for hypercholesterolemia (AOR = 2.85 and 6.95 for TC  $200-240$  and  $\geq 240$  mg/dL vs.  $<200$  mg/dL, both  $P<0.01$ ) and hyper-LDL-cholesterolemia (AOR = 2.08 and 4.17 for LDL cholesterol  $130-159$  and  $\geq 160$  mg/dL vs.  $<130$  mg/dL, both  $P<0.05$ ).

**Conclusions:** TC increased when thyroidectomized female DTC patients kept TSH levels within normal range. Thus, these patients had higher risks for hypercholesterolemia and hyper-LDL-cholesterolemia. Risks for metabolic derangements by insufficient levothyroxine replacement should be considered when the less-intense TSH suppression is adopted in postoperative DTC patients.

## Estimating the growth rate of lung metastases in differentiated thyroid carcinoma: Response Evaluation Criteria in Solid Tumors or doubling time?

**Eyun Song<sup>1</sup>, Jonghwa Ahn<sup>1</sup>, Min Ji Jeon<sup>1</sup>, Sang Min Lee<sup>1</sup>, Jeong Hyun Lee<sup>1</sup>, Tae Yong Kim<sup>1</sup>, Jung Hwan Baek<sup>1</sup>, Won Bae Kim<sup>1</sup>, Young Kee Shong<sup>1</sup>, Won Gu Kim<sup>1</sup>**

1. *Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*

**Background:** Estimating the growth rate of lung metastases in patients with metastases of differentiated thyroid carcinoma (DTC) is important as their clinical courses are associated with the progression of the lung metastases. This study aimed to evaluate survival outcomes using different criteria for estimating the tumor growth rate.

**Methods:** Patients with  $\geq 1$ cm lung metastases of DTC who underwent total thyroidectomy and high-dose radioactive iodine therapy were enrolled. The time to progressive disease (PD) by Response Evaluation Criteria in Solid Tumors (RECIST), tumor volume doubling time (TVDT) of the two dominant target lung lesions, and thyroglobulin doubling time (TgDT) were measured in each patient, and their association with disease-specific survival (DSS) was evaluated.

**Results:** Forty-four patients with target lung metastatic nodules with an initial maximal diameter of 1.3 cm (median) were followed up for a median of 6.8 years after the diagnosis of lung metastases. Based on RECIST, 12 patients (27.3%) showed fast tumor progression, with time to PD <1 year. When assessed by TVDT, 9 patients (20.5%) had TVDT  $\leq$ 1 year, showing rapid tumor progression. Seven of 33 patients (21.2%) who were negative for thyroglobulin antibody had TgDT <1 year. Growth rates assessed by all three criteria were significantly associated with DSS. However, TVDT had the highest predictive value for DSS, with a proportion of variation explained of 34.3%. Five-year DSS was 29.6% in patients with TVDT  $\leq$ 1 year, 50.0% in patients with time to PD <1 year, and 42.9% in patients with TgDT <1 year.

**Conclusions:** TVDT was the most powerful for predicting DSS, in comparison with RECIST and TgDT. Performing at least three serial chest computed tomography scans during the first year from the diagnosis of lung metastases can assist in early detection of patients with rapid tumor progression and provide objective guidance for initiation of systemic therapy.

## Role of FDG PETCT WB imaging in followup evaluation of Medullary thyroid carcinoma patients with high serum Calcitonin levels

SHANMUGA SUNDARAM<sup>1</sup>, Padma Subramanyam<sup>1</sup>

1. Amrita Institute Of Medical Sciences, Cochin, Kerala, India

AIM:

The aim of this study was to evaluate the value of <sup>18</sup>F fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PETCT) in restaging of medullary thyroid carcinoma (MTC) patients with rising calcitonin levels.

### METHODS:

30 patients (M: F = 11: 19, age 42 + 14 yrs) of treated MTC with high calcitonin levels scheduled for whole body FDG PETCT for restaging. Clinical examination was negative in all pts. 5 pts had suspected lymph nodes on neck ultrasound. Records were analysed with imaging findings. Patients undergoing nodal excision had histological proof as the gold standard in the confirmation of <sup>18</sup>F-FDG PETCT results. Patients were followed up for 12 months to look for persisting disease.

### RESULTS:

20/30 (66%) pts had positive FDG PETCT findings. FDG avid sites noted. 2/20 pts had disease in thyroid bed, 8/20 pts had cervical & or mediastinal lymph nodes, 5 pts had distant (lung, liver, skeletal) lesions while remaining 5 patients had both nodal, distant lesions. Histological confirmation was available in 10 pts with locoregional disease on PETCT by surgical excision or FNAC. FDG PETCT imaging yield was higher in patients with higher calcitonin levels (> 150 pg/ml), sensitivity was calculated as 90%. Mean SUV Max of metastatic nodal disease was  $5.12 \pm 1.17$  and  $3.8 \pm 1.02$ , respectively, the difference between the two groups was not statistically significant ( $P > 0.05$ ).

### CONCLUSION:

PETCT plays an important role in the evaluation of recurrent MTC, especially in patients with high calcitonin levels. Although the series is small, our study shows that PETCT is instrumental in further decision making.

## Does a whole body 18F FDG PETCT imaging have an incremental value in differentiated thyroid cancer patients with TENIS syndrome ?

PADMA SUBRAMANYAM<sup>1</sup>, Shanmuga Sundaram<sup>1</sup>

1. Amrita Institute Of Medical Sciences, Cochin, Kerala, India

### BACKGROUND:

Treated cases of Differentiated thyroid cancer(DTC) with Thyroglobulin(Tg) elevated, negative whole body I131 scan (TENIS) needs further evaluation.

### OBJECTIVE:

To determine if 18FDG PETCT WB imaging is useful in predicting occult disease in DTC patients with TENIS.

### METHODS:

51 treated DTC patients (post thyroidectomy, I131 therapy) having negative WB I131 scan but elevated stimulated Tg (> 10 ng/ml) were enrolled for 18FDG PETCT (TSH >30uIU/ml). Based on PET findings, patients had empirical I131 therapy/TKI or surgical exploration. Patients were followed up for 12 months. Correlation between FDG PETCT and Tg levels done and a threshold for Tg above which highest predictive value of PETCT determined.

### RESULTS:

Patient age, sex, histology, tumour size, extrathyroid extension, focality, N stage correlated and found to have not significantly associated with positive FDG PETCT results ( $P > 0.05$ ). AntiTgAb levels found to be statistically significant in predicting positive PET findings ( $P < 0.05$ ). Compared with TgAb level <150 IU/mL, univariate regression analysis showed that Odd Ratio value of TgAb level  $\geq$  150 IU/mL at diagnosis and span for progressively increased TgAb level were as much as 4.18 [CI:1.52–11.54] and 3.60 [CI:1.24–10.41] times for progressively increased TgAb level.

A threshold Tg value of > 25ng/ml found to predict highest PET positive disease burden

PET positive loco regional recurrence in 16%, pulmonary parenchymal lesions in 34 % & 3% skeletal lesions were found. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of PET to predict recurrence at follow-up were 68.8, 78.3, 86.8, 54.5 and 71.9%, respectively. Sensitivity, accuracy and PPV of PET increased with increasing Tg levels.

### CONCLUSION:

Our study shows that FDG PETCT is incremental in identification and predicting recurrence in DTC with TENIS syndrome. Predictive value of PET was highest at Tg > 25ng/ml with sensitivity of 76.2%, specificity of 100% to detect recurrence.

## A rare case of de-differentiation from papillary thyroid carcinoma to squamous cell carcinoma in an elderly patient – Predicting the unpredictable of thyroid cancer

**Yotsapon Dr. Thewjitcharoen<sup>1</sup>, Sirinate Dr. Krittiyawong<sup>1</sup>, Siriwan Ms. Butadej<sup>1</sup>, Soontaree Ms. Nakasatien<sup>1</sup>, Somsong Ms. Polchart<sup>1</sup>, Pairoj Asst.Prof. Junyangdikul<sup>2</sup>, Auchai Prof. Kanchanapituk<sup>1</sup>, Thep Prof. Himathongkam<sup>1</sup>**

1. Theptarin Hospital, Klongtoey, Bangkok, Thailand

2. Division of anatomical and clinical pathology, Samitivej Srinakarin Hospital, Bangkok, Thailand

**Background:** The unpredictability of thyroid cancer can be striking, as the disease may rapidly progress to death in some individuals. Herein, we reported a rare case of aggressive papillary thyroid cell carcinoma (PTC) in an elderly patient de-differentiated into squamous cell carcinoma (SCC).

**Clinical Case:** We describe a case of a 79-year-old Thai woman with a history of papillary thyroid carcinoma at right side of thyroid 3 years ago. The patient underwent total thyroidectomy at the initial hospital and received a high dose of radioactive iodine treatment at our hospital 1 month following the surgery and then lost to follow-up. Two years later she came back again with new development of right solid-cystic neck mass which was found to be recurrent PTC. Radical neck dissection was done and the second high dose of radioactive iodine treatment was given. However, she developed recurrent mass with tenderness at the site above previous solid cystic mass 6 months later. Re-exploration of neck mass revealed inflammatory midline mass 2 cm with enlargement of right lateral cervical lymph nodes. The histopathological examination of midline neck mass showed poorly differentiated SCC with lymphatic invasion. The intermingling of two morphologically distinct tumors typical PTC and poorly differentiated SCC had been identified in 1 out of 14 excised cervical lymph nodes. Immunohistochemistry revealed diffuse CK5/6, P63, and PAX8 positivity in the squamous cells suggesting a transformation process, and not a collision tumor. The patient decided to watchful waiting without further treatments. She is still in stable condition at 24 months later.

**Conclusion:** This case clearly demonstrated that SCC transformed from a pre-existing PTC. The clinician should consider a possible transformation of papillary thyroid cancer into more aggressive histological types in elderly patients who present with rapidly progressive clinical behavior. However, long-term survival can be expected for some patients.

## Hyperthyroidism due to metastatic T3-secreting follicular thyroid carcinoma: A case report and literature review

**Annabelle M Warren<sup>1</sup>, Mark Pace<sup>1</sup>, Suzanne Silberberg<sup>2</sup>, Leon Bach<sup>1</sup>, Duncan Topliss<sup>1</sup>**

1. The Alfred Hospital, Melbourne, VIC, Australia

2. Unaffiliated, Melbourne, VIC, Australia

**Background:** The identification of hyperthyroidism during work up of a thyroid nodule is generally regarded as reassuring for the presence of thyroid cancer.

**Case:** A 66-year-old woman with a longstanding multinodular goitre developed mild T3-toxicosis with TSH <0.01 mU/L (0.5-5.5mU/L), free T4 21.5 pmol/L (11.0-22.0 pmol/L) and free T3 7.3pmol/L (3.1-6.4pmol/L). Her goitre had enlarged and she had lost weight but she had no other symptoms or signs of hyperthyroidism.

Thyroid ultrasound revealed three 50-60 mm thyroid nodules, solid, isoechoic, smooth margins, wider-than-tall, with macrocalcification (TIRADS 4). A thyroid <sup>99m</sup>Tc radionuclide scan unexpectedly showed absent uptake.

She underwent total thyroidectomy. Histopathology revealed a 65 mm widely invasive follicular thyroid carcinoma with extensive capsular and vascular invasion. Repeat thyroid function 6 weeks post-operatively demonstrated worsening T3-toxicosis with TSH <0.01mU/L (0.5-5.5 mU/L), free T4 21.5 pmol/L (11.0-22.0 pmol/L) and free T3 12.8pmol/L (3.1-6.4 pmol/L). The patient developed anxiety and tachycardia. Levothyroxine therapy was ceased. Unstimulated serum thyroglobulin level was 23,902ug/L. Positron emission tomography and radioactive iodine (RAI) whole body scans demonstrated extensive iodine-avid and non-avid nodal, lung, and skeletal metastatic disease. Following administration of RAI, T3 levels declined and levothyroxine was recommenced.

**Discussion:** Thyrotoxicosis caused by thyroid hormone-producing functional metastatic thyroid cancer is rare, with 54 cases reported from 1946-2019 (1-4). Functional status is associated with widely metastatic disease, follicular thyroid carcinoma, prior structural thyroid abnormalities (e.g. multinodular goitre), higher serum thyroglobulin levels, and bony metastases more commonly than lung metastases (1, 5). By their nature, functioning metastases are sensitive to radioiodine. Several pathophysiological mechanisms of thyrotoxicosis caused by functional thyroid cancer are described (2, 6, 7).

**Conclusion:** Hyperthyroidism due to thyroid cancer is rare, but the presence of clinical or biochemical hyperthyroidism should not exclude the diagnostic possibility of thyroid cancer.

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## Influence of tumor size and Eastern Cooperative Oncology Group performance status (ECOG PS) at baseline on patient outcomes in lenvatinib-treated radioiodine-refractory differentiated thyroid cancer (RR-DTC)

Lori J. Wirth<sup>1</sup>, Sophie Leboulleux<sup>2</sup>, Naomi Kiyota<sup>3</sup>, Makoto Tahara<sup>4</sup>, Kei Muro<sup>5</sup>, Myung-Ju Ahn<sup>6</sup>, Yuichi Ando<sup>7</sup>, Matthew H. Taylor<sup>8</sup>, Shunji Takahashi<sup>9</sup>, Sung-Bae Kim<sup>10</sup>, Bruce Robinson<sup>11</sup>, Soamnauth Misir<sup>12</sup>, Corina E. Dutcus<sup>12</sup>, Ran Xie<sup>12</sup>, Prashant Joshi<sup>13</sup>, Brett G.M. Hughes<sup>14</sup>, Javier Aller<sup>15</sup>, Monika Krzyzanowska<sup>16</sup>, Jaume Capdevila<sup>17</sup>

1. Department of Medicine, Massachusetts General Hospital Cancer Center, Boston, Massachusetts, USA

2. Department of Nuclear Medicine, Gustave Roussy, Villejuif, France

3. Department of Medical Oncology and Hematology and Cancer Center, Kobe University Hospital, Kusunoki Cho, Chuo-ku, Japan

4. Division of Head and Neck Medical Oncology, National Cancer Center Hospital East, Kashiwa, Japan

5. Department of Clinical Oncology, Aichi Cancer Center Hospital, Nagoya, Japan

6. Department of Medicine, Samsung Medical Center Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

7. Department of Clinical Oncology and Chemotherapy, Nagoya University Hospital, Nagoya, Japan

8. Oregon Health & Science University, Portland, Oregon, USA

9. Department of Medical Oncology, The Cancer Institute Hospital of JFCR, Tokyo, Japan

10. Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

11. Cancer Genetics Laboratory, Kolling Institute of Medical Research, Sydney, Australia

12. Eisai Inc., Woodcliff Lake, New Jersey, USA

13. Formerly of Eisai Inc., Woodcliff Lake, New Jersey, USA

14. Cancer Care Services, The Royal Brisbane and Women's Hospital, University of Queensland, Queensland, Australia

15. Department of Endocrinology, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain

16. Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada

17. Medical Oncology Department, University Hospital Vall d'Hebron, Barcelona, Spain

### Background:

In SELECT, lenvatinib significantly improved progression-free survival (PFS) of patients with RR-DTC versus placebo (18.3 vs 3.6 months; hazard ratio [HR]: 0.21 [99% CI: 0.14, 0.31];  $P < 0.001$ ). Here we examine the treatment of RR-DTC with lenvatinib in relation to tumor size (sum of all targeted lesions) and ECOG PS.

### Methods:

In this post hoc analysis of SELECT with patients randomized to receive lenvatinib, Kaplan-Meier estimates of time to ECOG PS  $\geq 2$  were calculated for subgroups of patients according to baseline ECOG PS or tumor size. Objective response rate (ORR) and Kaplan-Meier estimates of overall survival (OS) and PFS according to ECOG PS (0 or 1) at baseline were calculated. Percentage change from baseline to postbaseline nadir in the sum of diameters of target lesions and percentage change over time according to ECOG PS at baseline (0 or 1) were assessed.

### Results:

Patients with ECOG PS 0 or 1 at baseline had similar demographic and disease characteristics. ORR was 78.5% and 51.0% for patients with ECOG PS 0 and 1 at baseline, respectively (odds ratio [95% CI]: 3.508 [2.018, 6.097]). Mean maximum percent decrease in tumor size was greater in patients with baseline ECOG PS 0 (-46.13%) versus patients with ECOG PS 1 (-37.16%). For patients with ECOG PS 1 at baseline, time to ECOG PS  $\geq 2$  was numerically shorter with tumor size  $> 60$  mm versus tumor size  $\leq 60$  mm (HR [95% CI]: 1.450 [0.708, 2.967]). Additional results are summarized in the **table**.

### Conclusions:

Among patients with RR-DTC, PFS, OS, ORR, and time to ECOG  $\geq 2$  were generally better for patients with lower ECOG PS or smaller tumor size at baseline. These results may indicate that it is beneficial to start lenvatinib in patients with RR-DTC early, before ECOG PS worsens and tumor size increases.

**Clinical trial information:** NCT01321554



Table

	Baseline population	Baseline ECOG PS	n	HR (95% CI)		
Time to ECOG PS ≥ 2	Tumor size*	≤ 35 mm	0 1	46 19	0.328 (0.114, 0.941)	
		> 35 – ≤ 60 mm	0 1	46 25	0.344 (0.092, 1.287)	
		> 60 – ≤ 92 mm	0 1	26 33	0.269 (0.088, 0.820)	
		> 92 mm	0 1	26 27	0.253 (0.051, 1.257)	
	Baseline population	Baseline ECOG PS	n	Median (months)	HR (95% CI)	
OS	ECOG PS 0 or 1	0 1	144 104	NE 22.1	0.420 (0.256, 0.690)	
PFS	ECOG PS 0 or 1	0 1	144 104	NE 12.6	0.523 (0.353, 0.775)	

\*Sum of all targeted lesions at baseline.

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## Diffuse Sclerosing Variant of Papillary Thyroid Carcinoma: Two Case Studies.

Wan-Chen Wu<sup>1</sup>, Min-Shiun Wu<sup>2</sup>, Jin-Ying Lu<sup>1</sup>

1. Internal Medicine, National Taiwan University Hospital, Taipei City, Taiwan

2. General Surgery, National Taiwan University Hospital, Taipei City, Taiwan

Diffuse sclerosing variant of papillary thyroid carcinoma (DSVPTC) is a rare variant, and the clinical characteristics were continuously debated. Here we described the clinical and sonographic characteristics of two cases.

The 17-year-old girl presented with neck swelling for one month, which was associated with mild dysphagia. Ultrasound showed bilateral multinodular goiter (MNG) and multiple cervical lymphadenopathies (LAPs). Blood test showed euthyroid with high anti-thyroid autoantibodies (ATA). The fine needle aspiration cytology (FNAC) showed positive for malignant cells. She received radical thyroidectomy and neck lymph node dissection (LND), and the pathology showed DSVPTC with multiple LN metastases, pT3N1b/cM0, stage I. 150mCi radioactive iodine (RAI) therapy was given, and the whole body scan (WBS) showed no residual functioning thyroid tissue and probably treatment response with minimal residual RAI-avid nodal metastasis at right thoracic inlet. There was no local recurrence or distant metastasis till 3-year follow-up.

Left neck mass was noted in health checkup in a 22-year-old male student. Blood test showed euthyroid and negative ATA. Ultrasound showed bilateral MNG and multiple cervical LAPs. The FNAC confirmed positive for malignant cells. He received radical thyroidectomy and neck LND, and the pathology showed DSVPTC with lymph node metastases, pT3N1b/cM0, stage I. He received 150mCi RAI therapy, and WBS showed probable RAI-avid malignancies at left cervical and upper mediastinal nodes. Local recurrent at left neck lymph nodes was noted one year later. He received neck LND again, and received another 150mCi RAI therapy. There was no local recurrence or distant metastasis till 4-year follow-up.

Both cases are diagnosed at young age. Both ultrasonography were characterized with diffusely involvement with scattered micro-calcification and multiple neck LAPs. Both pathology were characterized by diffusely involvement of bilateral thyroid with extensive squamous metaplasia, diffuse fibrosis, abundant lymphocytic infiltration and psammoma bodies, and positive extrathyroid extension and extranodal extension.

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## Effectiveness of retinoic acid redifferentiation therapy for radioiodine-refractory differentiated thyroid cancer based on 4 response-to-therapy categories

Joon-Kee Yoon<sup>1</sup>, Young-Sil An<sup>1</sup>, Su-Jin Lee<sup>1</sup>, Il-Hyun Kim<sup>1</sup>, Yoon-Sok Chung<sup>1</sup>, Jeonghun Lee<sup>1</sup>, Eu Young So<sup>1</sup>

1. Ajou University School of Medicine, Suwon, Kyeonggi-Do, South Korea

### OBJECTIVE

The aim of this study was to determine the effectiveness of retinoic acid redifferentiation therapy (RAT)-induced radioiodine therapy (RIT) for radioiodine-refractory thyroid cancer based on the 4 response-to-therapy categories.

### METHODS

A total of 20 patients (16 papillary, 4 follicular) with radioiodine-refractory thyroid cancers underwent 31 high-dose RITs after receiving 1.0-1.5 mg/kg retinoic acid for 6 weeks. Serum thyroglobulin levels were measured before and at the end of RAT, and 9 months after RIT. CT (chest or head/neck), whole body radioiodine scintigraphy, F-18 FDG PET/CT or spine MRI was performed to evaluate structural lesions.

### RESULTS

Fourteen patients (70%) had N1b lymph node metastasis, while 60% patients had distant metastases (10 lungs, 3 bones). Mean thyroglobulin level before RAT was 677 (13-4794) ng/mL. Mean cumulative radioiodine dose before RAT was 779 (100-2300) mCi, while mean radioiodine dose with RAT was 252 (150-400) mCi. Using 30% and 50% decrease as cutoffs for thyroglobulin level, 41.9% and 25.8% patients showed good response after RAT-induced RIT, respectively. Before RAT, there were 21 structural incomplete and 10 biochemical incomplete responses by 4 response-to-therapy categories, which changed

to 18 structural incomplete, 9 biochemical incomplete, 1 indeterminate and 3 excellent responses. All follicular thyroid cancer patients showed poor response. At the end of RAT, thyroglobulin level decreased in 57% (16/28) cases, of these, 63% (10/16) showed good response (> 30%) after RIT. Meanwhile, those with increased thyroglobulin level, 66% (8/12) showed poor response ( $p < 0.001$ ). Post-therapeutic whole body scintigraphy did not predict the response of RAT-induced RIT.

#### CONCLUSION

RAT-induced RIT changed response categories in some patients with radioiodine-refractory thyroid cancer. The decrease in thyroglobulin level at the end of RAT could predict the response of RAT-induced RIT.

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### Periprosthetic joint infection caused by Gram-positive versus Gram-negative bacteria, exerts adverse osteoclast-mediated effects on the bone: the possible role of thyroid stimulating hormone

Mei-Feng Chen<sup>1</sup>, Chih-Hsiang Chang<sup>2</sup>, Steve W. N. Ueng<sup>2</sup>, Yuhan Chang<sup>2</sup>

1. Bone and Joint Research Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan

2. Department of Orthopaedic Surgery, Chang Gung Memorial Hospital, Taoyuan, Taiwan

Periprosthetic joint infection (PJI) – the most common cause of knee arthroplasty failure – may result from Gram-positive (GP) or Gram-negative (GN) bacterial infections. The question as to whether PJI due to GP or GN bacteria can lead to different rates of aseptic loosening after reimplantation remains open. We sought to investigate this issue in a retrospective review of prospectively collected data obtained from 320 patients with bacterial PJI. The results revealed that, compared with GP infections, GN infections were associated with an increased risk of aseptic loosening. In order to shed more light on this phenomenon, mice underwent intrafemoral injection of lipopolysaccharide (LPS) from GN bacteria or lipoteichoic acid (LTA) from GP bacteria. We demonstrate that LPS – but not LTA – reduced both the number of trabeculae and bone mineral density. In addition, LPS-treated mice exhibited a reduced body weight, higher serum osteocalcin levels, and an increased number of osteoclasts. LPS accelerated monocyte differentiation into osteoclast-like cells, whereas LTA did not. Finally, ibudilast – a toll-like receptor (TLR)-4 antagonist – was found to inhibit LPS-induced bone loss and osteoclast activation in mice. Taken together, our data indicate that PJI caused by GN bacteria portends a higher risk of aseptic loosening after reimplantation – mainly because of LPS-mediated effects on osteoclast differentiation. On the other hand, recent research demonstrates that thyroid stimulating hormone (TSH) reduces osteoclastogenesis by TSH receptor G-protein-coupled receptor. Therefore, our next ongoing research is to explore the regulatory relationship between thyroid-stimulating hormone, TLR4, and osteoclast activation. Our findings may pave the way towards the development of new therapeutic strategies for PJI.

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### Overt hyperthyroidism and insulin resistance: a mini-systematic review

Wismandari Wisnu<sup>1</sup>, Leonardus Hidayat<sup>2</sup>, Jessica Marsigit<sup>2</sup>

1. Metabolic-Endocrinology Division, Internal Medicine Department, Cipto Mangunkusumo Hospital, Jakarta, DKI Jakarta, Indonesia

2. Faculty of Medicine, Universitas Indonesia, Central Jakarta, DKI JAKARTA, Indonesia

**Background:** Hyperthyroidism remains as one of the endocrinology disorders that have broad effect through the patient's body system. Recent evidence shows that hyperthyroidism could lead to impairment of insulin activity.

**Objective:** This mini systematic review addresses the correlation between overt hyperthyroidism that could lead into insulin resistance state.

**Methods:** Literature searching through four databases, which are PubMed®, Cochrane Library®, PROQUEST®, and SCOPUS® was performed. Articles were appraised for validity, importance, and applicability.

**Results:** There were six studies met the criteria before critical appraisal is conducted. Five of six studies showed significant linear correlation between overt hyperthyroidism and insulin resistance. Two studies also showed that overt hyperthyroidism altered insulin sensitivity in post-prandial state. Each of studies also studied any components which suggestively contributed to insulin resistance developing, i.e. glucagon and ghrelin hormone, glucose intolerance (as performed by OGTT)

**Conclusion:** Insulin resistance is commonly developed in patients with overt hyperthyroidism, either in fasting or postprandial state. Pathogenesis-related to development of insulin resistance as a consequence of overt hyperthyroidism should be determined briefly as there are many pathways have their role contributed to this condition.

## Involvement of somatic copy-number gains with the tumorigenesis of Thyrotropin-secreting pituitary adenomas.

Kazuhiko Horiguchi<sup>1</sup>, Kazuma Saito<sup>1</sup>, Masayuki Yoshioka<sup>1</sup>, Tetsuya Takamizawa<sup>1</sup>, Takashi Okamura<sup>1</sup>, Emi Ishida<sup>1</sup>, Shunichi Matsumoto<sup>1</sup>, Satoshi Yoshino<sup>1</sup>, Yasuyo Nakajima<sup>1</sup>, Eijiro Yamada<sup>1</sup>, Tsugumichi Saito<sup>1</sup>, Atsushi Ozawa<sup>1</sup>, Masahiko Tosaka<sup>2</sup>, Shozo Yamada<sup>3,4</sup>, Masanobu Yamada<sup>1</sup>, **Kazuhiko Horiguchi<sup>1</sup>**

1. Division of Endocrinology and Metabolism, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan
2. Department of Neurosurgery, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan
3. Department of Hypothalamic and Pituitary Surgery, Toranomon Hospital, Minato-KU, Tokyo, Japan
4. Moriyama neurological center hospital, Edogawa-Ku, Tokyo, Japan

**Objectives:** We have recently reported an analysis of genetic abnormalities in TSHomas using next-generation sequencer (J Clin Endocrinol Metab. 102:566-575, 2017). We found six somatic DNA variants as candidate driver mutations, but no variants were so far recurrent. However, SNP array analysis revealed multiple somatic focal and chromosomal arm-length copy-number abnormalities in 8 cases of TSHomas. The objective of this study is to investigate the involvement of this copy-number abnormalities with the tumorigenesis of TSHomas.

**Methods:** We performed a Single Nucleotide Polymorphism (SNP) array analysis of tumor DNA extracted from 12 TSHomas and 12 non-functional pituitary adenomas (NFPAs). SNP array analysis were also performed for DNA extracted from peripheral blood leukocytes of patients with 8 TSHomas. In addition, cDNA microarray analyses were performed using total RNA isolated from 4 TSHomas and 4 NFPAs.

**Results:** We observed that 75.0 % (9/12) of TSHoma samples were involved in at least one gain, copy neutral LOH (cnLOH) and loss event. In these copy-number abnormalities, copy number gain were fairly common compared to copy number loss, and chromosomal arm-length gain was found to be most frequent on chromosomes 4, 5, 7, 9 and 19. All copy-number changes examined were somatic changes, because no changes were found in blood samples. In contrast, only few focal copy number abnormalities were found in NFPAs, although only one case had a chromosomal arm-length loss. In TSHomas having copy number gain, the expression levels of all gene included in copy number gain region were not changed compared to the expression levels of the same genes without copy number gain. Microarray analysis revealed that the expression levels of the genes involved with chromosome segregation in TSHomas were not differed from those in NFPAs.

**Conclusion:** Somatic copy-number gains of chromosomes may be involved in the tumorigenesis of TSHomas.

## Clinical Profiles of Thyroglossal Duct Cyst in Adults: A 20-year Experience from Thyroid Clinic at Theptarin Hospital

**Yotsapon Dr. Thewjitcharoen<sup>1</sup>**, Siriwan Ms. Butadej<sup>1</sup>, Soontaree Ms. Nakasatien<sup>1</sup>, Ekgaluck Dr. Wanothayaroj<sup>1</sup>, Sriurai Dr. Porramatikul<sup>1</sup>, Sirinate Dr. Krittiyawong<sup>1</sup>, Thep Prof. Himathongkam<sup>1</sup>

1. Theptarin Hospital, Klongtoey, Bangkok, Thailand

**Background:** Thyroglossal duct cysts (TGDC) are the most common congenital midline neck mass typically diagnosed in patients before the age of 20; however, they can occur at any age and sometimes develop together with other thyroid disorders. Clinical profiles of adult TGDC are scarce due to uncommon occurrence in adults.

**Material and Method:** A retrospective 20-year (1998-2018) of TGDC was reviewed and analyzed at Theptarin Hospital, one of largest endocrine centers in Bangkok.

**Results:** A total of 28 TGDC patients (male 46.4 %, mean age 43.2±17.3 years, mean age at diagnosis 40.3±19.6 years) were seen in our hospital during study period. The mean cyst size was 3.0±2.3 cm (1.1-14.0 cm) and located in midline observed in 71.4% of patients followed by laterality in the left side in 21.4% of patients. Only 2 patients (7.1%) were presented with a lateral neck mass in the right side. Misdiagnosis of TGDC as thyroid nodules was found in 28.6% of patients with the median duration of misdiagnosis for 2.4 years. Concurrent various thyroid disorders were seen in 5 from 28 patients (17.9%). Definitive surgery was offered in only 64.3% of patients but only one patient underwent Sistrunk operation at our center. Two patients chose to treat with percutaneous ethanol injection (PEI) after excluding malignancy. However, only one patient completed the course of PEI treatments (5 times) with mean volume reduction at 70% at 6 months. No malignancy arising from TGDC was observed in our series.

**Conclusion:** Misdiagnosis of TGDC was still common in almost one-third of patients and laterality of TGDC to the left side could be found in one-fourth of adult patients, consistent with other reports. PEI treatment could be an alternative treatment in selected patients after excluding malignancy.

## Protective Effect of Alpha-lipoic acid on Salivary Dysfunction Following Radioiodine Therapy in a Mouse Model

**Junghwa Jung<sup>1</sup>**, Jin Hyun Kim<sup>1</sup>, Seung Hoon Woo<sup>1</sup>

1. Gyeongsang National University, Jinju-si, Gyeongsangnam-Do, South Korea

Radioiodine therapy is known to subject cellular components of salivary glands to oxidative stress leading to salivary gland dysfunction. However, the protective effects of antioxidants on RI-induced SG damage have not been well investigated. The aim of this study was investigated the radioprotective effects of Alpha-lipoic acid (ALA) administered prior to RI therapy in a mouse model of RI sialadenitis. Four-week-old female C57BL/6 mice (n=48) were divided into three groups; a normal control group, a RI-treated group (0.01 mCi/g mouse, orally), and an ALA and RI-treated group. Animals in these groups were divided into 3 subgroups and euthanized at 15, 30, and 90 days post-RI treatment. Salivary flow rates and lag times were measured, and morphologic and histologic examinations and TUNEL assays were performed. Changes in salivary <sup>99m</sup>Tc pertechnetate uptake and excretion were followed by single-photon emission computed tomography. Salivary lag times and flow rates in the RI + ALA group were faster than in the RI only group. There was no significant intergroup difference in the SG weight. The RI + ALA group exhibited more mucin-containing parenchyma and less fibrotic tissues than the RI only group. Salivary aquaporin 5 and myoepithelial cells were protected from radiation damage in the RI + ALA group. Low 8-OHdG and high superoxide dismutase 2 immunoreactivity was detected in the RI + ALA group when compared with the RI only group. Fewer apoptotic cells were observed in the RI + ALA compared to the RI only group in the TUNEL assay. The <sup>99m</sup>Tc pertechnetate excretion level recovered in the ALA group. Pretreatment with ALA before RI therapy is potentially beneficial in protecting against RI-induced salivary dysfunction.

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### Evaluation of two Estrous synchronization protocols for inducing fertile estrus in Achai Cows in Northern Pakistan

Muhammad S Qureshi<sup>1</sup>, Shah M Khan<sup>1</sup>, Hamayun Khan<sup>1</sup>, Younas Muhammad<sup>1</sup>, Umer Sadiq<sup>1</sup>, Ikramullah Khan<sup>1,2</sup>

1. The University of Agriculture, Peshawar, KP, Pakistan

2. Livestock and Dairy Development Department, Bannu, KP, Pakistan

**Objectives:** Achai cattle are found in hilly terrains of northern Pakistan. Accessibility of the veterinary practitioners for insemination of estrous cows at the herds, is difficult. Therefore, this study evaluated the efficiency of two different estrus synchronization protocols for estrus expression and overall conception rate.

**Methods:** Twelve lactating Achai cows (n=12) were divided into two groups with equal numbers and subjected to POP (Presynch Ovsynch) or MSS (Modified 7 day Select Synch) protocol. In POP protocol animals were administered with PGF<sub>2</sub>-α injections on day -38, -24 and -3. GnRH-1 was given on day -10 and 0 followed by TAI 16 hours later. Animals in MSS group were administered with a CIDR insert on day -7 concurrent with PGF<sub>2</sub>-α, GnRH-1 at day -5, removal of CIDR at day 0, concurrent with two doses of PGF<sub>2</sub>-α at 8 hr interval, GnRH-2 on day 3, followed by TAI after 16 hrs.

**Results:** Higher E<sub>2</sub> and lower P<sub>4</sub> concentrations were observed for MSS than POP group. Cows having higher level of E<sub>2</sub> and lower level of P<sub>4</sub> concentrations at the time of estrus, become pregnant (P<0.05). E<sub>2</sub> concentrations varied among pregnant and nonpregnant cows in both protocols (P<0.01). Highest concentration of E<sub>2</sub> was observed in pregnant cows in POP group. The P<sub>4</sub> concentration of pregnant cows in MSS was lowest at the time of insemination followed by pregnant cows in POP group compared to the nonpregnant cows in both the protocols (P<0.05). Maximum cows exhibited estrus in POP than MSS. Conception rate in TAI cows was higher in MSS than POP group. Overall conception rate was higher in MSS than the POP (66.66% vs 33.33; P<0.01).

**Conclusion:** It may be suggested that efficiency of MSS protocol was almost double as compared to POP; for estrus synchronization and conception in Achai cows.

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### Prevalence of thyroid disease in patients surgically treated for pituitary disease

Daham Kim<sup>1</sup>, Yongin Cho<sup>1</sup>, Cheol Ryong Ku<sup>1</sup>, Hyein Jung<sup>1</sup>, Ju Hyung Moon<sup>2</sup>, Eui Hyun Kim<sup>2</sup>, Dong Yeob Shin<sup>1</sup>, Sun Ho Kim<sup>3</sup>, Eun Jig Lee<sup>1</sup>

1. Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea

2. Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Korea

3. Department of Neurosurgery, Ewha Womans University College of Medicine, Seoul, Korea

Thyroid disease mainly has a thyroid origin but can occasionally have a pituitary origin. Clinicians face several challenges when these conditions occur together. We aimed to determine the prevalence of thyroid disorders in patients undergoing transsphenoidal adenomectomy (TSA) for pituitary disease. We reviewed the medical records of patients undergoing TSA for pituitary disease between 2008 and 2017 at Severance Hospital. Thyroid disorders were categorized using blood test results and medical history at the time of preoperative evaluation. Among 2202 patients, 44 (2.0%), 218 (9.9%), and 74 (3.4%) had hyperthyroidism, hypothyroidism, and post-thyroidectomy status before TSA, respectively. Among the 44 patients with hyperthyroidism, 30 (68.2%) had secondary hyperthyroidism. Among the 218 patients with hypothyroidism, 165 (75.7%) had secondary hypothyroidism. Secondary hypothyroidism was more common in patients with adrenocorticotropic hormone-secreting pituitary adenoma (adjusted odds ratio [aOR] 1.85), Rathke's cleft cysts (aOR 2.34), and craniopharyngioma (aOR 2.58) (all p<0.05) than in those with nonfunctioning pituitary adenoma. Contrastingly, thyroid cancer showed increased prevalence in patients with growth hormone- (aOR 3.17), prolactin- (aOR 3.66), and thyroid-stimulating hormone-secreting (aOR 6.28) pituitary adenomas (all p<0.05). Pituitary disease sometimes accompanies thyroid disorders; their characteristics vary according to the type of pituitary disease.

## Association between serum free T4 and anemia in euthyroid adults: A nationwide cross-sectional study

Mijin Kim<sup>1</sup>, Bo Hyun Kim<sup>1</sup>, Hyungi Lee<sup>2</sup>, Min Hee Jang<sup>1</sup>, Jeong Mi Kim<sup>1</sup>, Eun Heui Kim<sup>1</sup>, Yun Kyung Jeon<sup>1</sup>, Sang Soo Kim<sup>1</sup>, In Joo Kim<sup>1</sup>

1. Internal Medicine, Biomedical Research Institute, Pusan National University Hospital, Busan

2. ARO, Clinical Trial Center, Biomedical Research Institute, Pusan National University Hospital, Busan

**Background:** Although thyroid dysfunction is associated with anemia, data on the association between thyroid function and anemia in the euthyroid range are scarce. We aimed to evaluate the association between anemia and serum free T4 (fT4) and thyrotropin (TSH) levels in euthyroid adults.

**Methods:** Data of participants aged  $\geq 19$  years were obtained from the Korea National Health and Nutrition Examination Survey VI (2013–2015), which is a nationwide, population-based study. Anemia was defined as hemoglobin (Hb)  $< 13$  and  $< 12$  g/dL for men and women ( $< 11$  g/dL for pregnant women), respectively.

**Results:** Overall, 5,368 patients were included, of whom 6.2% had anemia, and more women (10.0%) had anemia than men (3.2%;  $P < 0.001$ ). In the multivariate analysis, serum fT4 levels, but not TSH levels, had an independently positive association with serum Hb levels in both men ( $P < 0.001$ ) and women ( $P < 0.001$ ). When we categorized participants into fT4 quartiles, those in the lowest quartile had 4% and 3% lower serum Hb levels than those in the highest quartile among men and women, respectively. In both men and women, serum Hb levels linearly reduced across decreasing serum fT4 quartile groups ( $P < 0.001$  and  $P < 0.001$ , respectively). Participants with low-normal fT4 had 5.6 ( $P < 0.001$ ) and 15.5 times ( $P < 0.001$ ) higher risks for developing anemia than those with high-normal fT4 among men and women, respectively.

**Conclusions:** A low-normal serum fT4 level was associated with a lower serum Hb level and a higher risk of anemia in euthyroid adults. These results suggest that the reference range of fT4 is not optimal with respect to anemia and that avoiding low-normal serum fT4 levels may help avoid anemia in both men and women.

## A significant association of muscle strength with thyroid function in overweight and obese population; a study of the sixth Korea National Health and Nutrition Examination Survey (KNHANES 2014-2015)

Min-Hee Kim<sup>1</sup>, Kwanhoon Jo<sup>1</sup>, Jeongmin Lee<sup>1</sup>, Dong-Jun Lim<sup>1</sup>, Jung-Min Lee<sup>1</sup>, Sang-Ah Jang<sup>1</sup>, Moo-Il Kang<sup>1</sup>

1. The Catholic University of Korea, Seoul, South Korea

### Background

In overt thyroid disease, neuromuscular deficit has been reported. As skeletal muscle is one of main targets of thyroid hormone signaling, an association of thyroid function and muscle strength could be expected. The aim of study is to evaluate the association of free thyroxine (FT4) and thyrotropin (TSH) with muscle strength, measured by hand grip strength, in national representative data.

### Methods

The study utilized the sixth edition of the Korea National Health and Nutrition Examination Survey (KNHANES 2014-2015), which represent general health and nutritional status of Koreans. After exclusion of subjects with age less than 19 years, free T4 level out of normal range, a history of thyroid disease or cerebral disease, restricted activity and incomplete data, a total of 3503 were recruited.

### Results

FT4 positively correlated with muscle strength ( $\beta$  coefficient = -12.84,  $p < 0.001$ ), while TSH did negatively ( $\beta$  coefficient = -0.37,  $p = 0.002$ ). After adjusting for several confounding factor such as age and BMI, statistical significance disappeared. However, subjects with BMI above 23, who were considered as overweight or obese in Asian, a negative correlation of TSH with muscle strength was found in a young age group (19-39 year-old) after adjustment for confounders ( $\beta$  coefficient = -0.56,  $p = 0.021$ ). In an old age group (40 or above 40 year-old), FT4 positively correlated with muscle strength ( $\beta$  coefficient = 3.24,  $p = 0.019$ ).

### Conclusion

In overweight and obese population, a significant association of thyroid function with muscle strength was observed in nationwide representative data. High TSH in a young age group and low FT4 in an old age group could be risk factors for decreased muscle strength.

## Correlation between Thyroid Hormone Replacement Therapy and Lipid Metabolism in Patients Treated with Total Thyroidectomy or Hemithyroidectomy: A Single Center Study.

**Jun Sung Moon<sup>1</sup>, Ji Sung Yoon<sup>1</sup>, Kyu Chang Won<sup>1</sup>, Hyoung Woo Lee<sup>1</sup>**

1. Yeungnam University College of Medicine, Daegu, Gyeongsangbuk-Do, South Korea

### Background

Hypothyroidism is closely related to dyslipidemia. There is still debate as to which patients need hormone replacement therapy (HRT) after hemithyroidectomy (HT), and little is known about the effect of HRT after HT. To address this, we aimed to investigate the effect of HRT in the incidence of dyslipidemia among three groups; TT with HRT (TT+HRT), HT with HRT (HT+HRT) or without HRT (HT-HRT).

### Methods

This was a retrospective cohort study, and 3,057 patients who underwent thyroidectomy at Yeungnam university hospital in 2011-2014 were included. We excluded subjects diagnosed as dyslipidemia, hypothyroidism before surgery and taking lipid-lowering agents. Dyslipidemia was defined as triglyceride  $\geq 200$ mg/dl, low-density-lipoprotein  $\geq 160$ mg/dl, total-cholesterol  $\geq 240$ mg/dl, or high-density-lipoprotein  $\leq 40$ mg/dl. Thyroid-stimulating hormone (TSH) level and lipid profiles were assessed annually for 5 years.

### Results

545 participants were finally enrolled and divided into 3 groups; TT+HRT (n=436), HT +HRT (n=37), and HT-HRT (n=72). The mean age was 52.96 and females were 87.9%. The occurrence rate of dyslipidemia in TT+HRT and HT+HRT was 28.7% (hazard ratio [HR] = 1.389; 95% confidence interval [CI] = 0.825-2.339, p=0.216) and 45.9% (HR = 2.634, 95% CI = 1.329-5.221, p=0.006), as compared with 22.2% in HT-HRT (p-for-trend=0.015). The mean TSH level at the end of follow-up was not significantly different (p=0.410).

### Conclusion

Even TSH level was not different between HT groups, HT+HRT group showed higher dyslipidemia incidence rate than HT-HRT group. These results suggest that surveillance for dyslipidemia may be necessary in patients with HT who need HRT.

## Thyroid hormone negatively regulates the pituitary NR4A1 without direct binding of thyroid hormone receptors on the gene.

**takashi okamura<sup>1</sup>, Yasuyo Nakajima<sup>1</sup>, Emi Ishida<sup>1</sup>, KAZUHIKO HORIGUCHI<sup>1</sup>, Shunichi Matsumoto<sup>1</sup>, Satoshi Yoshino<sup>1</sup>, Atsushi Ozawa<sup>1</sup>, Masanobu Yamada<sup>1</sup>, Takashi Okamura<sup>1</sup>**

1. Division of Endocrinology and Metabolism, Gunma University Graduate School of Medicine, Maebashi

**Objectives :** NR4A1 belongs to a superfamily of orphan nuclear receptors known as immediate-early response genes. We reported the regulation of pituitary *TSH $\beta$*  gene expression in TRH knockout mice by NR4A1, the mRNA level was stimulated by TRH in thyrotrophs. We determined whether *NR4A1* mRNA is regulated by thyroid hormone.

**Methods :** We examined the effects of the thyroid status on pituitary NR4A1 mRNA levels *in vivo*. We investigated whether thyroid hormone stimulated the promoter activity of the NR4A1 gene *in vitro* using CV-1 cells. To identify the region responsible for the T3-mediated suppression of the promoter activity, we generated a series of deletion mutants of the NR4A1 promoter and examined the effects of T3. We determined whether TR $\beta$  binds to the region responsible for the T3-induced suppression of the promoter activity using EMSA and ChIP assay.

**Results :** Pituitary *NR4A1* mRNA levels were decreased to 50% of control levels within 24 hr after single peritoneal injection of T4 in mice. In an *in vitro* experiment, the promoter activity of the *NR4A1* gene was increased by the overexpression of TR $\beta$ , and 10 nM T3 decreased its activity. An examination of a deletion mutant of the promoter region revealed that the region from -27 to +152 bp was responsible for the T3-induced suppression. An EMSA showed the lack of TR $\beta$  binding, whereas a ChIP assay demonstrated the recruitment of TR $\beta$  and a co-repressor, NCoR, in the region -147~+148 bp in the absence of T3, and T3 induced the release of TR $\beta$  and NCoR. Experiments on the overexpression and knockdown of NCoR, and using the mutant TRs supported the involvement of NCoR in the TR-induced stimulation.

**Conclusion :** These results demonstrate that thyroid hormone down-regulated basal *NR4A1* mRNA levels in the pituitary, and the direct binding of TR was not required.

## Complexities and conundrums in a case of type ii amiodarone-induced thyrotoxicosis

**Uyen Pham<sup>1</sup>, Brett Sillars<sup>1</sup>**

1. Sunshine Coast University Hospital, Birtinya, QLD, Australia

Amiodarone remains an important class III antiarrhythmic drug used in the medical treatment of malignant arrhythmias. However, it is associated with various adverse effects including thyroid dysfunction. We present a case of a 52 year old man

who presented with a 2 month history of weight loss, increased lethargy and dyspnoea more than 3 years after commencing amiodarone for recurrent ventricular tachycardia. His medical history was significant for idiopathic cardiomyopathy with ICD and previous stroke. Biochemical testing showed thyrotoxicosis with FT4 99, FT3 23 and TSH <0.05. Antibodies including anti-thyroid peroxidase, anti-thyroglobulin and TSH-receptor antibodies were negative. The patient was diagnosed with likely type II amiodarone-induced thyrotoxicosis and placed on oral corticosteroid therapy. Clinical and biochemical improvement was noted after 3 weeks. However, the patient represented 2 weeks later with acute confusion. Repeat testing revealed slightly worsened thyroid function. Extensive investigations for delirium did not reveal a clear infective cause although 14-3-3 protein was weakly positive on CSF examination. Creutzfeldt- Jakob disease was thought unlikely in the absence of other suggestive clinical features. Autoimmune screening and anti-neuronal antibodies were negative. CT head showed encephalomalacia and gliosis of the bilateral anterolateral superior temporal gyri with no acute pathology. His ICD unfortunately precluded further MR examination. The patient subsequently underwent total thyroidectomy with histopathology showing involuted follicular architecture with atrophy and fibrosis, consistent with type II amiodarone-induced thyrotoxicosis. The question of steroid responsive encephalopathy associated with thyrotoxicosis (SREAT) was raised by another team. This is an uncommon syndrome previously described in patients with Hashimoto's thyroiditis and remains a controversial entity without specific diagnostic markers. Multifactorial delirium in a susceptible brain was considered the most likely cause of the patient's unusual presentation in the setting of high dose corticosteroid therapy. Significant cognitive improvement was noted with gradual reduction in corticosteroid dose.

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## Getting to the heart of hyperthyroidism

**Vicka Poudyal<sup>1</sup>, Anthony Zimmermann<sup>1</sup>, Peak Mann Mah<sup>1</sup>**

*1. Lyell McEwin Hospital, Elizabeth Vale, SA, Australia*

### Introduction

We present a case of hyperthyroxinemia, decompensated idiopathic heart failure, and pituitary adenoma to discuss the diagnostic and management challenges in differentiating between thyroid stimulating hormone (TSH) producing pituitary adenoma and thyroid hormone resistance (RTH).

**Case:** A 58-year-old man with a three-year history of idiopathic non-ischaemic dilated cardiomyopathy with moderate to severe left ventricular failure, was brought into the emergency department with NYHA class IV heart failure. Family history remarkable for brother treated for Graves' disease with radioactive iodine over 20 years ago, subsequent reassessment for elevated TSH while on thyroxine replacement was found to have heterophile antibodies.

Our patient presented frail BMI 18 kg/m<sup>2</sup>, relative tachycardia (85 beats per minute on beta blockade), with a small palpable goitre. Laboratory investigations without iodinated contrast or biotin revealed elevated thyroid hormone levels (free T4: 44 pmol/L, free T3: 9.2 pmol/L) with an inappropriately normal TSH: 3.19 mIU/L (Roche). The result was reproducible on an alternative platform (Siemens), heterophile antibodies studies and dilution test negative. Elevated sex hormone binding globulin: 133 nmol/L (10-45 nmol/L). Alpha subunit: TSH ratio increased to 1.14 (<1). MRI revealed a 5mm right pituitary adenoma. TSH receptor antibody negative and other anterior pituitary hormones normal. Dynamic testing with a T3 suppression test was not appropriate due to his cardiac decompensation. SHBG, alpha subunit: TSH ratio and MRI were suggestive of a diagnosis of thyrotropin-secreting tumor (TSHoma).

Carbimazole was started due to concern that hyperthyroidism may be contributing to his decompensating cardiac failure while waiting for further investigation. Thyrotropin-releasing hormone stimulation test done withholding carbimazole for 10 days and family history both suggestive of RTH.

THRbgene pathogenic mutation (c.1357c>a) was found and he was diagnosed with RTH. Unfortunately, our patient within a day of discharge to a nursing home died suddenly. A limited post-mortem request has been requested.

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## Differences of Blood Cell Count in Different Thyroid Conditions

**Jongky Prajitno<sup>1</sup>, Soebagijo Adi Soelistijo<sup>1</sup>, Hermawan Soesanto<sup>1</sup>, Pradana Zaky Ramadhan<sup>2</sup>**

*1. Faculty of Medicine Airlangga University, Endocrine and Metabolic, Surabaya, East Java, Indonesia*

*2. Faculty of Medicine Airlangga University, Hematologi-Onkologi, Surabaya, East Java, Indonesia*

### Background

The thyroid gland produces thyroid hormones which play an important role in the metabolism and proliferation of blood cells. functional disorders of thyroid gland can cause disruption of thyroid hormone excretion and give a different effect on the components of blood cells, thus causing conditions of anemia, leukopenia, erythrocytosis, and thrombocytopenia. Even in some cases, even though it is very rare to report pancytopenia associated with thyroid gland dysfunction. Effects of thyroid hormones It was also known to affect the RBC index including MCV, MCH and MCHC in this study looking for differences in blood components in various hyper, hypo and normothyroid conditions.

### Purpose

To determines the differences of blood component in various thyroid hormone levels

### Methods

This study was conducted by collecting medical records in the private endocrine outpatient clinic during January-February 2019. Involved total 43 secondary data consisting of 5 (11.6%) male and 38 (88.4%) female. They were diagnosed with a thyroid gland disorder for the first time. Based on Thyroid hormones level, the samples were then classified according to hyperthyroidism, hypothyroidism and normothyroidism

### Results

The average Haemoglobin, platelet, MCH and MCHC values were higher in the hypothyroid group. Whereas for the average Erythrocytes was higher in the hyperthyroid group and WBC was higher in the normothyroid group.

There were significant differences in levels of haemoglobin, WBC, and MCHC in all three groups of thyroid hormones. whereas for platelets there was no statistically significant difference in the three groups

There was a significant difference in MCHC value between hyper-hypothyroid patients and normo-hypothyroid. The relationship between FT4 and Haemoglobin, MCV, and MCHC is negative. The relationship between FT4 and erythrocytes and MCH did not show a significant relationship

#### **Conclusion**

There were some differences in blood cell count component between various thyroid hormone status

### **A rare case of necrotizing granuloma of thyroid due to suspected non-tuberculous mycobacterium.**

**Swetha Rangaswamaiah<sup>1</sup>, Mahiban Thomas<sup>2</sup>, Emily Woolnough<sup>3</sup>, Kanakamani Jeyaraman<sup>1</sup>**

1. *Endocrinology, Royal Darwin Hospital, Darwin, Northern Territory, Australia*

2. *Surgery, Royal Darwin Hospital, Darwin, Northern Territory, Australia*

3. *Infectious diseases, Royal Darwin Hospital, Darwin, Northern Territory, Australia*

Background:

Granulomatous inflammation of thyroid has been reported with subacute thyroiditis, mycobacterium tuberculosis, blastomycosis, granulomatosis with polyangiitis, plasma cell granuloma and post surgical thyroiditis<sup>1-6</sup> We present a case of necrotizing granuloma of thyroid which was most likely caused by non-tuberculous mycobacteria.

Case presentation

A 62-year, immunocompetent, Caucasian lady presented with rapidly enlarging thyroid nodule for 3 months which had ulcerated. She had no symptoms of thyroid dysfunction and did not report recent respiratory infection. She had no associated neck pain, fever, weight loss or cough. She had no history of recent travel and no past history of or contact with tuberculosis. Physical examination revealed an ulcerating mass in right anterior neck triangle. MRI showed an enhancing dumbbell-shaped mass measuring 3.5x2.1x2.7 cm which appeared to arise from right thyroid lobe and was invading the sternothyroid muscle and platysma. She underwent right hemithyroidectomy. Histology showed extensive necrotizing granulomatous inflammation with occasional acid-fast bacilli. There were features suggestive of multinodular goitre in background thyroid tissue. Mycobacterial species were not detected by Polymerase Chain Reaction and mycobacterial culture was not performed as the specimen was paraffin-embedded. She did not receive anti-tubercular treatment and has remained symptom-free. Vasculitic screen was negative. She subsequently developed Mycobacterial abscessus cutaneous infection in her right leg ,3 months later requiring surgical excision.

Discussion: Our patient had no evidence of subacute thyroiditis or vasculitis. The lack of symptoms, absence of exposure or history of tuberculosis and negative PCR makes tuberculosis less likely in this case. The rapid progression of the thyroid lesion with spread to surrounding structures and skin, presence of acid fast bacilli on histology and subsequent cutaneous infection with mycobacterium abscessus makes non-tuberculous mycobacteria the most likely etiology.

Conclusion: Tuberculous and non-tuberculous mycobacterial infection of thyroid is rare but should be considered during evaluation and management of thyroid nodule.

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## Clinical and hormonal characteristics among patients with gestational trophoblastic disease in Hasan Sadikin General Hospital, Indonesia

**Ervita Yanuari Ritonga<sup>1</sup>, hikmat permana<sup>1</sup>, nanny nathalia soetedjo<sup>1</sup>**

*1. Hasan Sadikin General Hospital, Hasan Sadikin General Hospital, Bandung, Jawa Barat, Indonesia*

Background:

Gestational trophoblastic disease (GTD) is a rare complication of pregnancy, ranging from molar pregnancy to choriocarcinoma. Occasionally, GTD is complicated by hyperthyroidism, which may require treatment. This is thought to occur due to molecular mimicry between human chorionic gonadotrophin (HCG) and thyroid-stimulating hormone (TSH), and hence cross-reactivity with the TSH receptor. Hyperthyroidism develops in 5-10% cases of GTD and an incidence up to 78,8% is reported in Indonesia. Hyperthyroidism usually resolves as the GTD is successfully treated and correspondingly HCG levels normalise.

Objective:

This study aimed to determine clinical thyrotoxicosis and hormonal characteristics in patients with GTD in our hospital.

Methods:

A retrospective study was carried out from January 2013 to November 2017 in Hasan Sadikin General Hospital Indonesia. All the relevant data were collected from medical records.

Results :

Ninety three patients with GTD were identified, consisted of 64 complete moles, 24 partial moles, 1 persistent gestational trophoblastic tumor, 1 choriocarcinoma, and 3 invasive moles. Median distribution was at 34 years of age. Eighty-five percents patients had TSHs level <0.3 uIU/mL (both clinical and subclinical hyperthyroidism) and 15% had TSHs > 0.3 uIU/mL (no hyperthyroidism). Among patients with TSH level <0.3 uIU/mL,  $\beta$ hCG levels >300.000 uIU/mL, 100.000-300.000 uIU/mL, and <100.000uIU/mL were 43.33%, 23.33%, and 33.33% respectively.

Conclusion:

Concomitant biochemical thyroid disease in patients with GTD is relatively common. Measurement of thyroid function in patients with persistent GTD is important. Extremely high levels of HCG are typically required for the development of clinical hyperthyroidism as the relative potency of HCG for the TSH receptor is low. As a result, only a minority are clinically hyperthyroid and require treatment, although rarely the thyroid stimulation can have potentially lifethreatening consequences. However, a GTD may have a normal hCG level with suppressed TSHs.

Keywords: Gestational trophoblastic disease, hyperthyroidism

## Myxedema coma in a patient with subclinical hypothyroidism

**Shui-Boon Soh<sup>1</sup>, Tunn-Lin Tay<sup>1</sup>**

*1. Changi General Hospital, Singapore*

Myxedema coma (MC) is a rare manifestation of decompensated hypothyroidism, usually occurring in severe biochemical hypothyroidism. We describe an unusual case of myxedema coma in a patient with subclinical hypothyroidism.

A 55-year-old man with medical history of diabetes mellitus, hyperlipidemia, hypertension, chronic kidney disease and previous stroke was admitted for acute stroke. After 9 days of hospitalization, he was noted to have worsening drowsiness (Glasgow Coma Scale E4V1M4) as well as profound hypothermia (32.1°C) and bradycardia (41 beats/minute). New or worsening stroke was ruled out on brain imaging. He was not on new rate-control cardiac medication. There were no clinical evidence of sepsis, pericardial/pleural effusions and intestinal obstruction. His free triiodothyronine (fT3) was 2.6pmol/L (2.6 – 5.7), free thyroxine (fT4) 10.75pmol/L (10-20), and thyroid stimulating hormone (TSH) 8.18mIU/L (0.4-4.0). Thyroid peroxidase and thyroglobulin antibodies were both negative. Electrolytes were normal, apart from mild hyperphosphatemia of 1.85mmol/L (0.65-1.65) which was in keeping with his chronic kidney disease. Inflammatory markers were not elevated. Synacthen test showed peak cortisol response of 557nmol/L. MC was diagnosed based on clinical ground. He was commenced on intravenous thyroxine (IV T4) 200mcg, with intravenous glucocorticoid and continuous cardiac monitoring. IV T4 was continued at 300mcg the next day. There was significant clinical improvement to IV T4 therapy – drowsiness improved and body temperature and pulse rate increased to 36.0-37.4°C and 71-79 beats/minute respectively. IV T4 was subsequently converted to oral Levothyroxine 100mcg daily, and he was discharged to community hospital.

It is unusual for MC to occur in subclinical hypothyroidism. This case illustrates the importance of looking beyond thyroid function test in the evaluation for MC which remains a clinical diagnosis. Though not clearly understood, this may be explained by the inability of circulating thyroid hormones to accurately reflect the true tissue bioavailability in some patients.

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## Incidence and mortality of myocardial infarction and stroke in patients with hyperthyroidism: A nationwide cohort study in Korea

Seo Young Sohn<sup>1</sup>, Hyeong Sik Ahn<sup>2</sup>, Hyun Jung Kim<sup>2</sup>

1. Myongji Hospital, Goyang, Gyeonggi-Do, South Korea

2. Preventive Medicine, Korea University, Seoul, South Korea

**Background:** Hyperthyroidism is associated with various cardiovascular risk factors. However, the relationship between hyperthyroidism and myocardial infarction (MI) or stroke has not been fully elucidated; only a few studies have investigated the association of hyperthyroidism with survival after MI or stroke.

**Methods:** We included 59,021 hyperthyroid patients and 1,180,420 age- and sex-matched control cohort from the Korean National Health Insurance database. Blood pressure, body mass index, glucose and cholesterol level, and smoking history were obtained during National Health screening examination. We compared the incidence of MI, stroke, and survival after cardiovascular events between subjects with hyperthyroidism and control cohort.

**Results:** Subjects with hyperthyroidism had higher blood pressure, fasting glucose, and smoking rate, but lower cholesterol level and obesity rate compared with the control cohort. After adjusting these differences and atrial fibrillation, hyperthyroidism was associated with increased risk of MI and ischemic stroke in females. Adjusted hazard ratios (HRs) for MI and ischemic stroke in female subjects with hyperthyroidism was 1.22 (95% confidence interval (CI): 1.02-1.44) and 1.15 (95% CI: 1.05-1.26), respectively. However, these associations were not observed in males. The risk of hemorrhagic stroke was not different between subjects with hyperthyroidism and control. Adjusted HRs for mortality in subjects with hyperthyroidism who developed MI, ischemic stroke, and hemorrhagic stroke were 1.21 (95% CI, 0.93-1.56,  $P = 0.15$ ), 1.01 (95% CI, 0.86-1.20,  $P = 0.88$ ), and 1.21 (95% CI, 0.94-1.57,  $P = 0.14$ ).

**Conclusions:** Hyperthyroidism is associated with increased risk of MI and ischemic stroke, independent of cardiovascular risk factors, in females only. Hyperthyroidism did not significantly affect the mortality of cardiovascular events.

## Ethanol ablation of benign non-functioning thyroid nodules: more than 5-years follow-up results for 100 patients

Hyunju Yoo<sup>1</sup>, Dong Whan Choi<sup>2</sup>, Sae jeong Yang<sup>3</sup>, Jin Yong Sung<sup>3</sup>

1. Department of Pathology, Daerim St. Mary's Hospital, Seoul, South Korea

2. Department of Surgery, Daerim St. Mary's Hospital, Seoul, South Korea

3. Department of Internal Medicine, Daerim St. Mary's Hospital, Seoul, South Korea

**Objectives:** To evaluate the clinical outcomes and safety of ethanol ablation (EA) for benign non-functioning thyroid nodules over a 5-year follow-up.

**Materials and Methods:** We evaluated 100 benign non-functioning thyroid nodules of 98 patients (85 females, 13 males; mean age, 47.22±11.40 years) treated with EA and followed up more than 5 years. EA was performed using 18-gauge needle with 99% ethanol. Nodule volume and cosmetic and symptom scores were evaluated before treatment and during follow-up. Complications and factors related to efficacy were evaluated.

**Results:** The mean follow-up duration was 73.62±15.38 months. At last follow-up, the longest nodule diameter and the nodule volume significantly decreased (3.1±1.22 cm vs. 0.61±0.78 cm and 11.80±21.51 mL vs. 0.44±1.33 mL, respectively;  $p < 0.05$ ); a mean volume reduction of 96.19±8.35%. Both cosmetic and compressive symptoms significantly improved (3.71±0.46 vs. 1.17±0.40 and 2.85±1.34 vs. 0.01±0.10, respectively;  $p < 0.05$ ). Mean number of EA sessions was 1.07±0.29. 41 nodules (41.0%; 41/100) were completely disappeared on ultrasonography at mean 33.43±12.40 months after EA. The overall recurrence rate was 16.00% (16/100). Mean time of recurrence was 57.13±25.00 months. Additional radiofrequency ablation due to incompletely resolved clinical problems after EA was performed for 12 nodules (12.0%; 12/100). The overall complication rate was 0.02% (2/105).

**Conclusions:** EA was effective and safe in shrinking benign thyroid nodules and in controlling nodule-related problems over a 5-year follow-up. But, it is important to check recurrence during follow up periods and the additional treatment should be done at an appropriate time.

## Serum levels of fibroblast growth factor 21 in patients with hyperthyroidism or euthyroidism

**Fen-Yu Tseng<sup>1</sup>**

1. National Taiwan University Hospital, Taipei, TAIWAN, Taiwan

### Objective

Fibroblast growth factor 21 (FGF21) plays regulatory roles in glucose and lipid metabolism. Thyroid dysfunction may affect body weight, energy expenditure, and glucose metabolism. We evaluated serum FGF21 levels in patients with hyperthyroidism or euthyroidism.

### Subjects and methods

We recruited 30 newly-diagnosed hyperthyroid (HY) patients and treated them with anti-thyroid regimens as clinically indicated. Thirty euthyroid (EU) patients were recruited as controls. Laboratory parameters were measured at baseline and at 6 months. Associations between levels of FGF21 and free thyroxine (fT4), thyroid-stimulating hormone (TSH), log transformation of TSH (logTSH) or demographic, biochemical, and anthropometric data were analyzed.

### Results

There were no significant difference of FGF21 levels among the HY and EU patients, both at baseline ( $p = 0.217$ ) and at 6 months ( $p = 0.445$ ). Serum FGF21 levels had no associations with levels of fT4, TSH, or logTSH, both at baseline and at 6 months. However, levels of FGF21 had positive associations with age, fasting plasma glucose (FPG) and triglyceride (TG) at baseline ( $\beta = 3.99$ ,  $p = 0.005$ ;  $\beta = 4.69$ ,  $p = 0.008$ ; and  $\beta = 0.90$ ,  $p = 0.002$ , respectively) and at 6 months ( $\beta = 2.06$ ,  $p = 0.020$ ;  $\beta = 1.90$ ,  $p = 0.007$ ; and  $\beta = 0.49$ ,  $p = 0.004$ , respectively).

### Conclusions

Serum FGF21 levels are not associated with levels of fT4, TSH, or logTSH in patients with hyperthyroidism or euthyroidism. In this study, positive associations exist between levels of FGF21 and age, FPG or TG. Changes of serum FGF21 levels in the whole thyroid function spectrum remain to be investigated.

## Comparison of robot-assisted modified radical neck dissection using a bilateral axillary breast approach with a conventional open procedure after propensity score matching

**Se Hyun Paek<sup>1</sup>, Hyungju Kwon Kwon<sup>1</sup>, Kyung Ho Kang<sup>2</sup>, Sung Jun Park<sup>2</sup>**

1. EUMC, Seoul, South Korea

2. CAUH, Seoul, South Korea

**Background:** There is ongoing debate about whether or not robot-assisted thyroidectomy is appropriate for modified radical neck dissection (MRND). The purpose of this study was to compare the surgical outcomes of robot-assisted MRND with those of a conventional open procedure.

**Methods:** One hundred and forty-five patients who underwent total thyroidectomy, bilateral central neck dissection, and MRND (robotic,  $n = 28$ ; open,  $n = 117$ ) at our institution from June 2011 to June 2015 were enrolled in the study. The surgical completeness and complication rates in the robotic and open groups were retrospectively compared after 1:3 propensity score matching for age, sex, body mass index, tumor size, and extrathyroidal extension.

**Results:** The complication rates, including transient or permanent hypoparathyroidism and recurrent laryngeal nerve palsy, were comparable between the study groups ( $p > 0.05$ ). The operating time was significantly longer in the robotic group than in the open group ( $p < 0.001$ ). There was no significant difference in the number of retrieved lymph nodes, metastatic lymph nodes, or stimulated serum thyroglobulin level between the two groups ( $p = 0.733$ ,  $p = 0.663$ , and  $p = 0.285$ , respectively).

**Conclusions:** The surgical outcomes, including complication and completeness rates, were comparable between robot-assisted MRND using a bilateral axillary breast approach and conventional open surgery. Robot-assisted MRND can be recommended as an alternative to a conventional open procedure for thyroidectomy.

## Environmental Conditions Impact Stallion Fertility

**Róisín Griffin<sup>1</sup>, A Swegen<sup>1</sup>, J R Aitken<sup>1</sup>, Z Gibb<sup>1</sup>**

1. Priority Research Centre for Reproductive Science, University of Newcastle, NSW, Australia

Thoroughbred breeders experience undue pressure to achieve pregnancies close to the commencement of the breeding season, as yearling sales price and racing success are strongly affected by age. Periods of subfertility in stallions yield major cost and welfare concerns. The purpose of this study was to investigate environmental factors affecting stallion fertility. Dismount samples ( $n=486$ ) were collected weekly from 45 individual stallions, across four commercial stud farms. Samples were diluted (2:1, extender:semen), sperm concentration and motilities were recorded using a haemocytometer and iSperm™ device, then fixed in 2% PFA for morphological assessment. Stallion management data was collected, including per-cycle conception (PCC) rates, and libido scores (rated 0-5), and temperature and humidity loggers were installed in stables. A sub-population of nine stallions demonstrating susceptibility to stable temperature (ST) and humidity (SH) were identified based on correlations with PCC rates, and investigated further. Stepwise linear regression was performed using PCC as the response,

and total motility, abnormal head morphology, libido score, ST and SH (recorded the week of, one week preceding (-1) and two weeks preceding (-2)) as predictors ( $R^2= 0.79$ ), such that;

$$PCC = 3.21 + (-0.01 \times \text{Total Motility}) + (-0.01 \times \text{Head Abnormality}) + (0.12 \times \text{Libido}) + (-0.13 \times \text{Max Night ST}) + (-0.01 \times \text{Max Day ST}) + (-0.04 \times \text{Min Day ST}) + (0.03 \times \text{Min Night SH}) + (-0.01 \times \text{Min Day ST-1}) + (0.01 \times \text{Min Day SH-1}) + (-0.04 \times \text{Min Day ST-2}) + (-0.01 \times \text{Max Night SH-2}) + (0.02 \times \text{Max Day SH-2}).$$

Interestingly, libido and normal morphology were influenced by ST and SH ( $R^2= 0.79$  and  $R^2= 0.71$ , respectively), suggesting environmental conditions affect circulating testosterone levels; a hormone vital for normal spermatogenesis. Sub-fertility is a multifaceted issue, and this study demonstrates that non-targeted, comprehensive approaches are warranted for fertility investigations.