1

Studying neglected cell populations of the developing testis and their functions

Serge Nef[1]

1. University of Geneva, Geneva, PLEASE SELECT, Switzerland

Gonadal sex determination represents a unique model for studying cell fate decisions. However, a complete understanding of the different cell lineages forming the developing testis and ovary remains elusive. The widespread adoption of advanced sequencing technologies, such as scRNA-seq, has provided the field of developmental biology with an opportunity to discover previously unrecognized cell types, such as short-lived progenitors or rare cell lineages. By combining single cell transcriptomic analyses during the critical period of sex determination with *in vivo* lineage tracing, we will describe the specification and differentiation of several previously neglected gonadal cell lineages that give rise to multiple cell types such as rete testis cells, peritubular myoid cells, as well as fetal and adult Leydig cells.

2

Multiomics for premature ovarian insufficiency: gene discoveries and clinical impact

Elena Tucker[1, 2], **Sylvie Jaillard**[4, 3], **Shabnam Bakhshalizadeh**[1, 2], **Katrina M Bell**[1], **Daniella H Hock**[5], **Gorjana Robevska**[1], **Jocelyn van den Bergen**[1], **Brianna L Kline**[1], **Katie L Ayers**[1, 2], **Philippe Touraine**[6], **Chloe Hanna**[7, 1], **Sonia R Grover**[7], **Gary R Hime**[8], **David A Stroud**[1, 5, 9], **Andrew H Sinclair**[1, 2, 9]

1. Murdoch Children's Research Institute, Melbourne, Australia

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

3. Univ Rennes, CHU Rennes, INSERM, EHESP, IRSET (Institut de recherche en santé, environnement et travail), Rennes, France

4. CHU Rennes, Service de Cytogénétique et Biologie Cellulaire, Rennes, France

5. Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Australia

6. Department of Endocrinology and Reproductive Medicine, AP-HP, Sorbonne University Medicine, Centre de Référence des Maladies Endocriniennes Rares de la Croissance et du Développement, Centre des Pathologies Gynécologiques Rares, Paris, France

7. Department of Paediatric and Adolescent Gynaecology, Royal Children's Hospital, Melbourne, VIC, Australia

8. Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Australia

9. Victorian Clinical Genetics Services, Royal Children's Hospital, Parkville, Australia

Although genomic testing for many genetic disorders has become part of routine clinical care, this has not occurred for the management of female infertility, including premature ovarian insufficiency (POI). POI is a leading form of female infertility affecting up to 4% of women under the age of 40 and characterized by amenorrhea and elevated gonadotropins.

We have studied a diverse cohort of over 150 girls/women with POI using whole exome sequencing, identifying cause in >30%. We have validated causation using various approaches such as proteomic/transcriptomic analysis of patient cells, modelling in *Drosophila* or zebrafish and in-vitro functional assays. Our approach has led to multiple novel POI gene discoveries, such as *TP63*, *TFAM*, *MRPL50*, *HROB*, *REC8*, *GGPS1* and more.

Importantly, we have shown that genomic sequencing can alter and improve patient management and outcomes. For example, we identified causative variants in *NBN*, *EIF2B2* and *LARS2* in three different patients presenting with apparently "isolated" POI. These genes are usually associated with syndromic POI in the context of cancer predisposition, neurodegeneration and hearing loss respectively. In each case genomic sequencing identified syndromic POI before its full clinical manifestation. This enabled early intervention for associated co-morbidities, with the potential to improve patient outcomes.

Although genomic testing for infertility conditions such as POI has clinical utility, the fact that many causative genes have pleiotropic roles means that genetic diagnoses can have broad and unanticipated implications for patient health. Genomic counselling plays a critical role in the implementation of genomic testing for infertility to optimize health outcomes.

3

Rethinking the importance of men in assisted reproductive technology

Nicole McPherson[1]

1. University of Adelaide, Adelaide, SA, Australia

The male partner contribution is largely ignored when it comes to clinical assisted reproductive technology (ART) innovation. The processes and timing of semen collection, culture media and insemination have all been designed around what is best for the egg/female. Very little research has focussed how best to prepare sperm to maximise the chance of fertilisation success or defining the fertilisation potential of different sperm populations within the ejaculate. This remarkable neglect has resulted in the clinical inability to recapitulate or compensate for the natural sperm-selecting conditions of the female reproductive tract, a limitation that contributes to average fertilisation rates in IVF rarely surpassing 65%. Our idea is to identify key differences in sperm fate between natural conception and ART and to try and bridge the gap. One obvious difference is that clinical IVF does not recapitulate the physiological events of sperm maturation (hyperactivation and capacitation) and licensing that occur in the female reproductive tract prior to conception. The current protocols for sperm washing in IVF isolate motile sperm populations and remove the plasma fraction of the ejaculate, but no further sperm maturation or selection process have been widely implemented. Utilising known agents present in the female reproductive tract that induce sperm capacitation and

hyperactivation *in vivo* and novel sperm selection pressures (i.e. microgravity) we aim to redefine the importance of sperm in fertilisation and programming of a healthy embryo and the translation of these outcomes to improve ART pregnancy rates.

4

The role of mitochondria in programming placental and fetal growth

Joshua Fisher[1]

1. Hunter Medical Research Institute, School of Medicine and Public Health, College of Health, Medicine and Wellbeing, The University of Newcastle, NSW, Australia

Fetal growth restriction (FGR) affects approximately 10% of births in Australia each year, a statistic which equates to approximately 21,000 babies. FGR is defined as the inability of a fetus to reach its predetermined growth potential. Critically FGR directly contributes to ~5% of perinatal deaths in Australia. However, the mechanisms that underpin FGR remain elusive, with lifestyle, genetic, and maternal factors contributing to a broad range of insults collectively termed placental insufficiencies. Our recent work has established that mitochondria are essential for optimal placental function and appear dysregulated in FGR. We suggest this results in a lack the bioenergetic capacity to facilitate growth and development of the placenta and fetus. Our subsequent analysis established that single nucleotide polymorphisms within the nuclear genome present in gene regions that encode mitochondrial structure are correlated with decreased birthweight. A finding conserved in FGR placenta (n=15) at the gene and protein level. These findings have led us to develop a methodology of in-situ imaging of mitochondrial reticular networks, volume, and structure via cyro-electron microscopy. A technique which allows focus ion beam milling and imaging of placental sections 200nm in thickness, and the production of 3-dimensional reconstructions of placental villi using micro-CT and Serial block-face scanning electron microscopy. These projects have established the presence of mitochondrial dysfunction within the placenta of FGR, identified a potential mechanism of pathogenesis, and developed an innovative way to examine mitochondria within the placenta.

5

Coping with the cold: How does the ovine cervix respond to frozen sperm?

Jessica Rickard[1], S. Warr[1], S.P. de Graaf[1], t> Pini[2]

1. Faculty of Science, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia

2. School of Veterinary Science, The University of Queensland, QLD, Australia

The fertility of frozen ram sperm is limited following cervical artificial insemination due to its inability to successfully penetrate the ewes' cervix. However, if deposited in the uterus, its fertility is not compromised. The failed ability of frozen sperm to cross the ewe's cervix is thought to be related to freezing induced changes to sperm surface characteristics previously modified by seminal plasma at ejaculation. To date, extensive work has focused on characterising proteomic sperm traits prior to and following seminal plasma exposure and in vitro processing. However, it remains unclear how cervical tissue responds to different sperm phenotypes, and whether there is a transcriptomic response impeding cervical transit. As such, an ex vivo cell culture model was used to assess the transcriptomic response of cervical endometrial explants harvested from the reproductive tract of oestrus-synchronised ewes (*n*=6) to; epididymal sperm, epididymal sperm exposed to seminal plasma, frozen-thawed sperm and seminal plasma alone from rams (*n*=3). Explants were co-cultured under CO_2 for 6h. Analysis of differentially expressed genes by RNA sequencing revealed that sperm with exposure to seminal plasma significantly activated pathways related to integrin cell surface and extracellular matrix signalling, compared to epididymal sperm alone. Exposure of cervical explants to cryopreserved sperm activated NFKB signalling, multiple interleukin signalling pathways (IL-1, 2, 6, 8 and 15), miRNA biogenesis and estrogen receptor signalling. These results suggest that cervical gene expression is altered in response to spermatozoa and seminal plasma, and that cryopreservation may further modify this interaction. Identifying pathways of interest could highlight potential supplements for frozen sperm which could improve their ability to transit the cervix and achieve fertilisation following cervical artificial insemination.

6

Offspring and the Influence of Perfluoroalkyl And Polyfluoroalkyl Substance Exposure during critical developmental periods

Jacinta Martin[1, 2], Ewan Simpson[1, 2], John Schjenken[1, 2], Shannon Smyth[1, 2], Leah Calvert[1, 2], Amanda Anderson[1, 2], Ilana Bernstein[1, 2], Tessa Lord[1, 2], Mark Green[3], Shaun Roman[1, 4], Brett Nixon[1, 2]

1. Priority Research Centre in Reproductive Science (PRC), University of Newcastle, Callaghan, Newcastle, NSW, Australia

2. Infertility and Reproduction Program, Hunter Medical Research Institute, New Lambton Heights, Newcastle, NSW 2305, Australia, Newcastle, NSW

3. School of BioSciences, Faculty of Science, University of Melbourne, Melbourne, VIC, Australia

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

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a diverse family of fluorine-containing chemicals which possess unique chemical properties that render them near indestructible. The stability of these chemicals saw their widespread use, manufacture and distribution resulting in the pervasive exposure of humans and animals. As such PFAS are now considered a ubiquitously persistent environmental contaminant. Once absorbed into the body, PFAS bioaccumulates and can be transferred via the placenta and through milk during lactation. It is therefore not unsurprising that PFAS has been readily detected in human blood and breast milk, and in some occupationally exposed populations has been found in concentrations 1000-fold higher than the general population. Despite this, there remains no clear consensus on the biological impacts of PFAS exposure and consequently, the public demand for a definitive health risk assessment of PFAS continues to go unmet.

We therefore designed this study to assess the effects of gestational and post-natal exposure of a cocktail of nine PFAS chemicals, formulated to mimic that of environmental samples, on mouse development. Adult female mice were administered PFAS (or sham) via their drinking water three days prior to mating and continued to be administered PFAS from conception through to weaning (3 weeks post-birth). Plug and pregnancy rates were found to be unaffected by PFAS exposure, as was pregnancy duration. Litter size was however dramatically decreased for our PFAS dams ($P < 0.048$). Additionally, the pups born from PFAS exposed mothers were significantly heavier come weaning (day 21, $P < 0.0001$) and displayed altered reproductive millstones, as determined by vaginal opening, which were reflective of expedited puberty ($P < 0.016$). Intriguingly, we also saw that the pups of PFAS exposed mothers display altered behaviour, including reduced anxiety-like and increased risk-taking behaviours reflective of an attention-deficit disorder phenotype. This study, therefore, presents new evidence that PFAS impacts offspring health and development. Our longer-term studies will continue to explore the effects of these changes and will assess the integrity of the endocrine and reproductive systems given these preliminary results. We will also conduct more nuanced behavioural testing focusing on hyperactivity, learning, risk-taking and alcohol consumption behaviours.

7

The impacts of heat stress on bovine reproduction: A case for using whole-body heat challenges to understand the impacts of heat load on reproductive function in males

Angela M Lees[1], Andrea L Wallage[1], John B Gaughan[1], Stephen D Johnston[1]

1. The University of Queensland, Gatton, QUEENSLAND, Australia

Heat stress is already a significant economic and welfare concern in Australia's animal industries. However, there is limited evidence of the male's contribution to the economic impacts, despite being well established that heat stress reduces male reproductive function. The influences of heat stress on males have been attributed to the impact that hot conditions play on sperm production. Increased testicular temperature has profound impacts on sperm quality, including decreased sperm concentration and motility, increased morphological abnormalities, altered plasma membrane composition and reduced DNA integrity. The effects of increased testicular temperature on male reproduction are not immediately apparent, where abnormal sperm first appear in the ejaculate 1 to 2 weeks post-heat insult. Previous studies have demonstrated that heat stress can negatively impact male reproductive function. These studies have predominantly utilised a scrotal insulation technique, which directly impedes the thermoregulatory capacity of the scrotum. This technique also precludes the capacity of the behavioural and physiological responses that occur in an animals attempt to mitigate the impact of heat stress on the scrotum, resulting in an isolated heat challenge. This is an important concept as it removes the capability of the whole body to effectively evoke the conserved responses for coping with heat load conditions. As such, there are limited studies investigating the impact of heat stress on male reproductive function when the whole-body has been exposed to heat load. With this in mind, we developed a protocol for assessing scrotal thermoregulation in bulls during whole-body exposure to heat load conditions. We identified that the thermoregulatory capabilities of the scrotum are challenged during whole body exposure to heat load conditions. In the context of climate change, it is probable that there will be more persistent challenges placed on the thermoregulatory capabilities of the scrotum, increasing the deleterious effects of heat stress on males.

8

A fertility legacy: how environmental pollutants are affecting reproductive health

Deidre Mattiske[1]

1. University of Melbourne, Melbourne, VIC, Australia

Differences of Sexual Development (DSDs) are amongst the most common birth defects in humans. Alarmingly, the incidence of DSDs has increased significantly in the last few decades, and this rise is attributed to our increased exposure to endocrine disrupting chemicals (EDCs). Furthermore, some EDCs are predicted to have far reaching effects beyond the exposed individual, causing disease that persists over multiple generations. Our research examines the effects of estrogenic EDCs on reproductive development and fertility in both males and females over multiple generations. We have examined the effects of diethylstilbestrol (DES), a clinically relevant EDC, across four generations of mice. DES caused a significant reduction in anogenital distance, reproductive organ weights and fertility, and an increased incidence of DSDs through to the F4 generation. A significant difference in maternal and paternal lineages was also observed in males for body weight, seminal vesicle weight, seminiferous tubule diameter and hypospadias rate. This data has significant implications for multiple generations of DES descendants. Furthermore, the impacts described from exposure of pregnant mothers to EDCs raises concerns about the effects of exposures to multiple estrogenic EDCs present in our environment.

10

Understanding human reprogramming: A journey from epiblast to trophoblast and into iBlastoids

Jose Polo[1]

1. The University of Adelaide, Adelaide, SA, Australia

In 2007 Shinya Yamanaka demonstrated that human fibroblasts can be reverted back to a pluripotent state by the forced expression of the four transcription factors; *OCT4, SOX2, KLF4* and *cMYC*. These so-called induced pluripotent stem cells (iPSCs), like embryonic stem cells derived from the epiblast of blastocysts, can give rise to any cell type of the body. Furthermore, iPSCs carry the promise of personalised regenerative medicine and hold tremendous potential for applications such as cell replacement therapies, disease modelling and *in vitro* drug screening. However, the molecular mechanisms of these cellular transitions into primed or naive human-induced pluripotency remain poorly understood. To address this, we reconstructed the molecular reprogramming trajectories using single cell transcriptomics. This revealed that reprogramming into

the primed and naive human pluripotency states follows diverging and distinct trajectories. The integration of regulatory element usage with transcriptomics unveiled an unexpected role of trophectoderm (TE) lineage-associated transcription factors, as well as a subpopulation of cells that transiently upregulated a TE-like signature during reprogramming. We demonstrated that this TE state could be stabilised allowing the derivation of induced Trophoblast Stem Cells (iTSCs). Further inspection of these cell cultures also revealed the upregulation of a primitive endoderm-like signature in some of the cells. Unexpectedly, when all these cells are allowed to contact each other in a 3-dimensional system, they self-organise giving rise to blastocyst-like structures which we termed, iBlastoids. iBlastoids are capable of modelling *in vitro,* many molecular, morphological and functional aspects of embryonic development during the early stages of implantation.

11

Setting a foundation for future offspring: Polycomb-dependent epigenetic programming in the maternal germline

Patrick Western[1, 2], Sigrid Petautschnig[1, 2], Ruby Oberin[1, 2], Ellen Jarred[1, 2], Zhipeng Qu[3], Neil Youngson[1, 2, 4], Tesha Tsai[1, 2], Rheannon Blucher[1, 2], Heidi Bildsoe[1, 5], Steven Peterson[3], Mai Truong[6], Narelle McGregor[7], Emily Jaehne[8], Qing-hua Zhang[9], Jessica Stringer[9], William Gibson[10], John Carroll[9], David Gardner[6], Maarten van den Buuse[8], Natalie Sims[7], David Adelson[3]

1. Department of Molecular and Translational Science, Monash University, Clayton, Vic, Australia

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

3. Department of Bioinformatics and Computational Genetics, School of Biological Sciences, Adelaide University, Adelaide, SA, Australia

4. School of Biomedical Sciences, UNSW Sydney, Sydney, NSW, Australia

5. Centre for Reproductive Health, Hudson Institute for Medical Research, Clayton, Victoria, Australia

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

7. Bone Cell Biology and Disease Unit, St Vincent's Institute of Medical Research, Melbourne, Vic, Australia

8. School of Psychology and Public Health, La Trobe University, Melbourne, VIC, Australia

9. Biomedicine Discovery Institute, Monash University, Melbourne, VIC, Australia

10. Department of Medical Genetics, University of British Columbia and British Columbia Children's Hospital Research Institute, Vancouver, BC, Canada

Epigenetic modifications modulate cell differentiation partly by regulating transcription of developmental genes. While epigenetic programming in germ cells may be altered by environmental influences and affect offspring development, the mechanisms are poorly understood. Polycomb Repressive Complex 2 (PRC2) catalyses the epigenetic modification, H3K27me3 to repress developmental genes in many tissues. We demonstrated that transient PRC2 activity regulates establishment of H3K37me3 at developmentally important genes in growing oocytes. Loss of PRC2 in growing oocytes de-repressed 343 genes involved in development, neurogenesis, chondrogenesis and tissue patterning suggesting that PRC2 epigenetically regulates genes important for offspring development. Many of these genes contained H3K27me3 in human GV oocytes, demonstrating that PRC2 activity is conserved in human oocytes. Consistent with a role for PRC2 in maternal inheritance, post-implantation mouse fetal offspring from oocytes lacking PRC2 were developmentally delayed, but exhibited placental hyperplasia, accelerated growth late in fetal life, extended gestation and perinatal overgrowth, compared to genetically identical controls. Placental transcription and DNA methylation was significantly altered, including increased expression of the H3K27me3 imprinted gene and amino acid transporter, *Slc38a4*. While placentas contained increased numbers of glycogen enriched cells, late gestation fetal glucose levels were lower, but the fetal circulating metabolic profile was similar to controls. Fetal development was significantly affected as demonstrated by altered bone and brain development, and behaviour, in offspring. These outcomes are reminiscent of Cohen-Gibson/Weaver Syndromes caused by *de novo* germline mutations in human EED/EZH2 and characterised by overgrowth, skeletal abnormalities and learning deficits. Our work identifies a link between PRC2-dependent oocyte epigenetic programming and offspring development and indicates that this activity is conserved in human oocytes. Understanding these processes is critical for determining how epigenetic programming in oocytes regulates health and development of the next generation and how environmental influences, such as drugs or diet, could alter non-genetic inheritance in mammals.

12

Analysing genetic regulation of reproduction using a *Drosophila* model

Gary R Hime[1], Nicole A Siddall[1], Nicole Dominado[1], Franca Casagranda[1], Qiaolin Yang[1], Fernando Wijaya[1], Shabnam Bakhshalizadeh[1, 2, 3], Brendan Houston[4], Elena J Tucker[2, 3], Katie L Ayers[2, 3], Moira O'Bryan[4], Andrew H Sinclair[2, 3]

1. Anatomy and Physiology, University of Melbourne, Parkville, Vic, Australia

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

3. Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

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

The availability of genomic sequencing has identified many human genetic variants that have been linked to infertility. Studies to provide evidence for causation or molecular function can be costly and time consuming. Our lab has collaborated with other research teams to analyse specific genes and genetic variants using the vinegar fly, *Drosophila melanogaster*.

Drosophila has been a laboratory model organism for over 100 years and is now utilised by hundreds of research groups around the world to study genetic regulation of development and physiology. We have at our disposal a large number of genetic tools to conduct sophisticated experiments relating to gene function and have the capacity to rapidly mutate any of the 15,000 genes in the fly genome.

My research team has focussed on regulation of male reproduction over the last couple of decades and I will show examples of RNA-binding proteins, transcription factors and signalling molecules that we have shown to play roles in regulating male germline stem cell maintenance, differentiation, and subsequent meiotic and post-meiotic functions. I will also outline recent collaborative studies where we have mutated *Drosophila* orthologs of human genes associated with infertility and shown them to play conserved roles in maintaining gamete production. I will also show how we can generate *Drosophila* analogues of human genetic variants linked with reproductive phenotypes and thereby analyse gene function in a cost-effective and timely manner.

13

Understanding the mechanisms by which specific amino acids improve pre-implantation embryo development

Margot Day[1]

1. School of Medical Sciences, Sydney University, Camperdown, NSW, Australia

Despite substantial improvements to media used for in vitro development of pre-implantation embryos, there is still evidence that these embryos are under stresses that alter normal cellular processes, which impacts embryo viability. Cultured embryos have altered mTOR signalling, increased ROS and mitochondrial activity compared to those developed in vivo. Amino acids (AA) are generally added to culture media in groups. However, the transporters for AAs have multiple substrates which may cause competitive inhibition of uptake of beneficial AAs when all AAs are present. Addition of individual amino acids, such as L-proline (Pro), to media used for fertilisation and culture, at concentrations like that in reproductive fluids, improves blastocyst development and hatching. Pro transporters are present throughout pre-implantation development and the presence of other substrates (eg glycine) prevents the beneficial effects of Pro. Our data show that the mechanism of action of Pro involves mTOR pathway activity, as well as reduced ROS, probably due to direct ROS scavenging by Pro, metabolism of Pro to produce the antioxidant glutathione and a reduction in mitochondrial activity. Overall, our data suggest that the quieter metabolic status of embryos cultured in the presence of only Pro can improve embryo viability and that design of media composition needs to take into consideration the impact of combinations of AAs on the action of Pro and other beneficial AAs.

14

Transgender health and reproductive health; similarities and challenges

Ada Cheung[1]

1. Trans Health Research Group, Department of Medicine, The University of Melbourne, Heidelberg, VIC, Australia

Approximately 0.5-2% of the general population are trans and gender diverse, yet trans people are arguably the most marginalised and socioeconomically disadvantaged group in Australia with high rates of depression, suicidality and a dearth of research to guide optimal gender-affirming hormone and surgical interventions. In addition to these barriers, trans people have difficulty accessing basic healthcare due to lack of acceptance and knowledge among healthcare professionals.

A/Prof Cheung will provide an overview of her co-created research in transgender health, the impact of gender affirming hormone therapy on pelvic pain, fertility and bone health. She will also discuss the role that reproductive health clinicians and scientists play in providing affirming care and the similarities and challenges in reproductive and transgender health.

15

Challenging common assumptions regarding the reproductive health of trans women

Ken Pang[1]

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

Increasing numbers of transgender individuals are presenting for gender-affirming medical care. For trans women, gender-affirming hormone therapy (GAHT) promotes feminization but also inhibits spermatogenesis. In this presentation, I will present data that challenges two common assumptions in relation to the reproductive health of trans women. Firstly, there has been a widespread belief that trans adolescents are not interested in undertaking sperm cryopreservation for future reproductive purposes. However, we observed that – when given the opportunity – a majority of trans adolescents attempt to freeze their sperm prior to undertaking hormonal therapy, which indicates the importance of offering fertility preservation. Secondly, there is a common assumption that the inhibition of spermatogenesis that occurs secondary to GAHT is permanent, resulting in life-long infertility. However, in a recent longitudinal study, we observed the recovery of viable spermatozoa in nine trans women who stopped GAHT for reproductive purposes. In this way, these preliminary findings suggest that the negative impact of GAHT on spermatogenesis can be reversed, casting doubt on previous claims that GAHT in trans women inevitably leads to permanent infertility.

16

Doing Health and Wellbeing Research with Pacific Rainbow+ Communities in Aoteraoa-New Zealand: Insights from the Manalagi Project

Patrick Thomsen[1]

1. The University of Auckland, Auckland, New Zealand

The Manalagi Project is the first health and wellbeing project to be funded by the Health Research Council of New Zealand. Pacific Rainbow+ communities (LGBTTQIA+ MVPFAFF+) in Aotearoa-New Zealand occupy an intersectional space where racial/ethnic marginality intersects with gender-sex, sexuality diversity. This presents distinctive challenges to realising optimal health and wellbeing outcomes within Aoteraoa-New Zealand's healthcare and social support systems. Existing research demonstrates that discrimination (racism, homonegativity, heterosexism, transphobia etc) is a strong predictor of adverse mental health and increased risk related behaviour among Rainbow+ communities. This presentation will provide insights into how the Manalagi Project engaged with Pacific Rainbow+ communities in Aotearoa-New Zealand through its community design phase. During this phase, the Manalagi team developed a model of community engagement titled the Manalagi HCLC Model, that leans on the principles of Indigenous and Pacific research methodologies. The Manalagi HCLC Model aims to not only include Pacific Rainbow+ communities in the project design of the national survey, but also to empower our communities to leverage the Manalagi platform to advance their own projects and knowledge creation. This approach takes a holistic view of wellbeing that acknowledges the need to address societal status and visibility as integral contributors to the overall wellbeing of multiply-marginalised groups in Aotearoa-New Zealand.

17

Optimal Adrenal Vein Sampling Lateralization criteria for diagnosis of Unilateral Primary Aldosteronism using aldosterone concentration measured by LC-MS/MS post-ACTH stimulation

Moe Thuzar[1, 2], Kassem Zaal[2], Derrick Ma[2], Michael Morkos[2], Brett McWhinney[3], Jacobus Ungerer[3], Michael Stowasser[2]

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, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Background: Primary aldosteronism (PA) is characterized by excessive, autonomous secretion of aldosterone from one or both adrenal glands. Identification of unilateral disease and surgical resection can lead to cure, and is dependent on accurate criteria for lateralization of excess aldosterone during adrenal vein (AV) sampling (AVS). Current evidence for AVS lateralization cut-offs using plasma aldosterone concentration (PAC) measured by LC-MS/MS and post-surgical outcomes is limited.

Aim: To determine optimal cut-off criteria to define lateralization of PA on AVS based on PAC measured by LC-MS/MS post-ACTH stimulation and post-surgical outcomes data.

Methods: This study involved 61 subjects with PA who had PAC measured by LC-MS/MS post ACTH-stimulation during bilaterally-cannulated AVS, underwent unilateral adrenalectomy between July 2015-July 2021 and were followed up for ≥6 months. AVS lateralization parameters (lateralization index [LI; dominant $PAC/cortisol_{AV}$ ÷ non-dominant $PAC/cortisol_{AV}$], contralateral suppression index [CSI; non-dominant $PAC/cortisol_{AV}$ ÷ $PAC/cortisol_{Peripheral\ Vein}$], lateralization ratio [LR; dominant $PAC/cortisol_{AV}$ ÷ $PAC/cortisol_{Peripheral\ Vein}$]) and post-surgical outcome data were examined. Complete biochemical remission (CR) of PA post-surgery was defined using PASO International Consensus Criteria. Receiver operating characteristic (ROC) analysis was performed to determine optimal cut-offs for LI, CSI and LR based on cases who achieved CR of PA post-surgery (n=55) vs those who did not (n=5).

Results: Optimal cut-off LI during ACTH-stimulated AVS to identify unilateral PA cases who achieved CR post-surgery was 5.1 (AUC=0.92, P<0.001; 92.7 % sensitivity, 80.0% specificity) while the optimal cut-off for CSI was 0.6 (AUC=0.887, P<0.001; 82.1% sensitivity, 100.0% specificity), and for LR was 3.3 (AUC=0.695, P=0.202; 80.0% sensitivity, 80.0% specificity). Cases with LR of 2.0-3.3 also achieved CR post-surgery if it was associated with CSI <0.6.

Conclusion: LI of >5.1 provides high sensitivity and CSI <0.6 high specificity for identification of surgically curable unilateral PA during ACTH-stimulated AVS when PAC is analyzed by LC-MS/MS.

18

Unlocking the potential of CAR T cell immunotherapy for the treatment of prostate cancer

Laura H Porter[1], Joe J Zhu[2], Natalie L Lister[1], Sophie G Harrison[1], Shiva Keerthikumar[2], David L Goode[2], Rosalia Quezada Urban[2], David J Byrne[2], Arun Azad[2], Ian Vela[3], Michael S Hofman[2], Paul J Neeson[2], Phillip K Darcy[2], Joseph A Trapani[2], Renea A Taylor[1], Gail P Risbridger[1]

1. Monash University, Clayton, VIC, Australia

2. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

3. Queensland University of Technology, Brisbane, Queensland, Australia

Despite significant improvements in detection and treatment, advanced prostate cancer remains incurable. When androgen receptor (AR)-targeted therapies fail, it is challenging to find new treatments to eradicate resistant tumours. Immunotherapy with genetically engineered chimeric-antigen receptor (CAR) T cells is a profound advance in cancer therapy. However, the suppressive tumour microenvironment (TME) is a significant barrier to CAR T cell efficacy in solid tumours, including prostate cancer. Here, we assessed the effectiveness of Lewis Y (Le^Y) CAR T cell therapy using patient-derived xenografts (PDXs) of prostate cancer from the Monash Urology Research Alliance (MURAL). As Le^Y is not a predicted target antigen for prostate cancer treatment, we first assessed the expression of Le^Y on 800 prostate cancer patient specimens plus 49 patient-derived xenografts (PDXs). Le^Y was expressed on 12% of localized and 20% of metastatic tumours, and in 57% of PDXs. *In vitro,* Le^Y CAR T cells directly killed organoids derived from AR-positive or AR-null PDXs. *In vivo*, Le^Y CAR T cells alone did not affect tumour growth, but a single prior dose of carboplatin resulted in tumour eradication. Carboplatin induced a pro-inflammatory TME that facilitated early and durable CAR T cell infiltration, including an altered cancer-associated fibroblast phenotype, enhanced extracellular matrix degradation and M1 macrophage differentiation. In a PDX less sensitive to carboplatin treatment, carboplatin did not boost CAR T cell infiltration; however, a reduction in tumour burden was still observed

with increased T cell activation. Therefore, carboplatin improves the efficacy of CAR T cell treatment, with the extent of the response dependent on changes induced within the TME. As Le^Y expression was independent of AR expression, and both AR-positive and AR-null tumours were sensitive to Le^Y CAR T cells *in vitro*, Le^Y CAR T cells may provide a treatment option for patients who would not benefit from AR-directed therapies.

1. Porter, L.H., Zhu, J.J., Lister, N.L., Harrison, S.G., Keerthikumar, S., Goode, D.L., Quezada Urban, R., Byrne, D.J., Azad, A., Vela, I., Hofman, M.S., Neeson, P.J., Darcy, P.K., Trapani, J.A., Taylor, R.A. & Risbridger, G.P. Low-dose carboplatin modifies the tumor microenvironment to augment CAR T cell effcacy in human prostate cancer models. Nature Communications In Press(2023)

19

ESA Young Investigator Scientific Article Award (Clinical) – Oral abstract for ESA ASM

Nick Russell[1]

1. Department of Endocrinology, The Northern Hospital, Epping, VIC, Australia

Most men receiving androgen deprivation therapy (ADT) for prostate cancer experience hot flushes. Hot flushes cause discomfort and embarrassment, interrupt sleep, represent an unwelcome reminder of the prostate cancer diagnosis, and are reported to be an important mediator of ADT-associated decrements in self-reported quality of life. There are a range of commonly used treatments for ADT-induced hot flushes, but most have low efficacy, or limited evidence of efficacy.

Physiological evidence suggests that oestradiol withdrawal is the predominant cause of ADT-induced hot flushes, as is the case for hot flushes in perimenopausal women. In a 28-day pilot trial, transdermal oestradiol gel reduced hot flush frequency-severity scores in men receiving ADT for prostate cancer.

This paper reports the results of a 6-month randomised controlled trial in a new cohort of 78 men undergoing ADT for prostate cancer, hypothesising that this treatment would reduce hot flushes and thereby improve self-rated quality of life. Results will be discussed in detail.

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Incretin targeted therapies and cardiovascular risk

Stephen NIcholls[1, 2]

1. Monash Health, Clayton, VIC, Australia

2. Monash University, Clayton, VIC, Australia

Given the role of incretin hormones in regulation of postprandial glucose metabolism, there has been increasing interest in developing therapeutics that promote incretin function for the treatment of type 2 diabetes and obesity. Early clinical studies have demonstrated that administration of GLP-1 receptor agonists improve glycaemic control and promote weight loss with associated benefits in metabolic parameters and cardiovascular risk in the setting of both type 2 diabetes and obesity. Development of new therapeutics that target dual and triple incretin hormones appear to produce greater metabolic benefit, while their influence on cardiovascular risk is being evaluated in large clinical trials. The evidence from these clinical trials and implications for clinical practice will be reviewed.

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Genetic and cellular origins of adrenal primary aldosteronism

William Rainey[1]

1. Departments of Molecular & Integrative Physiology and Internal Medicine , The University of Michigan, USA

It has been just over 65 years since Jerome W. Conn published the clinical description of primary aldosteronism (PA) about a case with a unilateral aldosterone-producing adenoma (APA). PA has since been characterized by renin-independent adrenal aldosterone production causing hypertension and often hypokalemia. Chronic aldosterone excess leads to target organ damage exceeding that of essential hypertension, including left ventricular hypertrophy, stroke, and kidney impairment. Although PA was initially considered a rare endocrine disorder, it is currently recognized as the most common form of endocrine hypertension. Its estimated prevalence is 5-10% of hypertensive patients and 20% of patients with resistant hypertension. Recent studies suggest that PA has a wide spectrum of disease severity ranging from mild to overt, and that the early stages of disease can be seen in normotensive subjects who present with renin-independent aldosterone production. Because excess aldosterone causes peripheral organ damage, early detection of PA and appropriate treatment are highly recommended. However, PA appears to still be largely underdiagnosed.
Recent advances in genetic analysis methodologies and development of adrenal disease biomarkers have provided the tools to describe the molecular and histologic pathogenesis of this disease. The application of immunohistochemistry-guided capture of disease-causing lesions and the ability to sequence formalin-fixed paraffin-embedded (FFPE) archival adrenal material has resulted in the identification of somatic mutations in adrenals from both lateralized (in most cases unilateral disease) and bilateral PA cases. The vast majority of aldosterone-producing lesions in resected adrenals harbor aldosterone-driver somatic mutations in KCNJ5, ATP1A1, ATP2B3, CACNA1D, CACNA1H, CTNNB1, SLC301A1, CADM1 or CLCN2. Disease-causing mutations converge on intracellular calcium ion homeostasis whereby they cause inappropriate aldosterone production. Accumulating evidence suggests that patient age, race, and sex impact the somatic mutation spectrum causing PA. For example, somatic mutations in the KCNJ5 gene (encoding an inwardly rectifying K+ channel) are common in APAs from Asian

populations as well as women regardless of race, while mutations in the L-type calcium channel CACNA1D are more common men of African descent. In my seminar, I will review recent findings regarding somatic mutations causing PA, discuss newly discovered somatic mutations, and provide an update on the implications of these findings on personalized disease diagnosis. Acknowledgments: The work presented would not have been possible without collaboration and support from the University of Michigan Adrenal Research Team, collaborators within the American, Australian, Asian Adrenal Alliance (A5) and financial support from the NIH and American Heart Association.

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Anti-Müllerian Hormone Y (*AMHY*): the candidate sex determining gene of egg-laying mammals

Linda Shearwin-Whyatt[1], Jane Fenelon[2], Hongshi Yu[2], Andrew Major[3], James Galbraith[1], Keith Shearwin[1], Stephen Johnston[4], David Adelson[1], Craig Smith[3], Marilyn Renfree[2], Frank Grützner[1]

1. School of Biological Science, University of Adelaide, Adelaide, SA, Australia

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

3. Department of Anatomy and Developmental Biology, Monash University, Melbourne, Vic, Australia

4. Currumbin Wildlife Sanctuary, Currumbin, Qld, Australia

Egg-laying mammals (monotremes) lack the therian sex determination gene *SRY* and their sexual development pathway has not been characterised. The candidate sex determining gene of monotremes is a Y-localised copy of the Anti-Müllerian Hormone gene. AMH has conserved roles in vertebrate sexual development and has become the sex-determining gene in a range of non-mammalian vertebrates. In this work, we characterise the monotreme X- *and* Y-localised *AMH* gametologues and provide new evidence supporting *AMHY* as the male sex determining gene of monotremes. The *AMH* gametologues of platypus and echidna are located in the oldest strata of the X and Y chromosomes however, Y chromosome degradation has altered the genomic context of the monotreme *AMHY* gene compared to *AMHX* and therian *AMH* genes. We show that the *AMHX* and *AMHY* genes have undergone significant divergence at the promoter, gene and protein level but have retained the conserved features of mammalian TGF-β signalling molecules. Access to echidnas during early developmental stages has allowed us to investigate gene expression during monotreme sexual determination. We show that *AMHX* is expressed in the developing gonad of both males and females during the period of sexual determination, while *AMHY* is expressed throughout these fetal stages only in the developing testis. Expression of *AMHY* in the developing male gonad during sex determination provides strong support for its role in male sex determination. Functional analysis was carried out in the chicken by ectopic expression of the monotreme *AMHX* or *AMHY* genes in the chicken embryo. In this system, chicken AMH masculinizes the female chicken gonad, however the monotreme proteins did not affect gonadal development, likely due to an inability of the proteins to signal through the chicken AMH receptor. In conclusion, our results provide additional evidence in support of *AMHY* as the male sex determining gene in monotremes.

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Molecular controls of male germline maintenance and regeneration

Hue Mai La[1, 2], Ai-Leen Chan[1], Ashlee Hutchinson[1], Julien Legrand[3, 1], Fernando Rossello[2], Antonella Papa[4], Jinyue Liao[5], Robin M Hobbs[1, 6]

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

2. University of Melbourne, Melbourne, VIC, Australia

3. Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC, Australia

4. Monash University, Clayton, VIC, Australia

5. The Chinese University of Hong Kong, Hong Kong , China

6. Department of Molecular and Translational Sciences, Monash University, Clayton

Maintenance of male fertility is dependent on spermatogonial stem cells (SSCs) that self-renew and generate differentiating germ cells for production of spermatozoa. SSC function is dependent on growth factors produced within the testis microenvironment plus cellular factors that regulate gene expression within SSCs and modulate responses to growth factor stimulation. Despite the importance of SSCs for male fertility, the molecular mechanisms that regulate their function and maintenance remain incompletely understood. Further, SSC function and fertility can be compromised by exposure to genotoxic drugs but cellular pathways mediating the regenerative response of SSCs following germline damage are poorly studied. Our recent studies have focused on understanding the distinct roles played by growth factor-regulated signalling pathways in controlling SSC dynamics and function under homeostatic and regenerative conditions through use of mouse models and single cell approaches. We find that SSCs mediating germline regeneration adopt a unique cellular state associated with alterations in PI3K-mTORC1 signalling when compared to SSCs of steady-state tissue. Moreover, while chronic stimulation of PI3K-mTORC1 signalling is detrimental to SSC maintenance in undisturbed tissue, transient activation of this pathway is required to promote the SSC regenerative response. Importantly, concerted inhibition of growth factor signalling, including the PI3K-mTORC1 pathway, in SSCs results in pronounced defects in germline recovery after damage. In addition, the transcription factor and cell cycle regulator FOXM1 was found to integrate diverse signalling inputs to support SSC regenerative capacity. Combined, our data demonstrate key instructive roles for microenvironmental growth factors and PI3K-mTORC1 signalling in defining distinct functional states of homeostatic and regenerative SSCs.

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Generation of induced pluripotent stem cells and differentiation of primordial germ cells in the fat-tailed dunnart; an invaluable tool for next-generation marsupial conservation

Gerard A Tarulli[1], Dorthea K Hansen[1], Christina N Alsterberg[1], Nicola D Kolaitis[1], Patrick RS Tatt[1], Stephen R Frankenberg[1], Andrew J Pask[1]

1. University of Melbourne, Melbourne, VIC, Australia

Australian marsupial populations are in significant decline. While traditional approaches to conservation such as habitat protection and breeding programmes have proven useful in specific cases, next generation technologies are required to stem the tide of population losses. Such technologies include the generation of primordial germ cells (PGCs) from induced pluripotent stem cells (iPSCs), that may serve as starting material for *in vitro* gametogenesis, *in vitro* fertilisation, and testis transplantations.

This study generated iPSCs from primary dunnart fibroblasts using a transposase-based approach and dox-inducible OSKLM factors. Pluripotency of iPSCs was validated using transcriptomic analysis and embryoid body formation. Subsequently, PGC-differentiation protocols established in mouse or human iPSCs were applied. The extent of PGC differentiation was measured using qPCR for genes associated with pluripotency and PGC specification (POU5F1, NANOG, KLF4, PRDM1, PRDM14, KIT), as well as flow cytometry to assess cell surface expression of ITGA6.

Dunnart iPSCs demonstrated morphological features characteristic of a pluripotent state, and strongly activated transcript networks associated with pluripotency in other species. Embryoid bodies were readily formed, and expressed markers associated with differentiation into multiple germ layers, validating the functional pluripotency of dunnart iPSCs. Applying methods for differentiating mouse or human iPSCs into PGCs resulted in robust induction of PGC-specific genes in multiple dunnart iPSC lines. Consistent with results in human iPSCs, PGC differentiation increased the expression of ITGA6.

Altogether this study has established a method to reliably generate marsupial iPSC lines that exhibit critical hallmarks of pluripotency. These cells exhibit features of germline competence as evidenced by expression of PGC-specific markers in response to factors involved in mouse and human PGC differentiation. These findings provide an invaluable tool for development of next-generation conservation technologies that can be used to bolster population numbers and genetic diversity, as well as in marsupial de-extinction efforts.

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Variants in *SART3* underlie a syndromic condition with gonadal dysgenesis

Katie Ayers[1], Stefanie Eggers[2], Ben Rollo[3], Nadia Davidson[4], Nicole Siddall[5], Liang Zhao[6], Josephine Bowles[7], Katherine Smith[8], Karin Weiss[9], Ginevra Zanni[10], Lydie Burglen[11], Shay Ben Shachar[12], Jenny Rosensaft[13], Annick Rothschild[14], Anne Jorgensen[15], Ralf Schittenhelm[16], Cheng Huang[16], Gorjana Robevska[1], Jocelyn van den Bergen[1], Franca Casagranda[5], Justyna Cyza[1], Svenja Pachernegg[1], David Wright[3], Melanie Bahlo[4], Alicia Oshlack[17], Terrence O'Brien[3], Patrick Kwan[3], Peter Koopman[6], Gary Hime[5], Nadine Girard[5], Chen Hoffmann[18], Yuval Shilon[19], Amnon Zung[19], Enrico Bertini[10], Mathieu Milh[20], Bochra Ben Rhouma[21], Neila Belguith[22], Anu Bashamboo[23], Kenneth McElreavey[23], Ehud Banne[13], Naomi Weintrob[18], Bruria Ben Zeev[24], Andrew Sinclair[1]

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

2. Victorian Clinical Genetics Services, Melbourne, Australia

3. Department of Neuroscience, Central Clinical School, Monash University, Alfred Centre, Melbourne, Vic, Australia

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

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

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

7. The University of Queensland, Brisbane, Australia

8. AstraZeneca, Cambridge, UK

9. The genetics institute Rambam Health Care Campus, The Ruth and Bruce Rappaport Faculty of Medicine, , Technion-Israel Institute of Technology, , Haifa, Israel

10. Bambino Gesù Children's Hospital, IRCCS, , Rome, Italy

11. Centre de référence des malformations et maladies congénitales du cervelet, Et Laboratoire de Neurogénétique moléculaire, Département de génétique et embryologie médicale, APHP., Sorbonne Université, Hôpital Trousseau, , Paris, France

12. Tel-aviv Sourasky Medical Center, Tel-aviv, Israel

13. Genetics Institute, Kaplan Medical Center, Hebrew University Hadassah Medical School, Rehovot, Israel

14. Edmond and Lily Safra Children's Hospital, Chaim Sheba Medical Center, Ramat Gan, Israel

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

16. Monash Proteomics & Metabolomics Facility, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, , Monash University, Clayton, Australia

17. Peter Maccallum Cancer Center, Melbourne, VIC

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

19. Kaplan Medical Center, , Hebrew University Hadassah Medical School, Rehovot, Israel

20. Department of Neuroradiology. Timone Hospital. , Aix-Marseille Univ. APHM, Marseille, France

21. Higher Institute of Nursing Sciences of Gabes, University of Gabes, Gabes, Tunisia

22. Laboratory of Human Molecular Genetics, Faculty of Medicine of Sfax, Sfax University, Sfax, Tunisia

23. L'institut Pasteur, Paris, France

24. Sheba Medical Center, Tel Aviv, Israel

Differences of sex development (DSD) represent a major paediatric concern and can be associated with >200 congenital conditions. Clinical management of these conditions is challenging but can be guided by a genetic diagnosis to improve patient health and wellbeing.

Our group employs genomic sequencing of DNA from undiagnosed patients with syndromic DSD to find novel genetic causes. Recently we have identified recessive variants in the *Squamous cell carcinoma antigen recognized by T cells 3* (*SART3*) gene in nine individuals from six families. All affected individuals have intellectual disability, global developmental delay and a subset of brain anomalies. 46,XY children have gonadal dysgenesis (and have female or under-virilised male sex characteristics) whereas 46,XX children appear to have functional ovaries.

SART3 is an RNA-binding protein with numerous biological functions including recycling small nuclear RNAs to the spliceosome. SART3 is highly conserved across species. To test a role for *SART3* in gonadal development, we carried out knockdown experiments of the *Drosophila* orthologue *rnp4f*. This revealed a conserved role in embryonic neuronal development, and also disrupted testis but not ovarian function, consistent with patient findings. To test pathogenicity of the patient *SART3* variants we introduced these into human induced pluripotent stem cells (iPSCs). Variant iPSCs showed significant disruption of multiple signalling pathways and upregulation of spliceosome components. Using our recently developed stem cell-derived model of the gonad, we found that variant iPSCs had aberrant differentiation into gonadal cells and disruption to key testis signalling components. iPSCs also had disrupted differentiation into neuronal cells *in vitro*.

Collectively, these findings suggest that bi-allelic variants in *SART3* underlie a new syndromic DSD. We hope these findings will enable additional diagnoses and improve outcomes for individuals with syndromic differences of sex development.

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Exploring the intersection between metabolism and spermatogonial stem cell function

Katerina B Damyanova[1, 2], Ilana R Berntstein[1], Brett Nixon[1, 2], Tessa Lord[1, 2]

1. The University of Newcastle, Callaghan, NSW, Australia

2. Hunter Medical Research Institute, Infertility and Reproduction Research Program, Newcastle, New South Wales, Australia

Spermatogonial stem cells (SSCs) are a pivotal cell population responsible for maintaining lifelong spermatogenesis, however, gaps in knowledge exist in our understanding of the metabolic requirements for SSC maintenance. Recently, our research group identified that SSCs reside in hypoxic microenvironments in the testis (Bernstein et al., 2023; under review). We hypothesised that this low oxygen availability would drive glycolytic metabolism and be intertwined with SSC maintenance, as is the case with other adult stem cell types. A first-pass single-cell RNA sequencing analysis of mouse and human spermatogonia confirmed that SSCs possess a transcriptomic signature reminiscent of a cell residing in hypoxia and utilising glycolytic metabolism (e.g., *Aldoa*, *Pdk2, Gapdh, Myc*), while that of progenitor and differentiating spermatogonia reflected mitochondrial biogenesis and oxidative phosphorylation (e.g., *Ndufb7, Polg, Opa1, Cox11*). Follow-up analyses assessing mitochondrial copy number in SSCs and progenitors (isolated from neonatal *ID4-EGFP* transgenic mice) corroborated this, identifying an approaching-significant increase in copy number ($p=0.0639$) upon the SSC-to-progenitor transition. To explore any interconnection between these metabolic preferences and a hypoxic state, we employed pharmacological stabilisation of hypoxia-inducible factors using a prolyl hydroxylase inhibitor, Daprodustat. Daprodustat treatment of undifferentiated spermatogonia in culture decreased oxygen consumption and increased extracellular acidification rates indicative of a shift towards glycolytic metabolism. Importantly, Daprodustat treatment significantly improved the maintenance of SSCs *in vitro* ($p<0.05$), as demonstrated by a spermatogonial transplantation assay. These findings highlight a pivotal role for hypoxia in mediating metabolic adaptation and self-renewal of SSCs and suggest that mitochondrial biogenesis and a shift to aerobic metabolism are important components of spermatogonial differentiation. Additionally, this research provides compelling evidence for the therapeutic potential of manipulating hypoxic pathways to maintain and manipulate SSCs *in vitro*. This could have direct implications for the development of stem cell therapies aimed at reversing male infertility.

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Defining the fat-tailed dunnart (Smithopsis crassicaudata) seminiferous epithelial cycle and development of marsupial spermatogonial stem cells isolation, enrichment, and culture techniques.

Patrick R Tatt[1], Gerard A Tarulli[1], Katerina Damyanova[2], Stephen R Frankenberg[1], Tessa Lord[2], Andrew J Pask[1]

1. School of Biosciences, University of Melbourne, Melbourne, VIC, Australia

2. Biological Sciences, The University of Newcastle, Newcastle, NSW, Australia

The absence of advanced technologies such as genomic modification and artificial reproductive technologies (ART) limits marsupial research and conservation. We are working on filling this gap by generating stem cells lines, focusing on spermatogonial stem cells (SSCs), the undifferentiated progenitors of sperm. As SSCs have well-defined applications in genetic modification and *in vitro* gametogenesis, they provide a logical and valuable avenue to rapidly develop these technologies in marsupials. However, the lack of established markers for marsupial SSCs prevents the application of traditional enrichment techniques.

In this study, the fat-tailed dunnart (*Smithopsis crassicaudata*) seminiferous epithelial cycle was defined using histology, and key steps of SSC development were identified to optimise their enrichment. The timing of gonocyte migration to the basement membrane was defined using immunohistology targeting UCHL1 (PGP9.5), a marker of A-single/paired undifferentiated

spermatogonia. To enrich for live dunnart SSCs, testis tissue was enzymatically digested followed by differential plating, and fluorescence-activated cell sorting using custom dunnart antibodies specific for spermatogonia and germ cells (TSPAN8 and KIT). Quantitative real-time PCR was used to analyse the expression of stem cells markers (*GFRA1, POU5F1*), differentiating germ cells markers (*ESCO2*) and somatic cells (*GATA6, SOX9, NR2F2, CYP11A1*) within sorted populations.

UCHL1 staining demonstrated that dunnart SSC differentiation has occurred by day 110 in the dunnart. Therefore, to maximise SSC enrichment efficiency, dunnarts younger than 110 days old should be used. Increased germ cell markers and decreased somatic cell markers were observed in the non-adherent fraction of differentially plated single cell suspensions. Consistent with this, KIT-specific antibodies enriched for differentiating germ cells. All these findings will enable an increase in dunnart SSC enrichment. This will allow us to determine culture conditions and increase the efficiency of downstream applications such as SSC-based marsupial genomic editing, as well as ART techniques to enhance marsupial conservation efforts.

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A surge in cytoplasmic viscosity triggers nuclear remodelling required for Dux silencing and preimplantation embryo development

Yunan Ye[1], Hayden A Homer[1]

1. Centre for Clinical Research, The University of Queensland, Brisbane, QLD, Australia

Embryonic genome activation (EGA) marks the transition from dependence on maternal transcripts to an embryonic transcriptional program. The precise temporal regulation of gene expression, specifically the silencing of the Dux/MERVL program during late 2-cell interphase, is crucial for developmental progression in mouse embryos. How this finely tuned regulation is achieved within this specific window is poorly understood. Here, using particle-tracking microrheology throughout the mouse oocyte-to-embryo transition, we identify a surge in cytoplasmic viscosity specific to late 2-cell interphase brought about by high microtubule and endomembrane density. Importantly, preventing the rise in 2-cell viscosity severely impaired nuclear reorganisation resulting in a persistently open chromatin configuration and failure to silence Dux/MERVL. This in turn derailed embryo development beyond the 2- and 4-cell stages. Our findings reveal a mechanical role for the cytoplasm in regulating Dux/MERVL repression via nuclear remodelling during a temporally confined period in late 2-cell interphase.

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Multiplication of bovine embryos via blastomere separation techniques

Danielle Azoulay[1], Nicholas I. Cameron[2], Wilhelmus T.L. te Brake[2], Michael B. Morris[1], Margot L. Day[1]

1. School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia

2. Nbryo Pty Ltd, Brisbane, QLD, Australia

As the global population grows, the demand for dairy (bovine) and beef products rises. Efficient production of large numbers of high-quality livestock can help alleviate protein shortages. Embryo twinning offers a significant opportunity to amplify numbers of transferable embryos. Twinning can be artificially induced by separating blastomeres of cleavage-stage embryos and culturing them to create multiple blastocysts. Previously, blastocysts have been obtained from individual blastomeres from 2-cell, 4-cell and 8-cell stage embryos in bovine and other species (1,2,3,4,5). However, the maximum number of embryos produced from a single embryo was four (2,4).

This work aims to enhance bovine embryo twinning for production of viable calves. Blastomeres from 2-, 8-, 16-, or 32-cell embryos were separated and cultured to the blastocyst stage, individually, in pairs or quads. Blastomeres were either separated once (N=1 separation) or serially separated at the 2-cell stage, and then at each subsequent cleavage for three cleavages (N=4 separations). There was no difference in blastocyst formation from serial N=4 separations, from the 2-cell stage (19.5%) compared to N=1 separation at the 16-cell stage (17.6%). Pairs of blastomeres separated at 8-, 16-, and 32-cell stages developed to the blastocyst stage better than individual blastomeres, regardless of cell stage. Additionally, culture of quads of blastomeres, separated at the 32-cell stage, resulted in greater blastocyst development (61.4%) than pairs separated at the 8-cell (26.6%), 16-cell (47.2%) and 32-cell (29.1%) stages.

Preliminary embryo transfer studies, with blastocysts derived from either pairs or quads, resulted in 29.6% (n=27) and 36.4% (n=11) pregnancies and birth of 6 and 2 calves, respectively. In conclusion, blastomere separation techniques present an appealing approach for large scale production of embryos for improvement of genetic gains from high quality donors and sires.

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Elucidating the function of Sialic acid-binding immunoglobulin-like lectin-6 (SIGLEC6) in preeclampsia

Manju Kandel[2, 1], Lucy A Bartho[2, 1], Natalie J Hannan[2, 1], Ping Cannon[2, 1], Tuong-Vi Nguyen[2, 1], Natasha Pritchard[2, 1], Anna Nguyen[2, 1], Ciara N Murphy[2, 1], Georgia P Wong[2, 1], Stephen Tong[2, 1], Tu'uhevaha J Kaitu'u-Lino[2, 1]

1. Mercy Perinatal, Heidelberg, Victoria, Australia

2. University of Melbourne, Melbourne, VIC, Australia

SIGLEC6, a transmembrane receptor, is highly expressed in placenta in a human-specific manner. Our team (unpublished data) have identified SIGLEC6 as a novel biomarker of preeclampsia. We have shown SIGLEC6 is elevated in the maternal circulation preceding preeclampsia, among those diagnosed with preeclampsia, and is associated with increasing disease severity. The current study aimed to characterise SIGLEC6 and assess its potential role in disease pathogenesis using human (cyto)trophoblast stem cells (hTSCs), and primary Human Umbilical Vein Endothelial Cells (HUVECs).

SIGLEC6 mRNA expression was measured in three distinct placental cell types: cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast – using a first trimester hTSCs. We found that SIGLEC6 is expressed across all three cell types, with its highest expression observed in syncytiotrophoblast. Given preeclampsia is associated with placental hypoxia and inflammation, we next exposed syncytialised hTSCs to either hypoxia (1% Oxygen vs 8% Oxygen) or increasing doses of inflammatory cytokines IL-6 or TNFa. Hypoxia increased SIGLEC6 secretion (p=0.008) and mRNA expression (p=0.008) in syncytialised hTSCs. Similarly, there was an increase in SIGLEC6 secretion (IL-6: p=0.002, and TNFa: p=0.01) and mRNA expression (IL-6: p=0.002, and TNFa: p=0.009) with inflammation.

We next assessed whether the high levels of circulating SIGLEC6 observed in the maternal circulation with preeclampsia might induce endothelial dysfunction, a clinical feature of preeclampsia. Treatment of primary HUVECs with recombinant SIGLEC6 modestly increased VCAM1 (endothelial dysfunction marker, p=0.01), and HO-1 (cytoprotective molecule, p=0.03) mRNA expression, but had no effect on the mRNA expression of other endothelial dysfunction markers (ICAM-1 and ET-1), anti-angiogenic (sFlt-1 e15a and sFlt-1 i13), or pro-angiogenic (VEGFA, and PlGF) markers. Furthermore, increasing doses of SIGLEC6 had no effect on HUVEC proliferation.

We demonstrate that SIGLEC6 is induced by placental hypoxia and inflammation and that high circulating levels may induce endothelial dysfunction, which is a key characteristic of preeclampsia.

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Differences in gut microbiota composition and function are present before the development of symptoms of preeclampsia

Peter Sternes[1], Faisal Altemani[2], Helen L Barrett[3, 4], Leonie K Callaway[5, 6], David McIntyre[7], Gene Tyson[1], Marloes Dekker Nitert[2]

1. Centre for Microbiome Research, School of Biomedical Sciences, Queensland University of Technology, Wooloongabba, QLD, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

3. Obstetric Medicine, Royal Hospital for Women, Randwick, NSW, Australia

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

5. Women's and Newborns, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

6. Faculty of Medicine, The University of Queensland, St Lucia, Qld, Australia

7. Mater Medical Research Institute, South Brisbane, QLD, Australia

Preeclampsia, the presence of hypertension and dysfunction in one or more maternal organ systems, is a common pregnancy complication. Late-onset preeclampsia, which occurs after 34 weeks, is linked to metabolic and immune dysfunction. It is unclear if the altered composition of the gut microbiota in overt preeclampsia occurs prior to symptom development. Altered gut microbiota composition may reduce gut wall integrity, which increases the transfer of inflammatory molecules into the host or through reduced secretion of beneficial metabolites to the host. This study aims to investigate if changes to the gut microbiota occur before symptoms.

Ten participants in the Study of PRobiotics IN the prevention of GDM (SPRING), who developed late-onset preeclampsia (DPE) were matched with 24 participants who remained normotensive throughout pregnancy (C). Stool samples were collected at 28 weeks gestation and subjected to metagenomic sequencing. Metagenome assembled genomes were collected and combined with the Unified Human Gastrointestinal Genome collection and used for taxonomic assignment. Metagenome sequences were also mapped to the UniRef90 database and assigned to MetaCyc metabolic pathways.

The beta diversity of the gut microbiota in DPE women was significantly different from the C group (P=0.0024), with no differences in richness or evenness (alpha diversity) between the groups. DPE women demonstrated an enrichment of 61 species, particularly multiple members of the *Enterocloster* and *Blautia* genera but depletion of 33 species including multiple members of the *Collinsella* and the short-chain fatty acid-producing *Faecalibacterium* genera. Metabolically, 14 pathways were enriched in DPE women, including the glycosaminoglycan degradation pathway.

Our results indicate that there are changes to the gut microbiota composition in DPE women. Increased glycosaminoglycan degradation of the extracellular matrix components of the gut wall could contribute to decreased gut wall integrity and thereby increase inflammatory signalling in the mother thereby contributing to the development of symptoms.

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SPINT1: expression, regulation and function in the developing placenta

Ciara N Murphy[1, 2], Natalie J Hannan[1, 2], David Simmons[3], Tuong-Vi Nguyen[1, 2], Ping Cannon[1, 2], Manju Kandel[1, 2], Georgia P Wong[1, 2], Anna Nguyen[1, 2], Natasha Pritchard[2], Stephen Tong[1, 2], Tu'uhevaha J Kaitu'u-Lino[1, 2]

1. Obstetrics, Gynaecology & Newborn Health, The University of Melbourne, Melbourne, Victoria, Australia

2. Mercy Perinatal, Mercy Hospital for Women, Heidelberg, Victoria, Australia

3. School of Biomedical Sciences, The University of Queensland, St Lucia, QLD, Australia

Low Serine Peptidase Inhibitor Type I (SPINT1) accompanies fetal growth restriction (FGR) in humans. However, its significance for placental pathophysiology is not yet known. We investigated the expression, regulation, and function of SPINT1 in placentation.

SPINT1 immunohistochemistry and *in situ* hybridisation on placenta localised protein and mRNA to cytotrophoblast across gestation. Human (cyto)Trophoblast Stem Cells (hTSC) model first trimester placenta. We showed SPINT1 is expressed in hTSCs as well as differentiated lineages; syncytiotrophoblast and extravillous trophoblast (EVT), albeit at lower levels (~50% mRNA, $p<0.01$) in syncytiotrophoblast.

To explore SPINT1 regulation, protein and mRNA were measured in hTSCs subjected to: (a) Hypoxia (1% O_2), relative to first trimester (3%) and term (8%) normoxia. No significant differences were identified comparing 1% vs 3%, however, relative to 8%, hypoxia reduced *Spint1* by 40% ($p<0.01$) and protein secretion by 50% ($p<0.01$); (b) Knockdown of transcription factors that regulate SPINT1 in other tissues (siRNA; CDX2, GRHL2). This did not alter *Spint1* transcripts, although cellular and secreted protein reduced with siGRHL2 (<40%, $p<0.01$) and nearly doubled with siCDX2 ($p<0.01$), suggesting post-transcriptional modifications; (c) Inhibiting MMP-mediated secretion using Batimastat, which reduced SPINT1 secretion by 28% (10mM, $p<0.05$).

SPINT1 was silenced (siRNA) in hTSCs and the effect on cell function assessed. hTSC Proliferation (measured using xCELLigence) decreased after 96 hours ($p<0.05$). hTSC differentiation was impaired, with knockdown causing retained cytotrophoblast marker expression *TEAD4* (~140% control, $p<0.05$) in syncytiotrophoblast and 40% reduced EVT marker *HLA-G* ($p<0.05$) in EVTs. SPINT1 inhibits a range of proteases, however silencing it had no significant effect on proteolytic activity (fluorogenic substrate). Although, addition of small molecule SPINT mimetic led to enhanced protease inhibition after 12 hours (reduced 78%, $p<0.05$).

This work elucidates potential regulators of decreased SPINT1 observed in FGR and some effects this may have on placental development and subsequent function.

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New FKBPL-targeting treatment for preeclampsia rescues first trimester trophoblast cell proliferation and endothelial cell dysfunction

Sahar Ghorbanpour[1], Dinara Afrose[1], Philip Hansbro[1], Qian Peter Su[1], Mary Meltem Kavurma[2], Lana McClements[1]

1. University of Technology Sydney, Sydney, NSW, Australia

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

Preeclampsia is the leading cause of mortality and morbidity in pregnancy, and, still, it is a condition without a cure. Aberrant placental development and growth due to inappropriate trophoblast and/or endothelial cell (EC) function, are the underlying causes. FK506-binding protein like (FKBPL) is emerging as an important mechanism in the pathogenesis of preeclampsia[1] and a predictive and diagnostic biomarker[2]. In this study, we aimed to determine whether the FKBPL-based therapeutic mimetic peptide, AD-01, can abrogate impairment in trophoblast proliferation, oxidative stress and EC migration *in vitro,* in 2D and 3D microfluidic models of preeclampsia.

First trimester trophoblast cells, ACH-3Ps, were exposed to various preeclamptic stimuli in 2D, including hypoxia (dimethyloxalylglycine, DMOG, 1 mM), inflammation (tumour necrosis factor, TNF-α, 10 ng/ml) or mitochondrial dysfunction (Rho-6G, 1 µg/ml). AD-01 (100 mM), was added as a treatment at the same time, for 48 h. Trophoblast proliferation and uric acid concentrations were measured using MTT and Uric Acid Assay Kit, respectively. Human microvascular endothelial cells (HMEC-1) were treated ± FKBPL siRNA or exposed to inflammatory or hypoxic conditions using macrophage-condition medium (MCM) or DMOG (1mM), respectively, ± AD-01 (100mM). A 3D microfluidics chip was developed. EC migration and 3D cellular protein expression was determined.

AD-01 rescued the impaired ACH-3Ps proliferation induced by DMOG ($p<0.05$) or Rho-6G ($p<0.001$). AD-01 also abrogated the TNF-α-mediated uric acid increase ($p<0.001$). FKBPL protein expression was reduced following DMOG treatment ($p<0.05$), whereas AD-01 normalised both HIF-1α ($p<0.01$) and FKBPL expression ($p<0.001$). MCM increased both HIF-1α ($p<0.05$) and FKBPL ($p<0.01$) expression, whereas AD-01 abrogated this effect ($p<0.05$-0.001). *S*uppression of FKBPL with siRNA or via MCM/DMOG, stimulated HMEC-1 migration within the chip ($p<0.05$-0.001).

FKBPL-based therapeutic peptide, AD-01, restored the angiogenic imbalance via FKBPL, and could be a viable treatment option for preeclampsia, with dual utility capable of rescuing trophoblast and EC dysfunction.

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Maternal C1q deficiency: Implications for cardiovascular adaptations and pregnancy outcome.

Evangeline A Lovell[1], Shanna L Hosking[1], Holly M Groome[1], Matthew Chapman[2], Sven Y Surikow[2], Laura J Parry[3], Sarah A Robertson[1], Alison S Care[1]

1. Robinson Research Institute and School of Biomedicine, University of Adelaide, Adelaide, SA, Australia, University of Adelaide, Adelaide, SA, Australia

2. Cardiology Unit, Lyell McEwin Hospital, Northern Adelaide Local Health Network, Adelaide, SA, Australia

3. Robinson Research Institute and School of Biosciences, University of Adelaide, Adelaide, SA, Australia, University of Adelaide, Adelaide, SA, Australia

Cardiovascular complications of pregnancy such as preeclampsia predispose women to later life obesity, metabolic syndrome, and cardiovascular disease. Some women with preeclampsia exhibit low peripheral blood concentrations of complement regulatory protein C1q. C1q may be involved in the events of placental development, but its precise physiological role has not been investigated. We therefore hypothesised that maternal C1q deficiency could impair maternal cardiovascular function and lead to adverse pregnancy outcomes.

To investigate this, female mice with a null mutation in *C1qa* ($C1qa^{-/-}$ mice) were mated to BALB/c males. Wild-type C57BL/6 females mated to BALB/c males served as controls. Blood pressure was measured before and during pregnancy using tail cuff plethysmography. Uterine artery function was evaluated on day 9.5 post coitum (pc) using ultrasound bio-microscopy, and cardiac function was assessed on day 17.5pc using echocardiography.

There were no differences in mean arterial pressure (MAP) between $C1qa^{-/-}$ and wild-type mice before or during mid-pregnancy. However, in late-pregnancy, MAP decreased in wild-type mice, but not in $C1qa^{-/-}$ mice, indicative of impaired haemodynamic adaptation. In mid-pregnancy, uterine artery dysfunction was evident, with a 16% increase in uterine artery resistance index. Impaired uterine spiral artery remodelling was also observed, with a 41% reduction in artery lumen area, and an 11% reduction in uNK cell abundance ($P<0.05$). There was evidence of cardiac dysfunction in $C1qa^{-/-}$ dams in late-pregnancy with a 20% increase in cardiac weight, a 29% increase in left ventricular mass, and a 39% increase in cardiac output, compared to wild-type mice (all $P<0.05$).

These data demonstrate that maternal C1q deficiency has adverse consequences for maternal cardiovascular adaptations to pregnancy. This underscores the significance of C1q as a key immune regulator in cardiac function and vascular adaptations and identifies C1q as a candidate target for interventions to optimise pregnancy success and postpartum cardiovascular health.

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Eplerenone may offer a potential treatment for long-term complications of cardiovascular health following preeclampsia

Natalie K Binder[1, 2], Natasha de Alwis[1, 2], Bianca R Fato[1, 2], Morag J Young[3], Natalie J Hannan[1, 2]

1. Therapeutic Discovery and Vascular Function in Pregnancy Group, Department of Obstetrics & Gynaecology, University of Melbourne, Heidelberg, Victoria, Australia

2. Mercy Perinatal, Mercy Hospital for Women, Heidelberg, Victoria, Australia

3. Cardiovascular Endocrinology, Baker Heart & Diabetes Institute, Melbourne, Victoria, Australia

Preeclampsia is a devastating complication of pregnancy featuring profound injury to systemic vasculature, major organs, and the feto-placental unit, and kills approximately 70,000 pregnant people and 500,000 babies each year. Having a pregnancy complicated by preeclampsia results in serious increased (up to 5-fold) risk of subsequently developing cardiovascular disease. Here we model preeclampsia in the mouse via nitric oxide blockade and examine the effect of therapeutic intervention during pregnancy (esomeprazole; proton pump inhibitor) and postpartum (eplerenone; aldosterone antagonist) with respect to long-term indices of cardiovascular health.

CBA x C57BL/6 female mice consuming increased salt (0.9% NaCl drinking water), received 50mg/kg/day N(ω)-nitro-L-arginine methyl ester from pregnancy day (D)7.5 to block nitric oxide production and induce a preeclampsia-like phenotype. Mice were treated with 12.5mg/kg/day esomeprazole during pregnancy, alone or in combination with 55.5mg/kg/day eplerenone during the postpartum period. Maternal blood pressure was measured via tail cuff plethysmography throughout pregnancy (D13.5, D15.5, D17.5) and weekly postpartum (up to 10 weeks). Fetal growth was assessed at birth, and the mesenteric arcade collected for assessment of vasoactivity (via wire myography) at 5- and 10-weeks postpartum.

This model generates a preeclampsia-like pregnancy with maternal hypertension (elevated blood pressure at D17.5 of gestation) and significant fetal growth restriction. We also observed impaired maternal vasoactivity at 5-weeks postpartum. Esomeprazole treatment during gestation modestly reduced maternal hypertension, but did not rescue fetal growth, nor improve vasoactivity at 5- or 10-weeks postpartum compared to control (vehicle treated). Postpartum eplerenone treatment (± esomeprazole during gestation) resulted in significant improvements in maternal vasoactivity, directly demonstrating reduced vasoconstriction to phenylephrine at 5-weeks postpartum and enhanced vasorelaxation to acetylcholine at 10-weeks postpartum, suggestive of improved postpartum cardiovascular indices.

In our mouse model of preeclampsia with postpartum investigation of cardiovascular health, administration of eplerenone postpartum significantly improves vasoactivity at 5-weeks following a preeclampsia-like pregnancy.

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Characterisation of maternal plasma pregnancy zone protein as a potential biomarker for preeclampsia risk

Demi K Georgiou[1, 2], Jordan H Cater[3, 4], Noralyn B Manucat-Tan[1, 2], Dylan McCullough[1, 2], Shalem Y Leemaqz[1, 2], Tanja Jankovic-Karasoulos[1, 2], Claire T Roberts[1, 2], Amy R Wyatt[1, 2]

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

2. Flinders Health and Medical Research Institute, Flinders University, Bedford Park, South Australia, Australia

3. School of Chemistry and Biomolecular Science, University of Wollongong, Wollongong, New South Wales, Australia

4. Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, New South Wales, Australia

Pregnancy involves dramatic changes to the maternal proteome that are essential for the growth and development of babies. Remarkably, very little is known about the importance of pregnancy zone protein (PZP), a major pregnancy-associated plasma protein that's been shown to stabilise misfolded proteins *in vitro* (1). A few studies have reported an association between decreased maternal plasma PZP in preeclampsia (PE) (2, 3), but conflicting data has also been reported (4).

We quantified PZP in maternal plasma by ELISA (N=32 normotensive, N=31 PE) at 34 weeks' gestation. PZP was abundant in third trimester (mean 222.6 µg/mL, SD 182.2. µg/mL) but substantially less than reported previously (2, 5). PZP was also significantly lower in women with PE compared to normotensive pregnancy (median 123.1 µg/mL, IQR 50.1–247.5 µg/mL; 252.4 µg/mL, 88.3–405.1 µg/mL, respectively; *p=.016*; matched by maternal age, gestational age, and BMI). Interestingly, a moderate positive correlation between PZP level and parity was identified in normotensive pregnancy (rs=0.461, *p=.009),* but *not* in PE, suggesting that parity is an important consideration.

To address this, and other methodological limitations that potentially confound the association between PZP and PE reported in prior studies, we are currently analysing maternal PZP levels in two large nulliparous pregnancy cohorts, SCOPE and STOP (Table 1). Our preliminary analyses suggest that in the first trimester the odds of a woman having PE *increase* as PZP concentration increases.

Early identification of PE risk is essential for enhancing outcomes for mother and baby, but this remains a key clinical challenge. Increased protein misfolding has been shown in PE (6, 7, 8, 9), so elevated PZP concentration may indicate an adaptive response to physiological stress. A deeper understanding of PZP actions in pregnancy and the factors influencing its changing abundance across gestation is needed to uncover its potential as a prognostic biomarker.

Table 1: Pregnancy outcomes and sample size for STOP and SCOPE samples in which PZP concentration has been measured.

Pregnancy Outcome	**n**		
	SCOPE	**STOP**	**Total**
Preeclampsia	79	119	198
Gestational hypertension	64	78	142
Small for gestational age	67	103	170
Spontaneous preterm birth	44	53	97
Gestational diabetes	28	134	162
Other complication	117	67	184
Uncomplicated	464	696	1,160
Total	863	1,250	2,113

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Investigating side-population marker expression in placental insufficiency

Georgia P Wong[1, 2], Natalie J Hannan[1, 2], Ping Cannon[1, 2], Manju Kandel[1, 2], Anna Nguyen[1, 2], Tuong-Vi Nguyen[1, 2], Natasha Pritchard[1, 2], David G Simmons[3], Tu'uhevaha J Kaitu'u-Lino[1, 2]

1. Mercy Perinatal, Mercy Hospital for Women, Heidelberg, Victoria, Australia

2. Department of Obstetrics & Gynaecology, University of Melbourne/Mercy Hospital for Women, Heidelberg, Victoria, Australia

3. School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia

An emerging concept suggests dysregulated progenitor cell populations may contribute to placental insufficiency pathogenesis. Side-population cells are identified by their ability to efflux DNA dye, (Hoechst33342) and demonstrate progenitor properties. Recently, side-population cells were isolated from human placenta and identified as having enriched expression of 8 specific genes, relative to other cells (Gamage et al 2020, *Stem Cell Rev Rep*). We characterised these side-population markers in placentas from patients with preeclampsia or fetal growth restriction (FGR), and in differentiating human trophoblast stem cells (hTSCs).

mRNA expression of placental side-population markers *ELL2, GATA6, HK2, HLA-DPB1, CXCL8, INTS6, SERPINE3* and *UPP1* were measured in <34-week human placenta (n=78 preeclampsia, n=30 FGR, n=18 gestation-matched controls). *ELL2, HK2* and *CXCL8* mRNA were elevated in preeclamptic (p=0.0006,p<0.0001,p=0.0335 respectively) and FGR placentas (p=0.0065, p<0.0001,p=0.0001 respectively) versus controls. Conversely, *GATA6* was reduced in placentas from both preeclampsia and FGR (p=0.0014,p=0.0146 respectively).

To identify the cells expressing side-population markers, multiplex immunofluorescence was used in human placental sections (n=3 each group, 3 proteins/serial section). We next sought to identify whether the 4 side-population markers most dysregulated in preeclampsia/FGR were altered as hTSCs underwent differentiation into extra-villous trophoblast (EVTs) or fusion into syncytiotrophoblasts across 96h (n=5). EVT differentiation was confirmed by reduced hTSC marker, *TEAD4* (p<0.007); and elevated EVT marker *HLA-G* (p<0.026). *ELL2, GATA6* and *HK2* all increased with EVT differentiation (p=0.0015,p=0.0018,p<0.0257), whereas *CXCL8* was unaltered. Syncytiotrophoblast differentiation was confirmed at 96h by *TEAD4* loss (p<0.018) and elevated *SDC1* (p<0.0223). As cells sycnytialised, *CXCL8* and *GATA6* expression were unchanged while *ELL2* and *HK2* increased (p<0.0407,p<0.0414).

We have demonstrated that some of the 8 side-population marker genes are dysregulated with placental insufficiency, and how they change with placental cell differentiation. Our ongoing analysis of multiplex immunofluorescence is likely to provide insight into the location and frequency of this progenitor population.

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The effects of environmental endocrine disruptors on testicular differentiation in the tammar wallaby

Joao Cunha Cyrino[1], Marilyn Renfree[1], Geoff Shaw[1], Yu Chen[1]

1. School of BioSciences, Parkville/Melbourne, VIC, Australia

The environmental endocrine disruptor, bisphenol A (BPA), found in plastics and thermal printer paper, is a xenoestrogen that affects steroid receptor signalling and synthesis and may contribute to the increasing incidence of disorders of sex development (DSDs) in vertebrates. The study investigated the effects of BPA on testicular differentiation in the tammar wallaby (*Macropus eugenii*), a marsupial whose young are born before gonadal sex differentiation (1).

Pouch young were orally treated daily with BPA (50 µg/kg) from day 0-10 postpartum (pp) (covers early male gonadal differentiation window) and collected at day 10. Another group was treated from day 20-40 pp during the male programming window (2) and sampled at day 40 pp. A third group was treated daily with fulvestrant, an estrogen receptor degrader, (1 mg/kg) between day 20-90 pp and examined at day 150 pp during urethral closure.

BPA treatment from day 0-10 pp decreased the number of SOX9-positive Sertoli cells and blocked its nuclear translocation in testes. BPA treatment from day 20-40 pp significantly decreased the number of Sertoli cells in the seminiferous tubules and also significantly downregulated the expression of genes that are important in regulating Leydig cell differentiation (*DHH* and *PTCH1*), Sertoli cell differentiation (*SOX9*) and androgen synthesis (*STAR, CYP17A1* and *POR*). These data suggest that the xenoestrogen BPA interferes tammar testicular differentiation consistent with previous studies (3-5). Fulvestrant treatment downregulated *17βHSD3* expression, the gene for a key enzyme that converts androstenedione to testosterone and together with 3α-HSD, converts androsterone to dihydrotestosterone (2), suggesting that estrogen signalling might be important in maintaining androgen synthesis during testicular differentiation, as shown previously (4-5). We conclude that these results are due to the interference with the balance of androgen and oestrogen in developing male pouch young.

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Forcing fate decisions in the male germline: Cripto promotes pluripotency and malignancy of germ cells

Cassy Spiller[1], Guillaume Burnet[2], Clare Crisp[1], Chen-Wei Allen Feng[1], Josephine Bowles[1, 3]

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

2. Department of Developmental and Stem Cell Biology, Institut Pasteur, Paris, France

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

In mice, primordial germ cells (PGCs) originate from the posterior epiblast at around embryonic day (E) 6.5. Contrary to their somatic counterparts, PGCs possess an active pluripotency network and maintain the capacity to give rise to any cell type. Following migration to the developing gonad, the PGC pluripotency program is progressively shut down as they commit to the pro-spermatogonia fate during a short developmental window (E11.5 to E14.5). PGCs that fail to effectively differentiate, by maintaining pluripotency, harbour malignant potential and are considered the origin of testicular germ cell tumours in humans.

We aimed to explore the role of *Cripto* in this developmental process. Cripto is a co-receptor for Nodal signalling and is initially expressed in PGCs until E11.5, when its expression decreases sharply as PGCs lose pluripotency. Because we found that CRIPTO is overexpressed in pluripotent human testicular germ cell tumours (incl. seminomas, embryonic carcinoma), we hypothesised that maintenance of Cripto expression in PGCs might drive malignant transformation.

We generated a novel, germ cell specific, CRE recombinase mouse line (*Ddx4-iCre*) to over-express *Cripto* in PGCs from E11.5. We found that Cripto overexpression in PGCs leads to the maintenance of a pluripotent state. Furthermore, Cripto-overexpressing (Cripto-OE) PGCs failed to correctly differentiate, eventually leading to germ cell depletion by the time of birth. Although the Cripto-OE^{PGC} model held great potential to replicate the human pathology of testicular germ cell tumours, the complete lack of germ cells in Cripto-OE^{PGC} adult mice hindered our ability to investigate whether testicular tumours would form. To circumvent the germ cell loss, we placed our Cripto-OE model on a BAX-KO background (Bax is a pro-apoptotic gene). Here, as early as 8 weeks of age, we observed Cripto-OE germ cells persisting in homogenous clusters that morphologically resemble human intra-tubular seminomas.

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Interferon epsilon as a novel anti-viral agent in the testis: Insights into mechanism of action

Rukmali Wijayarathna[1, 2], Eveline de Geus[2, 3], Rosemary Genovese[1], Linden J Gearing[2, 3], Michelle Tate[2, 3], Paul J Hertzog[2, 3], Mark P Hedger[1, 2]

1. Centre For Reproductive Health, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

2. Dept. of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Melbourne, Victoria, Australia

3. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

The testis is susceptible to viral infections, which can impair fertility. Spermatogenic cells were thought to lack anti-viral defences, including interferon (IFN) or IFN-stimulated gene (ISG) expression. Challenging this dogma, we discovered that interferon-epsilon (IFNε), a type-I IFN first identified in female reproductive epithelia, is constitutively expressed by spermatogenic cells and macrophages in mouse and human testes. Moreover, mice lacking IFNε are more susceptible to viral epididymo-orchitis. The mechanisms of IFNε-mediated anti-viral protection in the testis were examined in this study.

A human Sertoli cell line (HSerc, ScienCell) was infected with Zika virus at a multiplicity of infection (MOI) of 5 or 10. Cultures were treated with 100IU recombinant human IFNε 12h before infection (prophylactic-IFNε) or 1h after infection (therapeutic-IFNε), or diluent alone (controls). Cells and media were harvested 24h or 48h post-infection for RNAseq, qPCR, and plaque assays.

IFNε treatment increased Sertoli cell anti-viral responses and reduced viral infection, with prophylactic-IFNε being more effective than therapeutic-IFNε treatment. Plaque assays and viral RNA qPCR showed that prophylactic-IFNε reduced viral load by approximately 98%. Therapeutic-IFNε reduced viral RNA by 70% and infectious virus by 97%. Sertoli cells expressed IFNAR1 and 2 receptors required for IFNε signalling. At 24h, both IFNε-treatments significantly increased anti-viral effector genes (*ISG15*, *OAS1*, *IFI35*, *RSAD2*), reduced induction of pro-inflammatory cytokines (*CXCL10*, *CXCL11*), and supported expression of Sertoli cell functional genes (*INHA*). Notably, anti-viral and inflammatory responses were relatively lower at 48h after IFNε treatment, compared with 24h, attributable to the reduction in viral load. Genes for IFNβ and IFNλ, but not IFNα, were induced by the virus at the time points examined.

These data indicate that IFNε induces anti-viral effector responses and reduces inflammatory responses in Sertoli cells, and demonstrates the importance of constitutive expression of IFNε in the testis to limit viral infection and inflammatory damage.

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Human seminal fluid extracellular vesicles induce immune responses in female cervical cells *in vitro*

Cottrell T Tamessar[1, 2], Chishan Burch[1, 2], Tegan Bryde[1, 2], Shanu Parameswaran[1, 2], Judith Weidenhofer[3], Hui-ming Zhang[4], Sarah A Robertson[5], Geoffry N De Iuliis[1, 2], Elizabeth G Bromfield[1, 2], David J Sharkey[5], Brett Nixon[1, 2], John E Schjenken[1, 2]

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

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

3. School of Biomedical Sciences and Pharmacy, College of Health, Medicine and Wellbeing, University of Newcastle, Ourimbah, NSW, Australia

4. Central Analytical Facility, Research and Innovation Division, The University of Newcastle, Newcastle, NSW, Australia

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

Seminal fluid (SF) interacts with epithelial cells lining the female reproductive tract to activate proinflammatory responses that facilitate pregnancy. Several SF factors, including transforming growth factor-β (TGF-β), have been identified as signalling agents but do not fully explain the female response. Seminal fluid extracellular vesicles (SFEVs) likely have signalling potential given their suggested roles in immune regulation; however, their signalling capacity remains elusive. To investigate the impact of SFEVs on female reproductive tract signalling, we utilised a well-established human ectocervical epithelial (Ect1) cell culture model. Human SFEVs were isolated from SF using density-gradient ultracentrifugation and incubated with Ect1 cells for 8 h prior to assessing gene expression profiles by transcriptomics (n=4/group) and qPCR (n=8-9/group). Untreated Ect1 cells were used as a control. Transcriptomic data were analysed using Ingenuity Pathway Analysis. Following 8 h co-incubation, SFEVs altered gene expression profiles in Ect1 cells (>1.5FC, 216 genes induced, 211 suppressed, FDR<0.05). Gene expression changes and signalling pathways were predominantly associated with the inflammatory response and were confirmed by qPCR. These included *IL1A* ($p \leq 0.01$, 2.6FC), *IL6* ($p \leq 0.01$, 2.8FC) and *CXCL2* ($p \leq 0.01$, 2.1FC), which were induced in Ect1 cells following SFEV treatment. Additional comparative analysis between SF and SFEV treated Ect1 cells indicated *IL1A* (SFEV:2.6FC, SF:2.1FC) and *IL6* (SFEV:2.8FC, SF:3.0FC) were induced to equivalent levels in SFEV and SF treated cells. In contrast, genes such as *CXCL2* (SFEV:2.1FC, SF:5.8FC) appeared primarily regulated by other components of SF. Bioinformatic prediction of SFEV signalling agents identified TGF-β (Z-score=4.8, $p<0.05$) and NFkB (Z-score=5.78, $p<0.05$) as potential SFEV cargo responsible for inducing changes in Ect1 gene expression. Our ongoing research delves into the potential delivery mechanisms of these signalling agents from SFEVs to Ect1 cells. This study highlights that SFEVs serve an important role in shaping the immune environment of the female reproductive tract to promote reproductive success.

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Elevated ambient temperature alters mouse seminal vesicle parameters and the subsequent female response to seminal fluid after mating

Hon Yeung (Dexter) Chan[1], David J Sharkey[1], David A Skerrett-Byrne[2, 3], Shanu Parameswaran[2, 3], Amanda L Anderson[2, 3], Lily A MacDougall[2, 3], Shannon P Smyth[2, 3, 4], Sarah A Robertson[1], Brett Nixon[2, 3], John E Schjenken[2, 3]

1. Robinson Research Institute & School of Biomedicine, University of Adelaide, Adelaide, SA 5005, Australia

2. Hunter Medical Research Institute, Infertility and Reproductive Research Program, New Lambton Heights, NSW 2305, Australia

3. Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, Discipline of Biological Sciences, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia

4. School of BioSciences, Faculty of Science, Bio21 Institute, University of Melbourne, Parkville, VIC 3010, Australia

Extreme weather events, such as heatwaves, are forecast to increase globally over the coming decades, posing a risk to reproductive health. Our studies show that male reproductive capacity is determined not only by sperm, but also the plasma fraction (seminal plasma, SP) derived primarily from seminal vesicles. In this study, we investigated whether a sub-chronic elevation in ambient temperature can modify seminal vesicle morphology, seminal plasma composition, and/or the subsequent female response to seminal fluid after mating. Adult male Swiss mice were exposed to either control (CT, 21°C) or 'heatwave' conditions (HT, 8 hours at 35°C followed by 16 hours at 25°C) for 7 days. Seminal vesicles (SV) were excised for morphological assessment and SV fluid (SVF) was collected, with the major SP signalling factor transforming growth factor beta (TGFB) family measured using Luminex microbead assay. The endometrial response to SP was assessed 8 hours following mating using qPCR. There were no overt changes to seminal vesicle morphology, and heat exposure did not alter the weight of seminal vesicles, or the amount of proteins in SVF. However, heat exposure led to reductions in epithelial cell height (15%, p=0.03) and mucosal folding (23%, p=0.07), and was accompanied by a 1.48-fold increase in TGFB1 (p=0.05) in SVF of HT compared to CT males (n=6-8/group). Additionally, increased expression of the seminal fluid induced cytokines *Csf1* (4-fold, p=0.01), *Csf2* (22-fold, p=0.02) was observed in the endometrium of female Swiss mice following mating with HT compared to CT males (n=6-8/group). These data indicate that seminal plasma composition is responsive to ambient temperature, and that altered composition of SP in response to a sub-chronic temperature elevation can alter the nature of the female reproductive tract immune response after mating, which in turn has potential to impact fetal development and neonatal outcomes.

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The Role of Progesterone signalling in Penis Development and Hypospadias

Luke C Govers[1], Deidre M Mattiske[1], Melanie K Stewart[1], Jay R Black[2], Darryl L Russell[3], Andrew J Pask[1]

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

2. Geography, Earth and Atmospheric science, The University of Melbourne, Melbourne, Victoria, Australia

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

Hypospadias is the most common persistent congenital abnormality in males affecting 1 in every 125 live male births. Despite this already high incidence, the incidence of hypospadias has been increasing at 1% per annum across Australia and the rest of the world. This rapid increase in the incidence of this problematic phenotype has been largely attributed to the disruption of normal hormonal signalling in the reproductive tract during development, due to our increasing exposure to Endocrine disrupting chemicals (EDCs). Over the last decade we have been investigating how EDCs induce hypospadias and other differences in sexual development (DSDs). However, we still lack fundamental knowledge of the normal balance of hormones which drive male penis development. It has been our aim to address this fundamental gap. Our research has shifted the androgen centric description of penis development to one which focuses on the balance of androgen and oestrogen required to achieve normal penile development. Here we describe another novel role for hormones in penis development. Using micro-CT scanning we have provided the first in depth analyses of the anatomy of the adult penis in the progesterone receptor knockout (PRKO) mouse. We show that males that lack the progesterone receptor (PR) have an increased risk of hypospadias and show other structural abnormalities of the penis. This not only confirms a critical role for progesterone signalling in the penis but implicates this pathway as yet another potential target of endocrine disruption in the increased incidence of male DSDs.

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The koala *Phascolarctos cinereus* prostate A comprehensive histological and immunohistochemical investigation

Yolande Campbell[1], Stephen Johnston[1], Chiara Palmieri[1], Sara Pagliarani [1], Jo Gordon[1]

1. University of Queensland, Gatton, QLD, Australia

The prostate of the koala (*Phascolarctos cinereus*), and of marsupials more generally, is the primary contributor of seminal fluid, but comparatively little is known about its microanatomy and/or biochemistry. This study explored evidence of parenchymal segmentation of the koala prostate. The prostate of three sexually mature koalas, euthanised for welfare reasons, were processed for histopathology, histochemistry (Masson's trichrome, Alcian Blue, PAS staining) and immunohistochemistry (IHC) using basal (p63, CK14) and luminal (CK8/18, PSA, AR) markers. Results confirmed clear tissue segmentation of the koala prostate into three zones, anterior, central and posterior, characterised by differences in the proportion of glandular tissue, as well as the thickness of collagen fibres; there were also distinct differences in the morphology of secretions produced in each zone. Based on immunohistochemistry, the koala prostate showed evidence of both basal proliferative and luminal secretory cells. The ratio of basal to luminal cells varied across the three segments, with the central segment housing the highest density of basal cells. Globular bodies produced in the anterior zone were shown to possess the same markers as those that have been described for human prostasomes. This study appears to be the first to comprehensively document the marsupial prostate in terms of microanatomy and corresponding immunohistochemistry. While further biochemical analysis, such as proteomics of each segment will better define the relative functions of each tissue, the data presented here is consistent with the hypothesis that the koala prostate potentially represents an example of an ontological stage in the evolutionary differentiation of male eutherian accessory glands.

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VEGF signalling activates MEK1/2 to regulate vasculature patterning in the fetal mouse testis

Rheannon O Blucher[1], Rachel S Lim[2], Matthew E Ritchie[2], Patrick S Western[1]

1. Centre for Reproductive Health, Hudson Institute of Medical Research and Department of Molecular and Translational Science, Monash University, Clayton, Victoria 3168, Australia

2. Epigenetics and Development Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia

Abnormalities in testis development are strongly associated with reproductive health conditions including infertility and testis cancer. In mouse testes, the transcription factor, SOX9 and fibroblast growth factor 9 (FGF9) promote Sertoli cell development, while vascular endothelial growth factor (VEGF) signalling is essential for testicular vasculature. These growth factors signal through downstream pathways such as the MAPK signalling cascade, which is essential for cell proliferation and differentiation, but little is known about MAPK signalling during fetal testis development. We explored MAPK functions in embryonic day (E)12.5 Oct4GFP transgenic mouse testes cultured with MEK1/2 inhibitor for 24 or 72 hours. The MEK1/2 target, phospho-ERK1/2 (pERK1/2), was detected in Sertoli and endothelial cells of controls, but not in MEK1/2 inhibited samples. Flow cytometric and immunofluorescence analyses revealed that MEK1/2 inhibition reduced Sertoli cell proliferation and Sertoli cell localisation to the testis cord basement membrane. RNA sequencing in isolated gonadal somatic cells identified 116 and 114 differentially expressed genes after 24 and 72 hours of MEK1/2 inhibition. Ingenuity Pathway Analysis revealed a striking association of MEK1/2 signalling with angiogenesis, vasculogenesis and cell migration, including a failure to properly express vascular remodelling transcription factors, *Sox7* and *Sox17,* VEGF receptor genes, cell adhesion factor gene, *Cd31* and other endothelial markers. Consistent with this, section and wholemount immunofluorescence revealed that CD31, SOX7 and SOX17 protein was lost in MEK1/2 inhibited samples. Similarly, VEGF receptor inhibition reduced CD31, SOX7 and SOX17. Moreover, while VEGF inhibition eliminated pERK1/2 in endothelial cells, pERK1/2 was maintained in Sertoli cells. Our data suggests that VEGF signalling activates MEK1/2 to promote vasculature patterning in the testis and that MEK1/2 signalling independently regulates Sertoli cell proliferation and organisation. As these processes are essential for testis function, this model provides important insights into testicular patterning events that are likely to affect lifelong male reproductive health.

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Clinical interpretation of BMD may lead to new endocrine diagnosis and improved outcomes

Joanna Potts[1]

1. Private Practice, Nowra, NSW, Australia

Clinical interpretation of bone mineral density (BMD) can identify endocrine conditions and lead to effective treatment. In turn, effective treatment can lead to improvement in BMD. This statement will be highlighted by exploring three case studies. 1. A suspected, but difficult to confirm Cushing's disease, successful surgical remission 2. Severe osteoporosis in 53 year old woman with low Z-score, investigation and diagnosis of Coeliac disease and 3. Disproportionately low BMD measurement of non-dominant distal radius 33% flagging investigation and subsequent treatment of primary hyperparathyroidism. These cases will draw attention to the ability of BMD to identify underlying (secondary) causes of osteoporosis when interpreted by the (experienced) clinician. With the initiation of appropriate treatment BMD can significantly improve and the outcome for the person raised.

Raising the outcomes in endocrine nursing.

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Case study- Water deprivation test- Where the true detective work begins

Maresa Derbyshire[1]

1. St Vincent's Public Hospital, Melbourne, Fitzroy, VIC, Australia

A Water Deprivation Test (WDT) is a lengthy Endocrine procedure to diagnose Arginine Vasopressin Deficiency (AVP-D). The test requires strict supervision for patient safety, due to the risk of severe dehydration, and to ensure there is no tampering of the samples or water drinking by the patient.

From midnight patients are required to abstain from all fluids, complete a fluid balance chart, collect and measure urine samples. The following day, overnight samples are sent to pathology, with weight, urine and blood samples collected hourly. Results for Urine Osmolality (Osm), Plasma Sodium and Plasma Osm are assessed to determine the need to continue testing or discharge the patient.

An illustrative case high lightening the importance of strict patient supervision during the WDT is presented below.

A 30yr old female referred to the St Vincent's Hospital, Endocrine Testing Area (ETA) for a repeat WDT due to inconsistent results in New Zealand. The patient travelled 4hrs to St Vincent's the day prior to collect necessary equipment for the test and baseline weight.

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The Rise & Fall of Serum Prolactin – To Rest or Not To Rest?

Roghaya Fatouros[1], Julie Hetherington[1, 2], Ashish Gargya[1, 3], Albert Hsieh[1, 4]

1. Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

2. Susan Wakil School of Nursing and Midwifery, University of Sydney, Sydney, NSW, Australia

3. Faculty of Medicine & Health, University of New South Wales, Sydney, NSW, Australia

4. Central Clinical School, Faculty of Medicine, University of Sydney, Sydney, NSW, Australia

Pathological hyperprolactinaemia may be associated with hypogonadotropic hypogonadism. There are many physiological causes of hyperprolactinaemia such as pregnancy and stress. A single elevated non-rested prolactin level may lead to overdiagnosis and unnecessary pituitary imaging [1]. We conducted a study to demonstrate that a time-based rested prolactin testing reduces the overdiagnosis of hyperprolactinaemia.

Study patients were recruited from January 2019 to July 2023 through Royal Prince Alfred Hospital (RPAH) Endocrinology if there was a single elevated serum prolactin level. Patients were referred from RPAH Fertility Unit and Endocrinologists. Exclusion criteria included symptomatic or medication-induced hyperprolactinaemia, history of pituitary disorder and macroprolactinaemia.

Thirty-two patients were included (29 females, 3 males). Mean age was 33.7 (±8.5 SD) years. Fifty-three percent of the cohort were Caucasian. The pre-testing prolactin mean was 975mIU/L (±531 SD). Nine out of 32 patients had a normal rested prolactin (≤500mIU/L) at baseline. At completion, 18 patients had a prolactin level >500mIU/L. Sub-analysis indicated four more patients would have likely achieved a prolactin level ≤500mIU/L if rested longer.

[1] Whyte MB, et al. Importance of cannulated prolactin test in the definition of hyperprolactinaemia. Pituitary 2015:18(3);319-25.

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Preventing Adrenal crisis - is it more than just patient education?

Lisa Shepherd[1]

1. University Hospitals Birmingham NHS Foundation Trust, Sutton Coldfield, WEST MIDLANDS, United Kingdom

People with Adrenal Insufficiency are educated to manage their condition, medication, and stressful episodes. Despite this, there is evidence that knowledge is not always applied during times of acute need and adrenal crisis still occur. Since managing one's medication is behaviour based, interventions designed to change behaviour may assist people with adrenal insufficiency to adopt the correct regime. Healthcare interventions that increase medication adherence tend to be education based and support the assumption that improving knowledge leads to optimal adherence. To date, such interventions have focused on increasing patients' knowledge about their condition, how and when to take medication and the consequence of not managing their medications. While much is known about interventions designed to enhance medication adherence there is very little available evidence to inform and help people with Adrenal Insufficiency to manage their medication regimens. Behavioural theory can aid the investigation why this may be the case and can help close the knowledge–behaviour gap to aid development of an intervention to prevent and manage adrenal crisis.

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Assessing ciclesonide a glucocorticoid receptor prodrug to improve respiratory outcomes in preterm birth but spare neurodevelopmental deficits

Kelly L Short[1], **Rutu S Dhavan**[1], **Judy Ng**[1], **Megan J Wallace**[1, 2], **Timothy J Cole**[1]

1. Department of Biochemistry & Molecular Biology, Monash Biomedicine Discovery Institute , Clayton , Vic, Australia

2. Hudson Institute of Medical research , Clayton , Vic, Australia

In Australia 8.5% of babies are born preterm (<37 weeks) and 1.6% (5000 babies) are born very preterm (>32 weeks). Complications from preterm birth account for 40% of neonatal deaths in Australia. Potent synthetic glucocorticoids (GCs) such as betamethasone or dexamethasone are routinely given antenatally to accelerate fetal lung maturation reducing both preterm infant morbidity and mortality. Synthetic GCs are up to 20x more active than endogenous GCs and risk long-term side-effects in many other organs, particularly in the developing brain of the neonate. We have assessed a novel steroid prodrug called ciclesonide for efficiency in the fetal respiratory system, compared to betamethasone and dexamethasone using both primary fetal mouse lung cells and mouse models of preterm birth *in vivo*. The prodrug ciclesonide is activated *in vivo* to an active agonist des-ciclesonide by tissue-specific carboxylesterase (CES) enzymes. We have recently demonstrated that ciclesonide is activated *in vivo* in most peripheral organs including the lung and in contrast to dexamethasone had no effects in the postnatal mouse brain[1]. To compare the regulatory effects of ciclesonide and des-ciclesonide to dexamethasone and betamethasone we treated cultured primary mouse lung fibroblasts for 6 hours. Changes in gene expression were assessed using microarray analysis and RT-qPCR. We demonstrated that des-ciclesonide, dexamethasone and betamethasone induced and repressed the expression of a similar profile of GR-regulated respiratory genes including *Fkbp5*, *Crispld2*, *Tgm2* and *Zbtb16*. Preliminary results comparing ciclesonide to dexamethasone as an antenatal steroid treatment of pregnant mice at E15.5 and E16.5, 48 hours before birth, showed that ciclesonide in contrast to dexamethasone had no growth retardation effects on fetal pups at E18.5. Fetal mice will be analysed with markers for accelerated lung development and assessed for developmental changes in neurodevelopment.

1. 1. Jaumotte et al. 2021 Neurobiology of Disease

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Vale Richard D. Gordon and the role of Adrenal Venous Sampling in Optimizing Management of Primary Aldosteronism

Michael Stowasser[1]

1. University of Qld School of Medicine, Woolloongabba, Qld, Australia

Richard D. Gordon, an Australian pioneer of endocrinology and hypertension research, recently passed away after a brief illness, aged 89. Born in Brisbane, Gordon's training as an endocrinologist included research fellowships in Melbourne, Nashville (Vanderbilt University) under Grant Liddle and the University of Adelaide. In addition to clinical work, these fellowships heavily involved laboratory bench work, setting up, validating and trouble-shooting new assays, which added an invaluable dimension to his critical judgement and expertise as a clinical scientist. Gordon returned to Brisbane in 1970 and established Endocrine Units at Greenslopes and Princess Alexandra Hospitals and a Hypertension Unit (later Endocrine Hypertension Research Centre) at Greenslopes Hospital. During over 50 years of clinical investigation, Gordon's scientific contributions included: (1) description of the circadian rhythm for renin; (2) defining the role of the sympathetic nervous system in the regulation of renin and aldosterone in man; (3) demonstrating salt-sensitivity and the variable phenotype of a new syndrome of hypertension and hyperkalemia that bears his name; (4) drawing attention to and defining in detail a variety of aldosterone-producing adenoma responsive to angiotensin; (5) describing a new variety of familial primary aldosteronism (PA) (familial hyperaldosteronism type II); and (6) showing that PA is much more common than previously thought, and the commonest potentially curable cause of hypertension.

Among patients with PA, unilateral adrenalectomy (ADX) for those with unilateral forms affords superior outcomes in terms of cardiovascular morbidity and quality of life compared with specific medical treatment with mineralocorticoid receptor antagonists (MRAs). Adrenal venous sampling (AVS) is superior to computed tomography (CT) for differentiating unilateral from bilateral PA. AV cannulation can be difficult, but success rates can be markedly improved by using a dedicated operator, ACTH stimulation, contrast CT and POC cortisol testing. If AVS is contraindicated or declined, CT can be combined with other ancillary information (e.g. age, severity of PA, hypokalemia) to decide re ADX vs medical treatment. ^{18}F aldosterone synthase ligand PET-CT and steroid profiling are in development and hold promise as alternatives to, or in combination with, AVS. Until then, subtype differentiation should include CT to seek obvious mass lesions (including those large enough to warrant removal because of their malignant potential) and to localize adrenal veins AND AVS as the criterion standard for differentiating unilateral forms from bilateral forms. Australian and New Zealand guidelines for AVS are currently in development.

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The "Point of our Care" is to do it once and do it well - how point of care cortisol kits have revolutionised our adrenal vein sampling service

Julie Hetherington[1], **Dr Albert Hsieh**[2], **Dr Nimalie Perera**[3]

1. Royal Prince Alfred Hospital, Camperdown Sydney, NSW, Australia

2. Susan Wakil School of Nursing and Midwifery, University of Sydney, Sydney, NSW, Australia

3. Central Clinical School, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

Adrenal vein sampling (AVS) is an important diagnostic test in the management of patients with primary aldosteronism (PA). Providing a successful service for patients requires a multidisciplinary team (MDT) with a dedicated coordinator. Since 2006, the Endocrine Clinical Nurse Consultant at Royal Prince Alfred Hospital (RPAH) has coordinated over 220 AVS procedures. The majority of AVS has been sequential sampling both pre and post stimulation from 2021 onward. This is to reduce the possibility of false negative results when only post stimulation AVS is performed[1].

Despite streamlining the patient preparation and having dedicated interventionalists, it can still be difficult to cannulate both adrenal veins. The success rate of bilateral cannulation from 2006 to mid-2019 at RPAH was 75% (n=77). Following the introduction of the intra-procedural rapid semiquantitative cortisol testing point of care (POC) strips (Quick Cortisol Kit Q-CTZ-1000; Trust Medical Corporation)[2], the success rate to date has increased to 95% (n=120) (Figure 1). However, this does not mean that the AVS cannulation is straightforward.

Interpreting POC sticks when cortisol levels >2000nmol/L are usually clear-cut. This is a typical level when ACTH stimulated AVS is performed. Using a post stimulation selectivity index (SI), an adrenal: peripheral cortisol ratio of 3:1, is generally easy to achieve when adrenal veins have been correctly cannulated. When sampling is performed pre-ACTH stimulation, the interpretation of the lower cortisol levels can be more challenging. The greatest challenge has been interpreting POC sticks in pre-ACTH stimulation samples when cortisol levels are <1000nmol/L.

This presentation will describe challenging cases, strategies to improve the quality of the venous sample and POC sticks interpretation when time of the day and catheter tip placement which can significantly impact the results.

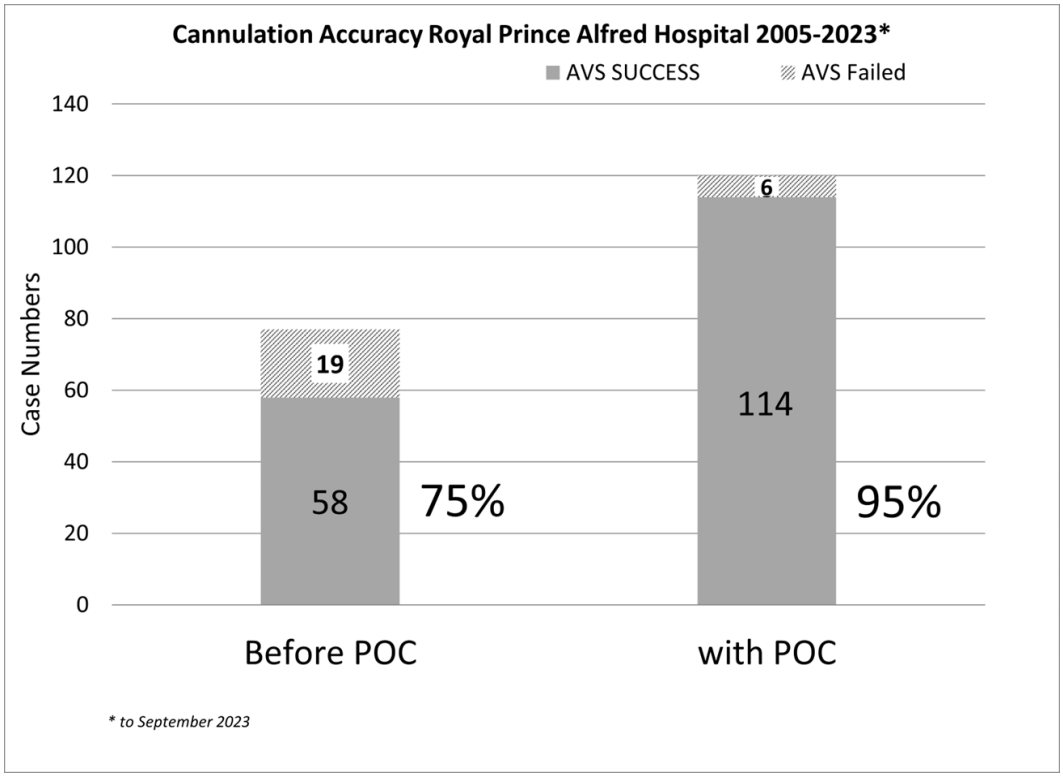

Figure 1

1. Violari EG, Arici M, Singh CK, Caetano CM, Georgiades CS, Grady J, Tendler BR, Shichman SJ, Malchoff CD. Adrenal vein sampling with and without cosyntropin stimulation for detection of surgically remediable aldosteronism. Endocrinol Diabetes Metab. 2019 Mar 7;2(2):e00066. doi: 10.1002/edm2.66.
2. Umapathysivam, M. M., Morgan, B., Bischoff, C., Hayes, A., Wilks, M., Stowasser, M., & Torpy, D. J. (2023). Intraprocedural cortisol testing improves adrenal vein cannulation success and diagnostic accuracy in assessment of primary aldosteronism, in a medium throughput centre. Journal of Human Hypertension, 37(9), 783-787.

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The Advantages and Limitations of Mass-Spectrometry-Based Methods in Endocrinology

Brett McWhinney[1]

1. Department of Chemical Pathology, Pathology Queensland, Brisbane, QLD, Australia

Historically, mass spectrometry had been of limited use in clinical laboratories; however, the combination of electron spray ionisation (ESI) methods with tandem mass spectrometry has opened up this technology to the clinical laboratory arena. Tandem mass spectrometry found its way into clinical laboratories in the early 1990s, with the analysis of acylcarnitines and amino acids from neonatal blood spots. During the past decade, liquid chromatography tandem mass spectrometry (LC-MS/MS) has played an increasingly important role in clinical analysis.

Over this time LC-MS/MS has had to overcome a number of challenges and there are still others remaining. It is now routinely used in laboratories with applications for therapeutic drug monitoring (TDM), endocrinology, toxicology and pharmacology. The majority of LC-MS/MS methods used in the clinical lab can be placed under the IVD test category, since they were "home brewed." The labs implementing this technology are expected to develop the test and undertake a thorough validation before putting these tests into routine use. The introduction of MS also brings with it's own set of MS specific issues that need to be monitored and addressed.

Due to the increased sensitivity, specificity and versatility of MS, endocrinology has been an area that has seen significant improvements in its transition to MS based methods. Advancements in Mass Spectrometry-based methods allow measurement of multiple steroids within a single sample and in matrices other than serum/plasma, including dried blood spots (DBS) and saliva. Also, recent developments have made the quantitation of free steroid hormones less laborious.

This talk will present an overview of the advantages and limitations of MS-based methods in endocrinology for clinical laboratories. The associated challenges and processes to overcome them will be discussed.

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Diagnostic Advances in Phaeochromocytoma/Paraganglioma

Roderick Clifton-Bligh[1]

1. Department of Endocrinology, Royal North Shore Hospital and Kolling Institute, University of Sydney, Sydney, NSW, Australia

Phaeochromocytomas (PCs) are adrenal chromaffin cell tumours; paragangliomas (PGLs) are derived either from parasympathetic paraganglia of the skull base and neck (HNPGLs: glomus caroticum, jugulare, tympanicum and vagale) and anterior/middle mediastinum, or from sympathetic-associated chromaffin paraganglia in the abdomen, pelvis and (rarely) the posterior mediastinum. PCs and PGLs (collectively, PPGLs) present in myriad ways, often dependent upon their specific genetic alteration (either germline or somatic). Several diagnostic advances in PC/PGLs converge on the basic principle of *a priori* assessment: this presentation will use clinical cases to highlight how baseline probabilities influence choice of testing across a range of modalities– biochemical, structural, functional, histological and genetic - and how these different tests are integrated to a final diagnosis. Specific diagnostic advances highlighted will include: adjusted thresholds for fractionated metanephrines measured by LC/MS-MS, according to baseline risk of disease; utility of plasma 3-methoxytyramine for HNPGLs and/or metastatic disease; careful use of pre- and post-contrast densities on CT imaging; use of MR angiography for HNPGLs; diffusion-weighted imaging in B mode (DWI-B) in whole body MRI; MR spectroscopy to assess succinate content in PPGL; the choice of PET tracers (^{18}F-FDG, ^{68}Ga-DOTATATE or ^{18}F-DOPA) depending on tumour genetics; imaging surveillance in asymptomatic mutation carriers; increased range of immunohistochemistry (SDHB, SDHA, FH, 2SC) to assist genetic interpretation; genetic testing pathways, including multipanel/exome germline sequencing or tumour-first testing; and the advent of liquid biopsies.

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Burning issues in male reproduction

Mark Hedger[1]

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

The male reproductive and immune systems co-exist in a finely-balanced relationship. The spermatogenic cells are highly antigenic, and immune reactions against these cells and their supporting epithelia leads to inflammation of the reproductive tract, hypogonadism and infertility. Even worse, the male reproductive tract is open to the external environment, and is susceptible to a broad range of bacterial and viral infections, which demand an effective immune response. These infections may be transmitted through sexual activity (*Neisseria gonorrhoeae, Chlamydia trachomatis*) but more commonly, threats can arise from commensal organisms or blood-borne pathogens (*E. coli*, Coxsackievirus, Mumps, SARS-CoV). In fact, the balance is so fine that inflammation due to systemic infections or even inflammatory disease in other tissues compromises male reproduction, not least because inflammatory cytokines and other mediators inhibit the hypothalamic-pituitary-testis axis at all levels. So, how does the male reproductive system protect itself from the damaging effects of infection and inflammation, and what actually happens when things go wrong? In the past three decades, our understanding of these issues has increased dramatically. We now know that so-called “immunological privilege” of the testis involves active immunosuppression of antigen-presentation and T cell activation, while mechanisms of peripheral immune tolerance provide protection in the epididymis and remainder of the genital tract. These tissues possess a complex network of resident immune cells and immunoregulatory molecular pathways that serve the dual purpose of constraining immunity, while providing some degree of protection against infection. These complex mechanisms represent a fundamental, but frequently neglected, aspect of male reproductive health. In the face of the ever-expanding threat of infectious diseases, and emerging viruses more specifically, driven by climate change and global interconnectedness, and the as-yet unsolved mystery of rising male reproductive disorders and declining fertility world-wide, understanding these mechanisms has become crucial for preserving male health and well-being more broadly.

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Estradiol add-back in men undergoing androgen deprivation therapy

Nick Russell[1]

1. Austin Health, Heidelberg, VIC, Australia

Androgen deprivation therapy (ADT) lowers testosterone and estradiol to castrate concentrations in men. It is a common treatment for prostate cancer, which is androgen-dependent, and the most prevalent contemporary cause of severe hypogonadism in older men. Men receiving ADT experience accelerated osteoporosis leading to fractures, accumulation of fat mass, loss of muscle mass, sexual dysfunction, hot flushes, and, reportedly, adverse neuropsychological effects.

Accumulating evidence suggests that many of the biological actions of testosterone in men are dependent on endogenous aromatisation of testosterone to estradiol. This raises the prospect of whether adverse effects of ADT in men with prostate cancer could be mitigated by replacing estradiol (‘estradiol add-back’).

Two randomised controlled trials of transdermal estradiol add-back (over 4 weeks, and 6 months, respectively), were conducted in men undergoing ADT for prostate cancer. These trials were designed to test the hypotheses that transdermal estradiol would reduce the ADT-associated unbalanced and accelerated bone remodelling, leading to better maintenance of volumetric bone mineral density; reduce the ADT-associated increase in fat mass; reduce ADT-associated changes in cognition; and reduce hot flushes and thereby improve quality of life.

The results of the trials will be presented in detail. They provide high quality evidence for this intervention to be used clinically. They also offer new insights into the biological actions of estradiol in men by providing direct observations of estradiol effects, in the absence of testosterone.

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New insights into tumour-suppressive androgen signalling in prostate cancer

Luke Selth[1]

1. Flinders Health and Medical Research Institute, Flinders University, Adelaide, SA, Australia

Inhibiting the androgen receptor (AR), a hormone-activated transcription factor, with androgen deprivation therapy is the standard-of-care treatment for metastatic prostate cancer. Paradoxically, potent androgen-mediated activation of AR can also inhibit prostate cancer growth in some patients and experimental systems, but the mechanisms underlying this phenomenon remain to be fully elucidated. In this presentation, new insights into androgen-mediated tumour suppression will be described, including work from our laboratory demonstrating that AR can modulate innate and adaptive immune signalling. Emerging therapeutic strategies to harness the tumour-suppressor activities of AR will also be discussed.

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Advancing Understanding and Treatment of Granulosa Cell Tumours in Ovarian Cancer

Simon Chu[1]

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

Within the spectrum of ovarian tumours, approximately 8% are comprised of stromal and/or sex cord tumours, which originate from the connective tissue within the ovaries. These tumours are uniquely characterized by their ability to secrete hormones, thus contributing to hormonal imbalances commonly observed in patients. Among these, granulosa cell tumours (GCT) are the most prevalent, accounting for 5-10% of malignant ovarian cancers.

Adult GCT are characterised by a mutation in the FOXL2 gene (C134W), which is not present in the less common juvenile form (which represent 5% of GCT). These tumours closely mimic the morphological, biochemical and hormonal features of proliferating normal pre-ovulatory granulosa cells, including the production of estrogen and inhibin, with inhibin proving to be a valuable tumour marker. Despite their clinical significance, GCTs have received limited attention compared to other ovarian cancers, resulting in an incomplete understanding of their molecular pathogenesis.

A unique feature of GCT is their propensity for late recurrence, currently impossible to predict, along with an inability to determine a tumours behaviour leading to patient mortality. demise. Patients with recurrent or aggressive GCT have few therapeutic options, with approximately 80% succumbing to the disease. Treatments often rely on regimens designed for epithelial ovarian cancer and offer only limited benefit. Thus, there is a reliance on anecdotal reports to guide therapeutic decisions in the absence of robust evidence-based approaches.

In light of the relatively low incidence, limited specific therapeutic alternatives, and the tendency for late recurrence, patients with GCT have sought support from patient advocacy groups like the social media-based group “GCT Survivor Sisters”. Collaboratively, we are partnering with this group to guide our research efforts addressing critical questions that hold the potential to significantly impact patients and their families. This collaboration has unearthed a clear and consistent picture of key challenges of the disease, and are being addressed in five core themes of our research program, namely: 1) Social media and the patient experience; 2) Beyond FOXL2: decoding the genomic landscape of adult and juvenile GCT, 3) Elucidating the molecular pathogenesis of adult GCT, 4) Developing novel biomarker diagnostic assays, and 5) Combination therapy: A precision medicine approach for GCT.

In this symposium, we aim to shed light on some these crucial aspects of GCT research, ultimately driving us towards a better understanding of this understudied ovarian tumour, and, more importantly, improving the lives of those affected by it.

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Urogenital defects in births to women dispensed clomiphene citrate within a whole of population birth cohort.

Michael Davies[1], **Alice Rumbold**[2], **Lynette Moore**[3], **Renae Fernandez**[1], **Lynne Giles**[1], **Heather McElroy**[1], **Vivienne Moore**[1]

1. University of Adelaide, Adelaide, SA, Australia

2. SAHMRI, Adelaide, SA, Australia

3. SA Pathology, Adelaide, SA, Australia

Background & question

Clomiphene citrate (CC) has been used for ovulation induction since 1967 & is both inexpensive and largely effective. However, CC is a selective estrogen receptor modulator (SERM), with a long half-life and the potential for endocrine disruption during fetal development. The FDA (1965) found it to be embryocidal and teratogenic at high doses. The link between CC and human birth defects has previously been reported by ourselves and others. There has never been a prospective safety study in humans.

Whether the link with urogenital defects exists where contemporary prescribing and dispensing patterns are clearly ascertained has not been determined.

Secondly, the interaction of prescribed clomiphene citrate by fetal sex has not undergone formal statistical testing.

Methods

We employed a population-based cohort study of 161,295 births in South Australia including 2,264 singleton CC exposed live births between July 2003 and December 2011, including defects coded to ICD9 and BPA, and dispensing in the national Pharmaceutical Benefits Scheme (PBS). Multivariate analysis in STATA was used to calculate odds ratios.

Results

Births among women dispensed clomiphene citrate, compared to those without this exposure, had increased urogenital defects (odds ratio (OR) = **1.73, CI=1.34 - 2.24).**

This association was slightly reduced after adjustment for maternal characteristics and potential confounders: (OR = **1.66, CI=1.28 -, 2.15)**.

There was evidence of an interaction with fetal sex, such that males were at particular risk.

Sex-by-clomiphene interaction	
Male	**1.92 (1.47, 2.52)**
Female	0.88 (0.37, 2.14)
Test for interaction	$\chi^2(1) = 2.72$; p=0.09

Conclusion

Clomiphene citrate (CC) is associated with urogenital defects, particularly in males. This is important as CC continues to be widely used, is a WHO essential drug and a first line treatment globally, but with largely unmonitored use contrary to FDA mandated safety requirements. Further studies across jurisdictions and clinical application are warranted.

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Automated Human Embryo Morphokinetic Annotation on Single Static Images via Deep Learning

Hooman Misaghi[1], Nidhi Gowdra[2], Dorothy Sobieszdzuk[1], Lynsey Cree[1], Nicholas Knowlton[1]

1. University of Auckland, Grafton, NZ, New Zealand

2. Center for E-Research, University of Auckland, Auckland, NZ

Purpose: The advent of time-lapse incubators has expanded the scope and volume of information accessible to embryologists, facilitating more informed decisions regarding embryo viability. One predictor that is gaining promise is accurate estimations of mophokinetic changes. Unfortunately, manually labeling these events is both susceptible to embryologist variation and is resource-intensive. Addressing these challenges, we harnessed AI to automate annotations and foster transparency by open-sourcing our model.

Methods: We utilized imaging data from a Vitrolife EmbryoScope, capturing images every 20 minutes from 413 patients across five fertility clinics in New Zealand. This dataset encompassed 104,418 images from 1612 embryos. Manual annotation by a senior embryologist defined 13 morphokinetic stages, including tPNa, tPNf, t2, t3, t4, t5, t6-7, t8, t9+, tM, tB, tEB.

For automated morphokinetic estimation, we trained a Convolutional Neural Network (CNN) on the images. Data was partitioned into training (87.2%), testing (3.2%), and validation (9.6%) sets. The CNN employed a Resnet-50 backbone for feature extraction, which was combined with elapsed time since fertilization converted to a time vector. A transformer layer with a multi-headed attention mechanism estimated interrelationships between imaging features and time, culminating in predictions from a fully connected layer. The final time for predictions are made via a sliding window approach.

Results: The model exhibited an impressive overall accuracy of 81.2% and when taking embryologist subjectivity into account the model accuracy rose to 96%. This high performance is due to the fusion of images with timing information. This amalgamation enhanced the model's comprehension of both the image and temporal context, enabling more precise predictions than those derived solely from single images.

Conclusion: In conclusion, our study introduces a powerful deep-learning model that automates human embryo morphokinetic annotation, attaining high accuracy and showcasing promising implications for integration into the clinic.

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Kingdom of reproductive life; Core sperm proteome

David A Skerrett-Byrne[1, 2], Brett Nixon[1, 2], Tim L Karr[3, 4], Taylor Pini[5]

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

2. Infertility and Reproduction Research Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

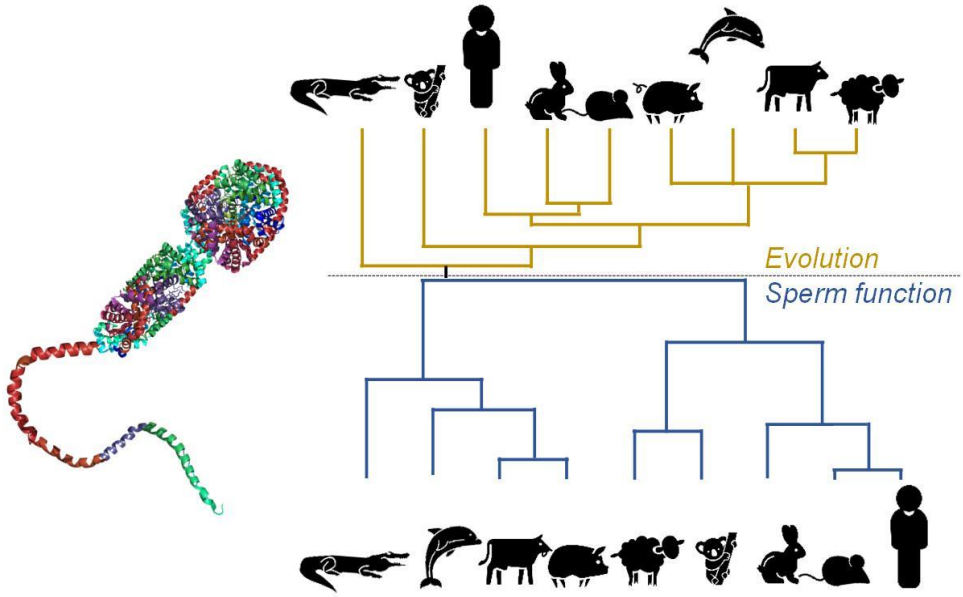
3. Biosciences Mass Spectrometry Core Research Facility, Knowledge Enterprise, Arizona State University, Arizona, USA

4. ASU-Banner Neurodegenerative Disease Research Center, The Biodesign Institute, Arizona, USA

5. School of Veterinary Science, , The University of Queensland, Gatton, QLD, Australia

Reproductive biology is often considered in the three siloed research areas, namely humans, domesticated animals and wildlife. Yet, there are common needs across these silos, notably treatment of subfertility, biomarkers for fertility/gamete selection, development of assisted reproductive technologies and effective contraception. To efficiently develop solutions applicable to all species, we must improve our understanding of the common biology underpinning reproductive processes. Accordingly, here, we performed an *in-silico* analysis of publicly available sperm proteomic data across 12 vertebrate species to consolidate these proteomic data and to define the core sperm proteome; a collection of highly conserved proteins that are critical for sperm structure and function. Over 2TB of RAW mass spectrometry data was sourced from ProteomeXchange and processed through a stringent and uniform search protocol to provide high confident protein identifications (FDR ≤ 0.01). A total of 13,853 proteins residing in the sperm of those species studied herein were identified, with the most significant contributions to this inventory being from humans and mice. Proteomic characterisation of non-traditional model species revealed >90% of their sperm proteins are currently curated as predicted to exist or inferred from homology, indicating that experimental evidence for their existence remains poorly defined. Despite variations in sperm proteome size, pathway analyses showcased functional relationships between species that differed from that of their evolutionary distances. Moreover, we report proteins that are highly conserved at the species (45 proteins) and order (135 proteins) taxonomic levels. Such proteins, in turn, mapped to critical pathways and molecular functions including protein folding and recycling, acrosome function and energy generation. In addition, we demonstrated that despite the fundamental biological role of spermatozoa, differing physiological

adaptations such as alterations in sperm metabolic preferences, external testes or even a history of selective breeding, are reflected in differences in the sperm proteome.



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Engineering functionalised surfaces that selectively capture suboptimal spermatozoa for applications in human IVF

Soraya Rasi Ghaemi[1], David Sharkey[1], Nicole McPherson[1], Krasimir Vasilev[2], Sarah Robertson[1]

1. Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia

2. Flinders University, South Australia, Adelaide, Australian Capital Territory, Australia

Selecting viable sperm for successful fertilisation and optimal developmental competence is an unmet challenge in reproductive medicine. There are limitations of methods based solely on motility and morphology criteria and this has prompted innovative approaches. Advances in understanding of the immunobiology of female reproductive tract sperm selection shows the role of immune cells in sperm selection. Knowledge of the relevant biomolecules provides novel avenues to develop surface chemistry-based approaches to emulate the molecular interactions between suboptimal sperm and immune cells. In pursuit of this objective, we have investigated plasma polymerisation approaches to recapitulate immune-mediated sequestration of suboptimal sperm on functionalised glass surfaces. We applied a plasma polymerised polyoxazoline (PPOx) film to glass using 2-methyl-2-oxazoline monomer. The PPOx film enabled the covalent binding of antibodies reacting with suboptimal sperm. Samples of human donor sperm (n=3-5) prepared by a standard 'swim-up' technique, or neat semen, were introduced to the activated surface and the rate of sperm attachment was measured. The characteristics of recovered unattached sperm were then assessed by flow cytometry to measure proportions of viable sperm (propidium iodide), apoptotic sperm (Annexin V expression), as well as levels of reactive oxygen species (CellROX Green), and sperm DNA fragmentation (HALO-sperm). We then applied similar surface functionalisation to antibody-coated glass channel-slides and showed that sperm recovered after introduction of neat semen exhibit superior functional characteristics compared with sperm prepared by standard swim-up or uncoated channelled slides. Sperm recovered from functionalised channel-slides exhibited a significantly lower Annexin V+ sperm subpopulation (6.3%±1.1%) compared with standard swim-up or uncoated channel slides (19%±4.1% and 15%±3.1%, respectively). The proportion of sperm exhibiting DNA fragmentation was also significantly decreased around (5.7%±1.2%) compared with the swim-up (11.5%±3.1%) samples.

In conclusion, our findings demonstrate the efficacy of surface functionalised antibody-coated channel-slides and potential in preparing high-quality sperm for applications in ART.

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Assessing the consequences of heightened pro-AMH processing on female reproduction in mouse.

Shreya Maskey[1, 2], William A Stocker[2], Adam Hagg[1], Amy L Winship[3], Craig A Harrison[2], Kelly L Walton[1, 2]

1. School of Biomedical Sciences, The University of Queesland, St. Lucia, Queesland, Australia

2. Department of Physiology, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

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

Within the mammalian ovary, anti-Müllerian hormone (AMH) is produced by granulosa cells of growing follicles and primarily acts to limit the activation of dormant primordial follicles. AMH is initially synthesised as a pro-AMH protein that undergoes proteolytic maturation to release the mature bioactive dimer, which binds and signals via AMH receptors. However, pro-AMH

cleavage is largely inefficient and as a result, the pro-AMH form is much more abundant in circulation compared to the mature form. This study hypothesised that enhancing pro-AMH cleavage efficiency would increase AMH bioactivity both *in vitro* and *in vivo*. Firstly, we used site-directed mutagenesis to enhance the furin cleavage site in pro-AMH (^{443}RTGR^{445} to ^{443}RKKR^{445}) and verified that these modifications improved the yield of bioactive mature AMH (in AMH responsive HEK293T cells). Next, we aimed to determine whether improving pro-AMH processing *in vivo* translated to enhanced AMH-mediated actions in the mouse ovary. Here, we used CRISPR/Cas9 to generate an $AMH^{RKKR/RKKR}$ mouse on a C57BL/6J background. Analyses of 12-week-old $AMH^{RKKR/RKKR}$ mice revealed that the ovaries were significantly lower in mass (-25%, $p<0.05$) relative to ovaries from littermate $AMH^{WT/WT}$ controls. Similarly, the ovaries from 24-week-old $AMH^{RKKR/RKKR}$ mice tended to be lower in mass compared to the ovaries from control $AMH^{WT/WT}$ females. Histological analyses hope to reveal the cause of the shrunken ovaries in the $AMH^{RKKR/RKKR}$ mice. Despite the overt differences in ovarian masses, oestrus cycling, female fertility, and serum AMH and inhibin B levels were unaltered across genotypes. Furthermore, no overt differences in the testes were observed in adult male $AMH^{RKKR/RKKR}$ mice compared with $AMH^{WT/WT}$ controls. Our findings to date support that the optimisation of pro-AMH processing alters the size of reproductive organs in female mice, and ongoing investigations hope to unveil the mechanisms driving this phenotype.

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Characterising the impact of environmental toxicant exposure on mammalian ovaries

Ngoc Tho Tony TN Nguyen[1], Zaahida Abdul Jalil[1], Bethany Finger[2], Qiaochu Wang[1], Xuebai Cai[1], Amy Winship[1], Nadeen Zerafa[1], Lauren Alesi[1], Yujie Cao[1], Jodi Flaws[3], Mark Green[2], Jessica Stringer[1], Karla Hutt[1]

1. Monash University, Melbourne, Victoria, Australia

2. Melbourne University, Melbourne, Victoria, Australia

3. University of Illinois Urbana-Champaign, Champaign, Illinois, United States

Background:

In Australia, heavy use of the herbicide atrazine has led to widespread water and soil contamination. The reproductive health impacts of long-term low-level exposures to this environmental toxicant are unknown. Therefore, this study aimed to evaluate the effects of multi-generational exposure to environmental concentrations of atrazine on the ovary.

Method:

Female C57/Bl6 mice were continuously exposed to atrazine an environmentally relevant atrazine concentration (0.02 ng/ml) or control (DMSO vehicle control) via drinking water for 2 generations and ovaries were collected from generation 2 mice at 6 months of age (n=6/treatment/age). The numbers of healthy and atretic follicles were determined, and DNA, protein and lipid oxidative damage was assessed by immunostaining for 8-oxo-G, 4-HNE and NTY, respectively. In addition, oxidative stress related genes Sod1, Sod2, Ucp2, Cat and Gpx3 were examined using qRT-PCR.

Results:

The number of primordial follicles were significantly decreased, and atretic antral follicles increased, in 6-month-old atrazine-exposed generation 2 mice compared to controls, while no changes in the number of healthy primary, secondary, and antral were observed. The percentages of follicles positive for markers of oxidative stress induced damages in DNA, protein, lipid oxidation, were significantly increased in atrazine-exposed generation 2 mice compared to controls, as well as for late-stage apoptosis. The mRNA levels for antioxidant genes *Cat* and *Gpx3* were also altered in atrazine-exposed generation 2 mice compared to controls.

Conclusion:

These data suggest that continuous multi-generational exposure of mice to low environmental levels of atrazine prematurely depletes the ovarian reserve, and increases follicular atresia, possibly through the induction of intra-ovarian oxidative stress. Understanding the impact of pervasive environmental toxicants, like atrazine, on female health is crucial to prevent reproductive disorders and diseases in current and future generations.

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Conditional loss of *Brca1* in oocytes causes accelerated ovarian ageing and subfertility in mice

Amy Winship[1], Lauren Alesi[1], Jessica Stringer[1], Yasmin Lewis[1], Lisa Tu[1], Elyse Swindells[1], Saranya Giridharan[1], Wilcox Cao[1], Xuebi Cai[1], Nadeen Zerafa[1], Martha Hickey[2, 3], Karla Hutt[1]

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

2. Gynaecology Research Centre, Royal Women's Hospital, Parkville

3. Department of Obstetrics and Gynaecology, The University of Melbourne, Parkville

An estimated one in 350 women carry germline *BRCA1/2* mutations, which confer increased risk of developing breast and ovarian cancer and may also contribute to subfertility. In this study we addressed a longstanding question in the field regarding the functional consequences of BRCA1 inactivation in oocytes. We generated mice with conditional gene deletion of *Brca1* using *Gdf9*-Cre recombinase (WT: $Brca1^{fl/fl}Gdf9^{+/+}$; cKO: $Brca1^{fl/fl}Gdf9^{cre/+}$). After a comprehensive fertile lifespan breeding trial, the average number of pups per female across all litters was significantly reduced in *Brca1* cKO (9.4 pups ± 0.7) compared to WT animals (11.6 pups ± 0.4, p=0.0196), indicating that conditional loss of *Brca1* in oocytes leads to subfertility. To determine whether reduced oocyte number contributed, ovarian follicles were enumerated throughout the lifespan. Females of each genotype were endowed with equal numbers of total follicles at postnatal day (PN)5 (range 5392-5562 total follicles/ovary). However, by PN300, the ovarian reserve of primordial follicles was significantly reduced by 47% in *Brca1* cKO animals (141 ± 17) versus WT (264 ± 27; p=0.0007). In advanced reproductively aged mice at PN300, oocyte *in vitro* maturation was reduced by 50% in *Brca1* cKO mice compared to WT, demonstrating defective oocyte quality. Serum anti-Müllerian hormone (AMH) concentrations (which is the gold-standard indirect marker of the ovarian reserve used in clinical practice), were not predictive of reduced primordial follicle number in *Brca1* cKO mice versus WT. Furthermore, we found no correlation between follicle number or density and serum AMH concentrations in matched samples from premenopausal women with *BRCA1/2* mutations. Together, our data demonstrate that BRCA1 is a key regulator of oocyte number and quality in females and suggest that AMH is not a reliable marker of the ovarian reserve in this context.

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Nicotinic acid pre-maturation treatment improves the developmental potential of equine oocytes for cloned embryo production

Jenin Cortez[1, 2], Kylie Hardwicke[2], Danton Cervi[2], Christopher G Grupen[1]

1. Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camden, NSW, Australia

2. Catalina Equine Reproduction Centre, North Richmond, NSW, Australia

Reproductive aging in mares is associated with a decrease in the quality and quantity of oocytes, which is the limiting factor in the production of embryos in vitro. Nicotinic acid (NA) is a key precursor of nicotinamide adenine dinucleotide (NAD+), which is involved in energy metabolism, cell survival, and DNA repair. Studies in mice and pigs have shown that NAD+-elevating treatments during in vitro maturation (IVM) improve oocyte developmental competence [1,2]. This study aimed to evaluate the effect of NA treatment on equine oocyte quality by assessing the development of embryos produced by somatic cell nuclear transfer (SCNT). Cumulus-oocyte complexes (COCs) collected from slaughterhouse-derived ovaries were washed and transferred to cryovials filled with a 1:1 mixture of DMEM-F12 and TCM-199 containing 10% FCS and kept at 20-22°C. Groups were either untreated (control) or treated with 50 and 200 μM NA for 18 h. Immature oocytes from the three groups were then washed, transferred to maturation medium, and incubated for a further 18 h at 38.5°C in 5% CO_2. A total of 694 oocytes were matured in 6 replicates. The methods used for IVM, SCNT, and embryo culture were identical for all three groups. The rates of meiotic maturation, fusion, cleavage, and embryonic development were evaluated. Data were subjected to ANOVA and Tukey's test. The rates of maturation, couplet fusion and cleavage were similar for the three groups (P>0.05). Day 7 blastocyst formation rates were greater for the treatment groups, 50 (27.1 ± 1.4%) and 200 μM NA (32.9 ± 3.0%), compared to the control group (19.9 ± 1.7%; P<0.05). The results show that NA supplementation improved the development of equine cloned embryos. Further studies are needed to reveal the cellular benefit of elevating NAD+ during the pre-maturation period and to determine embryo viability in vivo after transfer to recipient mares.

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Clinical challenges in bone (Clinical)

Christian Girgis[1]

1. University of Sydney, Darlinghurst, NSW, Australia

A number of challenges have emerged in the treatment of osteoporosis. This talk will take a case-based approach to discuss three such challenges: 1) denosumab withdrawal; 2) denosumab sequencing to an osteoanabolic agent; and 3) treatment considerations following an atypical femur fracture.

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Advances in Medical Treatment in Thyroid Eye Disease

Jwu Jin Khong[1]

1. The Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia

Background/Aim: Medical treatment for Thyroid Eye Disease (TED) has become more specific and targeted as pathophysiology is better understood. Over the last decade, clinical trials informed and led changes in management of TED.

This review aimed to explore the efficacy of emerging treatments with a focus on immunotherapies and to summarise future pipelines in new drug trial.

Methods: A narrative review of the literature was performed. Two online databases, Medline and Embase, were searched using keywords for TED, immunotherapy and clinical trials. Inclusion criteria were human clinical trials, immunotherapy, dated from 2012 to 2022 in English. This research also reviewed data from two international clinical trials registries (ClinicalTrials.gov & European Clinical Trials database). Inclusion criteria were current, recruiting, upcoming and completed clinical trials in immunotherapy for TED.

Results: Monoclonal antibodies are increasingly used in the treatment of thyroid eye disease. Teprotumumab has shown significant efficacy in reducing proptosis, inactivation of disease, improving diplopia and quality of life in patients with active TED. Tocilizumab showed superior response in reducing orbital inflammation, especially in steroid resistant cases, while rituximab showed mixed results for TED inactivation. Statins demonstrated early efficacy in improving overall treatment response in combination with intravenous corticosteroid in active TED with hypercholesterolaemia, with a safe side-effect profile. Emerging monoclonal antibodies K1-70 was safe and well tolerated, demonstrated dose dependent induction of hypothyroidism and early evidence of proptosis reduction, while Belimumab showed clinical activity score reduction and favourable side-effect profiles in early phase trial. The novel Long-Circulating PEGylated Liposomal Glucocorticoid also showed significant inflammation improvement without typical steroid side effects. New drug trials targeting novel molecular moiety includes anti-IGF-1R, anti-VEGF-A, anti-neonatal Fc receptor, anti-IL 17A and mTOR inhibitor via subcutaneous and oral formulations are currently underway.

Conclusions: Immunotherapy shows the capability to reduce severity and inflammation in TED, with several large-scale and rigorous studies performed. Therapies in the future will likely involve combination therapies, dosing optimisation and novel/more targeted administration to maximise efficacy and reduce the burden of adverse events.

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Update on prediction and prevention of type 1 diabetes

John Wentworth[1]

1. WEHI, Parkville, VIC, Australia

Early diagnosis of type 1 diabetes through autoantibody screening decreases the risk of critical illness and provides opportunities to use immunotherapy to delay disease progression. This talk will focus on Type1Screen and other local screening efforts and the ATIC consortium, which is running immunotherapy trials throughout Australia.

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Antigen-specific tolerising immunotherapy for autoimmune diabetes: trials and biomarkers

Ranjeny Thomas[1]

1. University of Queensland Diamantina Institute, Princess Alexandra Hospital, Brisbane, QLD, Australia

Tolerising immunotherapy including antigen-specific immunotherapy (ASI) holds great promise for autoimmune diseases, where there are limited treatment options and/or scope for prevention. These diseases include rheumatoid arthritis (RA) and type 1 diabetes (T1D). Preclinical success for this approach has been demonstrated *in vivo*, and promising evidence for various strategies that restore immune regulation is beginning to emerge from clinical trials in people at risk and after onset of autoimmune diseases. Hurdles include the development of robust prognostic and predictive biomarkers for therapeutic outcome and identification of the most appropriate population and timing of intervention. ASI promise greater specificity and safety than non-ASI, and thus great potential for autoimmune disease interception in high-risk individuals. I will discuss my personal journey developing and trialling innovative therapeutics for tolerising ASI in autoimmune diseases, including the development and use of biomarkers. I will demonstrate how the results of our clinical studies and trials in RA and T1D open new insights into the concept of tolerance, immunotherapeutic mechanisms of action, innovative use of biomarkers in clinical trials and therapeutic implementation of ASI.

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Understanding the role of thyroid antibodies in mediating complications of pregnancy

James Cuffe[1]

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

Thyroid antibodies are detectable in approximately 25% of pregnant women. These antibodies may contribute to thyroid dysfunction and changes to thyroid hormone concentrations. However, in the majority of women with high thyroid antibodies levels, no other changes to thyroid parameters can be detected. We and others have demonstrated that thyroid peroxidase antibodies (TPOab) or thyroglobulin antibodies (TGab) increase the risk of a range of pregnancy complications such as gestational diabetes mellitus, miscarriage and preterm birth. As such, my laboratory has utilised a number of different approaches with the aim of investigating how these antibodies may be contributing to poor pregnancy outcomes. Two different rat models of TGab positivity in pregnancy were generated. The first rat model was established to look at how increased TGab and low T4 together contributed to poor outcomes. Rats given methimazole to impair thyroid hormone production had elevated TSH and TGab. These rats developed diabetes like symptoms in pregnancy, had placental dysfunction, fetal growth restriction and had changes to most metabolic parameters investigated. The second model was setup to investigate outcomes when thyroid autoimmunity is directly stimulated. Rats were immunised with thyroglobulin five times over the course of seven weeks before pregnancy was induced. These TGab positive rats had minimal thyroid pathology or changes to metabolic parameters. Instead, these TGab positive rats had impaired oestrous cycling, reduced survival of male fetuses and changes to fetal brain

weights. We are currently using a rat model of TPOab positivity to determine how different antibody types may cause different outcomes. Overall, rat models have proved useful in being able to start to dissect the actions of thyroid antibodies on pregnancy outcomes. There are a number of adverse pregnancy outcomes that seem to be directly linked to thyroid antibody concentrations and these models will be used in the future to test potential therapeutic interventions.

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Mitochondrial activator BGP-15 protects sperm from DNA damage during sperm recovery for Assisted Reproductive Technology

Macarena Gonzalez[1], Haley Connaughton[1], Rebecca Robker[1], Michael Barry[1, 2], Ryan Rose[1, 2]

1. The University of Adelaide, Adelaide

2. Genea Fertility SA, St. Andrew's Hospital, Adelaide

The recovery of sperm for Assisted Reproductive Technology (ART) results in significant DNA damage. Elevated levels of DNA damage in sperm have been linked to poor-quality embryos and increased pregnancy loss after ART. Therefore, in this study, we aimed to investigate potential of the new mitochondrial activator BGP-15 to prevent sperm DNA damage in an *in vitro* ejaculate over time. Additionally, we sought to determine whether BGP-15 can preserve sperm quality during various sperm recovery techniques used in ART.

First, we incubated donated human semen specimens untreated or treated with BGP-15, then examined sperm motility and DNA damage (DNA fragmentation and oxidation). Semen samples were then processed using different clinical sperm recovery methods: simple wash (W), swim-up (SU) and density gradient centrifugation (DGC); and the purified sperm underwent further analysis including; motility, vitality, ROS levels, mitochondrial membrane integrity and mitochondrial membrane potential (MMP), and DNA damage.

Semen samples incubated with BGP-15 demonstrated improved sperm motility and reduced DNA damage levels. W samples had the highest sperm count, while SU had the highest vitality and DNA integrity, but the lowest MMP. DGC sperm had 11% increased MMP after BGP-15 treatment. Although BGP-15 treatment only reduced sperm DNA fragmentation in W samples, it reduced DNA oxidation by at least 30% in all recovery methods.

Our findings indicate that clinical manipulation of patient semen samples can negatively impact sperm quality, and different sperm recovery methods have unique effects that should be considered for each individual patient. Moreover, we show that the addition of BGP-15 into clinical processes helps preserve sperm quality, potentially leading to improved embryo quality and ART success.

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BMP signalling and RA-induced STRA8 ensure mitosis-to-meiosis transition in fetal mouse ovarian germ cells

Fiona KM Cheung[1], Chun-Wei (Allen) Feng[1], Cassy Spiller[1], Josephine Bowles[1]

1. School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, St Lucia, QLD, Australia

Successful generation of haploid gametes from diploid germ cells through meiotic division is a critical, if not the most important, step for successful sexual reproduction. Despite the importance of meiosis, molecular mechanisms governing its control remain poorly understood. The initiation of meiosis in germ cells depends on their exposure to extrinsic retinoic acid (RA); however, the sufficiency (or even the requirement) for RA in meiosis has been an on-going debate, and other factors from the gonadal somatic environment is likely to be involved.

Inspired by previous studies in primordial germ cell-like cells (PGCLCs) (1,2) that demonstrated the role of BMP signalling in female fate specification *in vitro*, we investigated the requirement for BMP signalling in mouse fetal germ cells using a transgenic mouse model that allows temporally controlled depletion of BMPR1A-mediated BMP signalling specifically in the germ cells (*Bmpr1a*$^{\Delta PGC}$). We verified, for the first time *in vivo*, that BMP signalling promotes meiotic progression in fetal ovarian germ cells. Initiation of *Stra8* expression was found to be independent upon BMP signalling, although aberrant cytoplasmic STRA8 was observed in *Bmpr1a*-deficient ovarian germ cells. Meiotic progression was delayed and mitotic exit was compromised in *Bmpr1a*-deficient germ cells. Differential gene expression among *Bmpr1a*$^{\Delta PGC}$, *Stra8*^{null}, and their control ovaries showed that in mouse fetal ovaries, BMP signalling and STRA8 are both responsible (directly or indirectly) for the downregulation of pluripotency factors and early primordial germ cell-related genes, but BMP signalling is required also for suppressing mitotic cell cycle genes and male germ cell-specific genes, and to regulate the dosage of meiotic progression genes. We conclude that in mouse fetal germ cells, BMP signalling and RA-induced STRA8 together ensure the loss of pluripotency, proper exit of mitotic cell cycle and entry of meiotic cycle, and the subsequent progression through meiosis.

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Low-level exposure to Bisphenol A and its alternatives has detrimental impacts on oocyte health

Alexandra E Peters[1, 2], Emmalee A Ford[1, 2, 3], Shaun D Roman[4], Elizabeth G Bromfield[6, 7, 5], Brett Nixon[6, 7], Kirsty G Pringle[1, 2], Jessie M Sutherland[1, 2]

1. School of Biomedical Science and Pharmacy, College of Health, Medicine, and Wellbeing, University of Newcastle, Callaghan, NSW, Australia

2. Mothers and Babies Research Program, Hunter Medical Research Institute , New Lambton Heights, NSW, Australia

3. The Research Centre, Family Planning Australia, Newington, NSW, Australia

4. NSW Health Pathology, Newcastle, NSW, Australia

5. School of BioSciences, Faculty of Science, Bio21 Institute, The University of Melbourne, Parkville, VIC, Australia

6. School of Environmental and Life Sciences, College of Engineering, Science, and Environment, University of Newcastle, Callaghan, NSW, Australia

7. Infertility and Reproduction Program, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia

Bisphenol A (BPA) is an endocrine disrupting chemical and component of plastic materials, including food packaging, ubiquitously contaminating ecosystems and human populations. BPA can elicit an array of damaging health effects and alarmingly, emerging 'BPA-free' alternatives mirror these harmful effects. Bisphenol exposure can negatively impact female fertility, damaging both the ovary and oocytes therein. Such damage can, in turn, diminish reproductive capacity, pregnancy success, and offspring health. Despite global government regulations in place to indicate 'safe' BPA exposure levels, these policies have neglected to consider the effects of BPA and BPA alternatives on oocyte health. To address this discrepancy, we conducted a scoping review to evaluate evidence on the effects of BPA/BPA alternatives on five standardised parameters of oocyte health. Four databases (Medline, Embase, Scopus, and Web of Science) were searched to capture studies assessing mammalian oocyte health post-bisphenol exposure. After screening, 106/3147 studies were included. Of the in vitro exposure studies, 96.2% (26/27) and 93.8% (15/16) found at least one adverse oocyte effect using BPA or BPA alternatives, respectively. These included increased meiotic cell cycle arrest, altered morphology, and abnormal meiotic spindle/chromosomal alignment. In vivo, 82.9% (29/35) of studies on BPA and 92.3% (12/13) on BPA alternatives documented adverse effects on follicle development, morphology, or spindle/chromosome alignment. Importantly, these effects were recorded using levels below those deemed 'safe' for human exposure. Over half (11/21) of all human observational studies correlated higher urinary BPA levels with reduced antral follicle counts or oocyte yield in IVF patients. These data highlight the detrimental impacts of low-level BPA and BPA alternative exposure, contributing to poor oocyte quality and reduced fertility. In addition, this study serves as a valuable resource to researchers, providing key recommendations on study design, reporting elements, and endpoint measures to strengthen future studies and promote revision of current guidelines.

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A biomimetic 3D platform for high-throughput sperm selection

Farin Yazdan Parast[1], Alecia Wong[1], Moira K. O'Bryan[2], Reza Nosrati[1]

1. Department of Mechanical and Aerospace Engineering, Monash University, Clayton, Victoria, Australia

2. School of BioSciences and Bio21 Institute, University of Melbourne, Parkville, Victoria, Australia

Infertility is a prevalent global health concern affecting one in six individuals [1], with male infertility contributing to about 45% of these cases [2]. Over the past four decades, assisted reproductive technologies (ART) have emerged to combat infertility. A key step in ART is the selection of high-quality sperm, which significantly impacts the treatment's success rate, live-birth rate, and offspring health [3]. However, current clinical sperm selection methods are largely manual, time-intensive, susceptible to operator errors and differ considerably from natural three-dimensional (3D) *in vivo* selection. Conventional methods have not been changed over the past 40 years, resulting in limited success rates for treatment cycles [4]. Here, we present a scalable, high throughput, and clinically relevant technology for selecting high-quality sperm via a 3D network of microchannels.

The 3D platform of the sperm selection device mimics the highly parallelized and 3D structure of the female reproductive tract and allows for high-throughput selection. The device was initially fabricated using a rapid prototyping method (3D printer) [5]. However, to facilitate clinical translation, the device design has been modified for injection moulding. Subsequently, a novel prototype composed of polystyrene was developed. During the selection time, motile sperm navigate through the microchannels to reach the outlet, while debris and non-motile cells are retained in the inlet. The selection throughput of the device is over 41%, significantly higher than previously developed microfluidic technologies. It retrieves over 1.6 million high-quality sperm in just 15 minutes which is more than sufficient for applications in IVF and IUI. Furthermore, the results indicated over 65% improvement in both DNA integrity and morphologically normal sperm.

In conclusion, we present a low-cost and rapid selection method, offering a promising possibility for conducting IUI and IVF procedures more frequently in fertility clinics.

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77

Proline improves development of mouse preimplantation embryos by protecting them against oxidative stress

Madeleine LM Hardy[1], Michael B Morris[1], Margot L Day[1]

1. The university of Sydney, Sydney, NSW, Australia

The demand for assisted reproductive technologies (ART) to create embryos in vitro has increased, and now constitutes 1 in 20 births in Australia. However, the success rates of ART have remained stagnant over the past two decades. *In vitro* cultured embryos exhibit poorer development compared to their *in vivo* counterparts, with slower growth and fewer reaching the blastocyst stage. Changes to the composition of culture media to promote *in vitro* embryo growth include the addition of specific amino acids. Proline, a non-essential amino acid, improves embryo cleavage and blastocyst development when included in mouse embryo culture medium without other amino acids. However, mechanisms by which proline works are poorly understood.

Live-cell imaging of 2-cell and 4-cell mouse embryos cultured in medium containing proline revealed a reduction in mitochondrial activity and reactive oxygen species (ROS). This decrease in mitochondrial activity and ROS was prevented by culture in tetrahydro-2-furoic acid (THFA), an inhibitor of proline oxidase, suggesting that the beneficial effect of proline on embryo development is dependent on proline metabolism.

Glutathione (GSH) is a major regulator of ROS and its production is, in part, dependent on proline metabolism. Live-cell imaging and liquid chromatography-mass spectrometry confirmed that proline metabolism increased both GSH levels and the GSH:GSSG ratio throughout preimplantation embryo development. This, in turn, reduces oxidative stress in the embryo and improves development.

In conclusion, proline improves *in vitro* embryo development by reducing oxidative stress, likely through increased GSH production and decreased mitochondrial activity. These findings should inform the development of improved embryo culture media used for ART.

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Immortalised mouse caput epididymal epithelial cells: a model to study how parental stress is remodelling the sperm epigenome

Jessica E Mulhall[2, 1], Natalie A Trigg[4, 3], Ilana R Berntstein[2, 1], Amanda L Anderson[2, 1], Heather C Murray[5, 6], Petra Sipilä[7], Tessa Lord[2, 1], John E Schjenken[2, 1], Brett Nixon[2, 1], David A Skerrett-Byrne[2, 1]

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

2. Infertility and Reproduction Research Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

3. Departments of Genetics and Pediatrics , Penn Epigenetics Institute, Institute of Regenerative Medicine, and Center for Research,University of Pennsylvania Perelman School of Medicine on Reproduction and Women's Health, Philadelphia, Pennsylvania, USA

4. Division of Neonatology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

5. Precision Medicine Research Program, Hunter Medical Research Institute,, Newcastle, NSW, Australia

6. School of Biomedical Sciences and Pharmacy, College of Health, Medicine and Wellbeing, University of Newcastle, Newcastle, NSW, Australia

7. Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology, and Turku Center for Disease Modeling, University of Turku, Turku, Finland

Male infertility and paternal preconception health are key factors influencing offspring development. Both are heavily shaped during the sperm's transit of the epididymis, a segmented tubule situated after the testes with distinct regions: initial segment, proximal caput, corpus, and distal cauda. The luminal milieu of the heavily convoluted epididymis is essential for the functional maturation of spermatozoa. This microenvironment is created by the combined secretory and resorptive activity of the epididymal epithelium, including the release of extracellular vesicles destined for maturing sperm cells, containing fertility modulating proteins and a myriad of small non-coding RNAs (sncRNAs), acting as conduits of epigenetic information. Our laboratory has previously identified the caput region as the primary site in shaping the final sncRNA profile of mature sperm, which altered following environmental stressors. To enable investigation of this intercellular communication nexus, we have applied our label-free proteomic platform to our immortalized mouse caput epididymal epithelial cell line (mECap18). We report the identification of >5,300 proteins, >75% of which were present in the proteome of *in-vivo* caput epididymal epithelial cells. Furthermore, key pathways associated with protein synthesis (e.g. EIF2 signalling) and cellular protection in the male reproductive tract (e.g. sirtuin signalling) were enriched in both proteomes. This comparison supports the utility of the mECap18 cell line as a tractable *in-vitro* model for studying caput epididymal epithelial cell function, and importantly the epididymis' mediation of paternal stress signatures to sperm. The glucocorticoid receptor (NR3C1), a transcription factor and known regulator of sncRNAs, is a promising orchestrater for this transmission; further study is necessary to understand this relationship. Using an acute stress exposure model on the mECap18, we have begun to investigate the effects of acute stress exposure on NR3C1 and the efficacy of therapeutic intervention to restore NR3C1 expression to pre-exposure levels.

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Vitamin C addition recovers antioxidant capacity, motility, vitality and DNA integrity lost with cryoprotectant addition, improving cryopreserved human semen parameters

Alena J Hungerford[1], Hassan W Bakos[1, 2], John J Aitken[1]

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

2. Memphasys Reproductive Biotechnology, Homebush West, NSW, Australia

The aim of this project was to examine the significance of oxidative stress in the etiology of sperm cryostorage injury and determine whether antioxidant supplementation might improve the efficacy of this process.

Human semen samples were initially tested (i) unprocessed, (ii) following addition of Quinn's Advantage™ Sperm Freeze medium, (iii) immediately after a freeze-thaw cycle and (iv) 3 h after being resuspended in culture medium. Tests included: basic sperm parameters, reactive oxygen species production (ROS), lipid peroxidation, DNA fragmentation, and antioxidant activity. To determine the value of antioxidant supplementation samples (n=15) were cryopreserved with resveratrol (0.625 - 320µM), vitamin C (0.1-1.6 mM) melatonin (2.2 mM) or *N*-acetylene cysteine (NAC; 0.1 mM).

Cryoprotectant addition significantly lowered motility and vitality as well as seminal antioxidant capacity ($P < 0.001$). Cryopreservation and subsequent incubation led to further deterioration in sperm motility, vitality, antioxidant activity, ROS production, lipid peroxidation and fragmented DNA ($P < 0.01$).

A significant improvement in vitality was found with 1.25 – 5 µM resveratrol addition ($P < 0.01$). Moreover, supplementation with ≥80 µM resveratrol significantly improved antioxidant status but proved cytotoxic to spermatozoa at this dose.

Both pre- and post-cryopreservation, vitamin C, but neither melatonin nor NAC, significantly improved motility and vitality. ($P < 0.05$). A subsequent dose-dependent study with vitamin C established an optimal dose of 0.4 mM, this antioxidant level significantly improved motility, vitality and DNA fragmentation levels post-cryopreservation ($P < 0.05$).

Cryopreservation is harmful to human spermatozoa because this process induces oxidative stress. Utilising vitamin C to enhance the level of antioxidant protection during cryopreservation, led to significant improvements in total and progressive motility ($P < 0.05$) and a significant decrease in DNA fragmentation levels ($P < 0.05$) post freeze. This information has the potential to inform the design of improved cryoprotectant media.

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Repeated delivery increases the risk of diabetes by impairing the proliferation of pancreatic β cells

Joon Ho Moon[1, 2], Joonyub Lee[2], Hail Kim[2]

1. Seoul National University, Seongnam, South Korea

2. Korea Advanced Institute of Science and Technology, Daejeon, South Korea

Pregnancy imposes a substantial metabolic burden on women. We have reported than lactation improves pancreatic β cell mass and function in postpartum women[1,2,3], but little is known about whether or how repeated delivery increase the risk of maternal postpartum diabetes. In this study, we assessed the metabolic impact of multiple pregnancies in humans and a rodent model. Mice underwent multiple pregnancies had increased adiposity and insulin resistance developed but insulin secretory function and compensatory pancreatic β cell proliferation were impaired in multiparous mice compared to age-matched virgin mice. The β cells of multiparous mice exhibited aging features including telomere shortening and increased expression of *Cdkn2a*. Single-cell RNA-seq analysis revealed that the β cells of multiparous mice exhibited upregulation of stress-related pathways and downregulation of cellular respiration- and oxidative phosphorylation-related pathways. In humans, women who delivered more than three times are more obese and their plasma glucose concentrations were elevated compared to women who had delivered three or fewer times, as assessed at 2 months postpartum. The disposition index, which is a measure of the insulin secretory function of β cells, decreased when women with higher parity gained body weight after delivery. Taken together, our findings indicate that multiple pregnancies induce cellular stress and aging features in β cells which impair their proliferative capacity to compensate for insulin resistance.

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Do chili compounds "Beige" human white fat that have been transplanted into mice and exhibit improvements in mouse metabolism.

Sarah Davis[1], Jenny Gunton[2]

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

2. Centre for Obesity, Endocrinology and Diabetes, Westmead Hospital, Westmead, NSW, Australia

INTRODUCTION: Obesity is a major health concern and increases risk of metabolic syndrome, type 2 diabetes, dyslipidemia, cardiovascular diseases and many cancers. Obesity occurs with decreased physical activity and increased caloric intake. Clinical management is still limited. Brown adipose tissue is a thermogenic organ which expresses uncoupling protein 1. When activated it increases energy expenditure by up to 20%. Recent evidence suggests that white adipose tissue can be 'browned' and have similar characteristics, called beige fat.

AIM: This project tested a "beiging" agent capsaicin to determine whether it could brown human fat and improve metabolism.

MATERIALS AND METHODS: We used a "Humanised Mouse Model" where human fat is inserted intra-abdominally in immune suppressed mice (to avoid rejection). Mice were fed ad libitum normal or a high fat diet (45% calories from fat) ± 0.03% capsaicin or capinoids. Metabolic studies were conducted before and after fat transplant and 12 weeks on diets. These studies included glucose and insulin tolerance tests and metabolic cages. mRNA and histology samples were taken.

RESULTS: Results show increases in energy expenditure (vCO_2 and vO_2) in mice fed capsaicin compared to their respective controls. High fat diet + capsaicin or capinoids showed improvements in glucose tolerance and insulin sensitivity. Capsaicin up-regulated uncoupling protein 1 in human fat.

CONCULSONS: These results indicate beiging of human white fat is possible and has the potential to improve metabolism. Components of chili have the potential to beige human white fat. A 20% increase in energy expenditure has the potential to cause clinically significant improvements in obesity. Further studies will examine combination therapies to optimise browning.

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Disrupted glucocorticoid receptor-mediated signalling causes a primary cilia defect in the fetal mouse renal tubule

Jianshen Lao[1], Kelly L Short[1], Judy Ng[1], Denny L Cottle[2], Timothy J Cole[1]

1. Department of Biochemistry & Molecular Biology, Monash Biomedicine Discovery Institute, Clayton, Vic, Australia

2. Department of Anatomy & Developmental Biology, Monash Biomedicine Discovery Institute, Clayton, Vic, Australia

Primary cilia are microtubule-based organelles that protrude from cell membranes to mediate diverse developmental signalling pathways and senses extracellular stimuli to maintain tissue homeostasis. We show that glucocorticoid (GC) signaling via the glucocorticoid receptor (GR) is a regulator of normal primary cilia formation in kidney renal tubules. RNA sequencing of mice with global deletion of the GR (GR-null) identified significant reduced expression of key ciliogenesis-related genes; *Ccp110* (fold -2.17), *Cep97* (fold -1.79), *Cep290* (fold -2.90), *Kif3a* (fold -1.82) and *Rpgr* (fold -1.87). Confocal microscopy revealed abnormal, stunted primary cilia in renal proximal tubules, collecting ducts and podocytes in GR-null or in conditional GR-deleted mice. Primary cilia length was significantly decreased in kidney proximal tubule cells in GR-null mice (5.21 ± 0.22μm) compared with wildtype controls (6.80 ± 0.59μm). In contrast, activation of GR signaling with the synthetic GC dexamethasone in mouse IMCD3 kidney tubule cells significantly increased primary cilia length (2.89 ± 0.04μm) compared with vehicle controls (2.46 ± 0.28μm), an effect blocked by the GR antagonist RU486. Together, these results demonstrate that GC signalling via the GR is required for normal primary ciliogenesis in the developing renal tubule and suggests that synthetic GR agonists may provide a novel therapeutic option for human ciliopathies such as those observed in forms of polycystic kidney disease.

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An Ode to the "Special Tests Sisters" Why EVERY Tertiary Endocrinology Department needs an Endocrine Nursing Service

Warrick Inder[1]

1. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, QLD, Australia

Endocrine nurses have been around in some institutions across Australia and New Zealand for decades. Yet still, many tertiary hospitals do not employ specialist endocrine nurses, despite the strong presence of diabetes educators and other specialist nurses throughout our health service. I contend that a true tertiary level endocrinology department is incomplete without a strong endocrine nursing service.

My first contact with endocrine nurses was in 1993 at Christchurch Hospital, when I was a first year advanced trainee. There were three half time nurses who shared the load. Our set up was designed for efficiency and proximity – the consultant and registrar offices, outpatient clinic rooms, Endocrine test centre, Endocrine / General medicine ward and Endocrine laboratory were situated within about a 20m walk. The special tests sisters were efficient, knowledgeable, unflappable, kind and most of all for a newly sprung endocrine registrar very supportive. There was no endocrine condition they had not seen. Their roles included routine blood sampling for the clinics, dynamic tests of endocrine function, a myriad of research projects led by endocrine legends like Eric Espiner and Rick Donald and patient education. They held your hand and guided you through the scary tests like ITTs!

After leaving Christchurch, I have worked with wonderful nursing colleagues at St Vincent's Hospital, Melbourne and Princess Alexandra Hospital, Brisbane. Over the time I worked at these hospitals, we developed a specialist endocrine nursing service which has streamlined dynamic testing and provided consistent and high quality education for patients with complex endocrine conditions.

In recent years, the place of the endocrine nurse in patient care has been recognised as an essential service by the Endocrine Society. Convincing cash-strapped hospital administrators is a challenge but one worth fighting for. I simply could not do my job without you!

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The changing role of UK Endocrine Nursing and its application to Australian and global practice

Lisa Shepherd[1]

1. University Hospitals Birmingham NHS Foundation Trust, Sutton Coldfield, WEST MIDLANDS, United Kingdom

The first edition of the UK Competency Framework for Adult Endocrine Nursing was published in 2013 following response to an absence of set training in adult nursing for the specialty. The second edition followed in 2015 building upon the previous edition's competencies by adding four additional competencies. The competency framework is beneficial for endocrine nurses, employers and patients. Not only has this proved invaluable in the UK, but it has also been acclaimed worldwide.

An updated online version is currently being developed, which incorporates Benner's model of practice and Advanced Practice Pillars. It will also provide access to training resources and mentorship.

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Enhancing the Benefits of Exercise Using a Novel HIF1a Mutant Mouse Line

Josephine Yu[1], Jenny Gunton[1, 3, 2]

1. WIMR, 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

Aims: Exercise is well-established to be beneficial for obesity and T2D. This is in part, due to the regulation of hypoxia inducible factors (HIFs). We have created a novel mouse model which targets the beneficial effects of HIF in metabolism (HIF1α-N813Q). We aim to explore the effects of exercise and high fat diet (HFD) feeding in our novel HIF1α mouse model and hypothesise that mice with this mutation will experience some protection against adverse consequences of HFD, and experience *greater* benefits when given access to exercise.

Methods: Male HIF1α-N813Q mutant mice and their wild-type (WT) littermates underwent a series of basal tests for assessment of metabolic status and muscle function: glucose tolerance test (GTT), fore-limb grip strength, insulin tolerance test (ITT) and endurance testing. Mice were then challenged with HFD (Diet 6B; 45% digestible energy from lipids) and given access to a running wheel (locked for controls).

Results: Baseline data from metabolic tests did not exhibit any differences between genotypes in glucose and insulin tolerance, and baseline endurance or grip strength. Body weight gain and fat mass were significantly reduced in mutant mice given access to a wheel but not the other groups (mutant and control sedentary, control with wheel). As expected, 4 weeks of HFD-feeding significantly worsened GTT response in all groups, except for N813Q-mutant exercise mice. N813Q-exercise mice had significantly enhanced grip strength ($p<0.05$) and significantly increased exercise endurance after only 2 weeks which further increased by 6 weeks. The increase was significantly greater than in control-exercise mice which only showed a smaller increase in endurance at 6 weeks.

Conclusions: Overall, the data show that our novel HIF1α mice experience enhanced improvements in both muscle functionality and metabolism. This suggests that targeting this pathway is a potential mechanism to substantially augment the beneficial effects of exercise.

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Nuclear receptor V-ErbA-related protein 2 (EAR2) is a novel driver of breast cancer proliferation

Fathima H S Yoosuf[1], Chantal Magne De[1], Colin D Clyne[1], Simon Chu[1], Peter J Fuller[1]

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

Oestrogen drives breast cancer, and hormone directed therapy is the mainstay of treatment. However, a third of patients do not respond or develop therapeutic resistance. Apart from ERα, PR and AR, the role of other nuclear receptors (NR) in breast cancer have been little explored. A previous study[(1)] on NR in breast cancer found that NR2F6 or EAR2 (V-Erb-A related protein 2) was uniquely upregulated in both ERα-positive and ERα-negative breast cancers, while all other NRs were universally downregulated. This suggests that EAR2 may be a driver of breast cancer proliferation.

Here, we have examined the role of EAR2 in breast cancer cell proliferation, invasion and migration after modulating EAR2 expression in two breast cancer cell lines.

The Tet-On gene expression system was used to overexpress EAR2 in MCF7 cells and Crispr/Cas9 gene editing to knockdown EAR2 expression in T47D cells. Proliferation was assessed using the xCELLigence Real Time Cell Analyser SP system as well as MTS assays. Cell migration was studied using the Incucyte Live Cell Imaging and Analysis System. RNA-seq analysis was performed and analysed using the Beijing Genomic Institute's Dr.Tom platform.

EAR2 overexpression increased proliferation while knockdown of EAR2 decreased proliferation and increased cell size. Cell migration was increased by EAR2 overexpression. In MCF7 cells overexpressing EAR2, 219 differentially expressed genes were identified, including 47 upregulated and 172 downregulated genes. The most highly expressed genes included *GALNT16, MACROD2, GPD1* and *WNT10B*, some of which have implicated in therapy-resistant breast cancer.

EAR2 appears to be a novel driver of breast cancer proliferation with a role in cancer progression in both ERα-positive and ERα-negative breast cancers. The finding that EAR2 upregulated *MACROD2* implicates EAR2 in tamoxifen resistance. These findings suggest that EAR2 could be an unexplored and novel target for breast cancer treatment.

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Placental-specific ciclesonide to des-ciclesonide conversion is not impacted by gestational age or placental sex

Ashley Meakin[1], Kathryn Gatford[2], Yu-Chin Lien[3], Michael Wiese[1], Rebecca Simmons[3], Janna Morrison[1]

1. University of South Australia, Adelaide, SA, Australia

2. The University of Adelaide, Adelaide, SA, Australia

3. Center for Research on Reproduction and Women's Health, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Background: Current clinical management of pregnancies at risk of preterm delivery includes maternal antenatal corticosteroid (ACS; dexamethasone or betamethasone) treatment. ACS activate the glucocorticoid receptor (GR) in all fetal tissues, maturing

the lungs at the cost of impaired brain development. This highlights a need for novel treatments. The prodrug ciclesonide (CIC) activates the GR only in tissues with specific enzymes, particularly carboxylesterase 1 and 2 (CES1, CES2), whose expression and activity are high in lungs but not the brain. However, the human placenta expresses CES, and might therefore convert CIC to its GR-activating metabolite des-CIC. This may preclude CIC use as a novel GR-agonist before preterm birth, since the fetus would be systemically exposed to des-CIC, causing GR activation in the brain and lung. We therefore investigated CES isoform expression and conversion of CIC to des-CIC in human placentas collected during the second trimester (Tri2), and at preterm and term birth.

Methods: Differential expression analysis was performed in Tri2 (n=27), preterm (n=34), and term (n=40) placentas using the DESeq2 *R*-package. A log fold change (logFC) of ±1 with a false discovery rate (FDR) of 0.05 was considered biologically significant. Conversion of CIC to des-CIC was measured in a subset of placenta samples (Tri2 n=7, preterm n=26, term n=20) using functional assays developed for Liquid Chromatography with tandem mass spectrometry. Data was analysed using KW-ANOVA.

Results: *CES1* was higher in Tri2 compared with preterm (logFC=1.31, FDR= 1.74E-09) and term (logFC=1.61, FDR= 7.86E-15) placentas. *CES2* expression did not differ between gestational ages. Human placenta converted CIC to des-CIC; however, activity was not impacted by gestational age.

Conclusion: Conversion of CIC to des-CIC by the human placenta highlights a need for preclinical studies to assess the efficacy of novel delivery methods to achieve GR activation in the developing lung but not brain.

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Androgen receptor expression dictates efficacy to Bipolar Androgen Therapy (BAT) in patient-derived models of advanced prostate cancer

Georgia Cuffe[1], Ashlee Clark[1], Michelle Richards[1], Renea Taylor[2, 3, 4, 5], Gail Risbridger[1, 3, 4, 5], Megan Crumbaker[6], Anthony Joshua[6], Mitchell Lawrence[1, 3, 4, 5]

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

2. Department of Physiology, Monash Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, VIC, Australia

3. Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

4. Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC, Australia

5. Cabrini Institute, Cabrini Health, Malvern, VIC, Australia

6. The Kinghorn Cancer Centre, St Vincent's Hospital, Sydney, NSW, Australia

Publish consent withheld

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Development of adrenal organoids after intracutaneous adrenal cell auto-transplantation for primary adrenal insufficiency in a porcine implanted biodegradable temporising matrix

Brigette M Clarke[1, 2, 3], Svjetlana Kireta[1, 3], Julie Johnston[1, 3], James Kollias[4], John Greenwood[5], Chris Christou[6], Patrick Coates[1, 3, 7], David Torpy[1, 2]

1. University of Adelaide , Adelaide, South Australia, Australia

2. Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, South Australia, Australia

3. Centre for Clinical and Experimental Transplantation, Royal Adelaide Hospital, Adelaide, South Australia, Australia

4. 287 Angas Street, Adelaide, South Australia, Australia

5. Royal Adelaide Hospital, Adelaide, SA, Australia

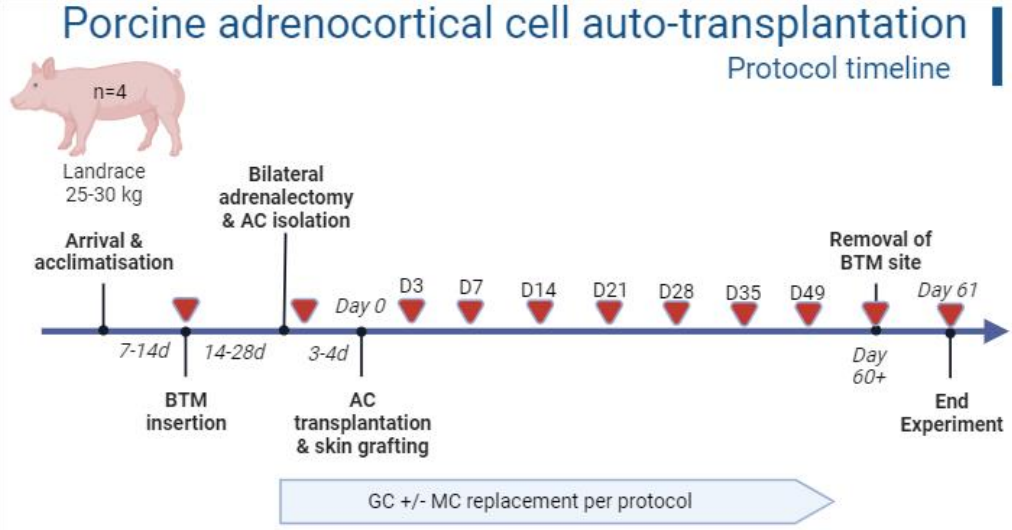
6. Preclinical Imaging and Research Laboratories, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

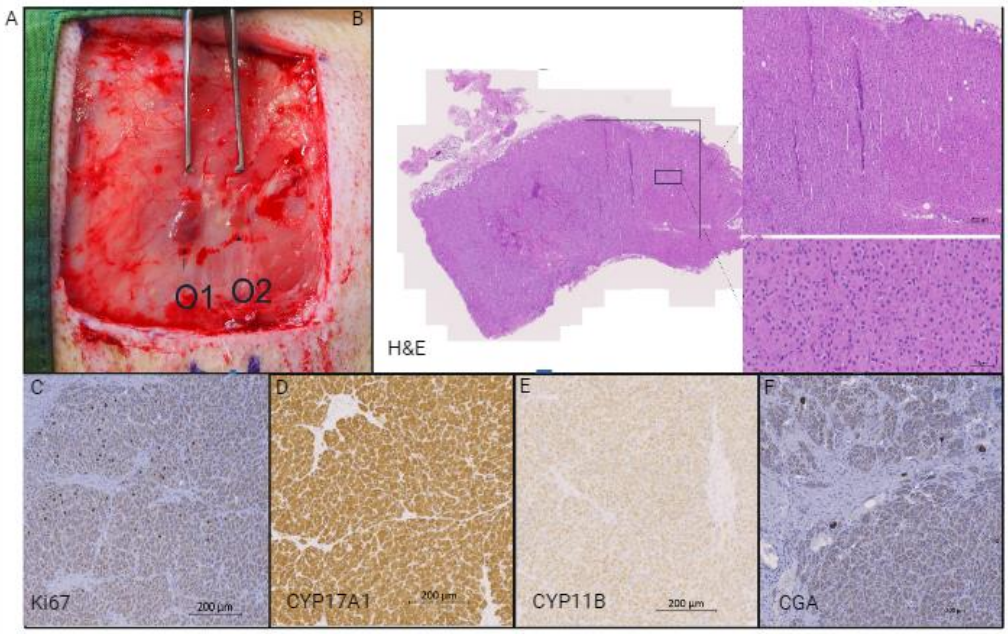
7. Central and Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, South Australia, Australia

Recognising the limitations of current therapies for primary adrenal insufficiency (PAI), novel treatments that better replicate dynamic physiologic corticosteroid secretion under ACTH control are required (1). The aim of this experiment was to evaluate the feasibility of adrenocortical cell transplantation (ACT) in a porcine model, adapting methods successfully used for intra-cutaneous pancreatic islet cell transplantation using a biodegradable temporising matrix (BTM) (2).

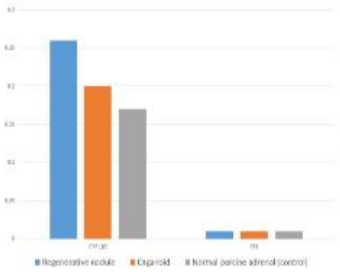
BTM material was engrafted and auto-ACT undertaken by bilateral adrenalectomy followed by isolation, culture and intracutaneous injection of adrenocortical cells (ACs) into the pre-prepared skin site (Figure). Pharmacologic corticosteroids were administered and blood sampling undertaken at serial time points. Clinical signs were monitored for adrenal insufficiency and corticosteroids weaned as tolerated. The graft was excised at end-point for analysis. Outcome measures included detection of ACs at the transplant site, systemic hormone levels and hydrocortisone independence.

Transplanted AC survival and proliferation was demonstrated macroscopically, histologically and by gene expression (Figure) in 1 of 3 transplant recipients. Post-transplantation, all subjects survived to the pre-determined end point, though corticosteroids were unable to be completely ceased. Interpretation of systemic hormone levels was confounded by identification of accessory adrenals and regenerative cortical tissue within the adrenal bed post-mortem. Symptoms of adrenal insufficiency varied dependent on the degree of remnant/regenerative adrenal tissue.





Findings at transplant removal: Adrenal organoids (O1 & O2) identified growing in line with transplant tracks (A). Histologic features of adrenal organoids: Structural arrangement of cells stained with H&E is similar to that of normal porcine adrenals (B). Tissue stains positively for cell proliferation marker Ki67 (C), adrenal steroidogenic enzymes CYP17A1 (D), CYP11B (E) & adrenal medullary cell marker chromogranin A (F)



RT-qPCR: Expression of CYP11B & SF1 genes in the transplant adrenal organoid as compared to regenerative nodule at the adrenal bed site post-mortem and normal porcine adrenal. RPL13 used as the reference housekeeping gene for analysis.

ACT in a large animal model has not previously been attempted, yet it is an important step towards clinical translation. These results demonstrate potential for ACT based on the development of adrenal organoids at the BTM site. However, the inability to achieve clinically relevant systemic hormone production suggests insufficient functioning, number or regeneration of transplanted cells. Further studies are required to optimise cell isolation and culture methods, develop means to objectively evaluate isolate quality prior to transplantation and determine cell yields required for clinically significant function.

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High monounsaturated fat diet reduces the lipotoxic effect on human islet function

Charmaine Cheung[1, 2], Wayne Hawthorne[1, 2], Jenny Gunton[1, 2]

1. University of sydney, Sydney, NSW, Australia

2. Westmead Institute for Medical Research, SYDNEY, NSW, Australia

Long-term consumption of high saturated-fat diet (HSFD) in animals causes β-cell deterioration due to lipotoxicity. Lipotoxicity in vivo in human islets is currently unproven.

AIM: To use "humanised mice" to examine effects of different dietary lipid composition on human islets in vivo.

METHODS: Immunodeficient RAG1-null mice (C57Bl/6 background) were studied. Recipient mice were made diabetic by Streptozotocin. 45 female mice received 2000IEQ human islets from 8 normal glucose tolerant donors. Mice with functioning grafts (random-fed BGL (rBGL) <10mmol/L, n=36) were then fed chow, high-saturated (HSFD, 45% calories from lipids) or high monounsaturated fat diets (MUFD, 45% of calories from lipids). Glucose tolerance tests (GTT) were performed before and during assigned diets.

RESULTS: Mice fed HSFD gained >10% of body-mass, rBGL were also increased by 16 weeks of diet. In contrast, MUFD mice had significantly lower weight-gain and rBGL which did not differ from chow mice.

By mixed model analysis with Tukey's correction for multiple comparisons, GTT was significantly worsen in HSFD mice versus chow ($p<0.0001$), but not MUFD vs chow. Comparing all transplants by mixed model with repeated measures, there was a significant difference between islet preparations, ($p<0.0001$), and diets ($p<0.001$) as well as a significant diet-donor-preparation interaction ($p=0.001$).

HSFD grafts had reduced final β-cell volume which was 46% lower than chow and 23% lower than MUFD. There was also an increase in glucagon staining (alpha-cells) and dual glucagon and insulin positive cells in HSFD grafts, indicated potential β-cell dedifferentiation or β to α cells trans-differentiation.

CONCLUSION: HSFD caused weight-gain and deteriorations on human islets despite all human islet-donors had normal glucose tolerance. Thus far, every human donor shows significant deterioration in GTT with HSFD. MUFD did not cause these deleterious effects. This work has important implications for diet after pancreas- or islet-transplantation and in people with diabetes.

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Discovering the early pregnancy placental dysfunction underlying preeclampsia: omics of chorionic villus samples

Ellen Menkhorst[1]

1. University of Melbourne, Melbourne, VIC, Australia

A healthy pregnancy is crucial for a healthy baby and a healthy start to life. Preeclampsia is a pregnancy-induced disorder unique to humans and a major cause of maternal and perinatal morbidity and mortality worldwide. Preeclampsia is a complex multi-system disease, diagnosed by sudden onset hypertension (>20 weeks gestation) and at least one other associated complication including proteinuria, placental or maternal organ dysfunction. Preeclampsia's impact extends beyond pregnancy: preeclampsia has significant long-term health consequences for both the mother and child including elevated risk of developing chronic kidney and cardiovascular disease.

Poor implantation and placentation in the first trimester of pregnancy are widely accepted to be the sentinel causes of pregnancy diseases including preeclampsia. Despite decades of research advances in the detection, prevention and treatment of preeclampsia, particularly term preeclampsia, have been limited by our inadequate understanding of its pathogenesis. Chorionic villus samples (CVS) are placental biopsies collected between 11-13 weeks gestation. These biopsies represent a unique opportunity to understand the early pregnancy placental dysfunction present in pregnancies that subsequently develop preeclampsia.

In a world-first, we have performed multiple omics analyses on CVS from pregnancies that developed preterm (<37 weeks) and term (>37 weeks gestation) preeclampsia as well as normotensive, uncomplicated pregnancies: 'bulk' (whole tissue) RNA sequencing (mRNA, short RNA) and quantitative label free proteomics as well as spatial transcriptomics. Using this innovative approach we have identified for the very first time an 'early pregnancy placental molecular signature' in placentas that subsequently develop preeclampsia. This study provides crucial insight into the early pregnancy placental dysfunction that precedes preeclampsia.

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Using a Multi-Omics Landscape of the Maternal-Fetal Interface to model the Longitudinal Pathogenesis of Early-Onset Pre-eclampsia

Olivia Nonn[1, 2, 3, 4]

1. Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

2. Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

3. Experimental and Clinical Research Center, a cooperation between the Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association and the Charité - Universitätsmedizin Berlin, Berlin, Germany

4. Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria

Pre-eclampsia (PE) is a syndrome that affects multiple organ systems and is the most severe hypertensive disorder in pregnancy. It frequently leads to preterm delivery, maternal and fetal morbidity and mortality and life-long complications. We currently lack efficient screening tools and early therapies to address PE.

To identify candidate biomarkers and operative pathways in early onset PE (eoPE, severe PE with onset before week 34), we performed spatio-temporal multi-omics profiling of human eoPE placentae and healthy controls, and validated targets in early gestation in a longitudinal clinical cohort. We used a single-nuclei RNA-sequencing combined with spatial proteo- and transcriptomics and mechanistic *in vitro* signalling analyses to bridge the gap from late pregnancy disease to early pregnancy pathomechanisms.

We discovered a key disruption in villous trophoblast differentiation, which is driven by the increase of transcriptional coactivator p300, that ultimately ends with a senescence-associated secretory phenotype (SASP) of trophoblasts in eoPE. We found a significant increase in the senescence markers in preeclamptic maternal serum in early gestation and late gestation, before the development of clinical symptoms, indicating that the placental syndrome drives systemic maternal syndrome, even before clinical manifestation of eoPE.

Our work describes a new disease progression model, starting with dysregulated transition in villous trophoblast differentiation. Our study identifies potential pathophysiology-relevant biomarkers for the early diagnosis of the disease as well as possible targets for interventions, which would be crucial steps toward protecting the mother and child from gestational mortality and morbidity and an increased risk of cardiovascular disease later in life.

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A primary human pipeline for screening, development, and translation of therapies for pregnancy complications

Natasha de Alwis[1]

1. Department of Obstetrics and Gynaecology, University of Melbourne, Carlton, VIC, Australia

Pregnancy complications including preeclampsia, fetal growth restriction, and preterm birth can have devastating short- and long-term impacts on the mother and child. Though affecting up to 20% of all pregnancies, these conditions are not completely understood, and there is a serious lack of therapies to effectively prevent or treat disease. Our team has developed novel preclinical screening approaches, utilising primary human tissue models and mouse models of disease to test innovative therapeutic strategies, which include examining new drugs and innovative delivery methods.

Vital to the design of our pipeline studies is our access to healthy and pathological human tissues collected from across gestation. These prized human tissues have allowed us to produce our own specialised models of pregnancy and disease, closely simulating human pregnancy in the laboratory. This includes outgrowth models using first trimester placental tissues, which allow us to study how the placenta responds to a therapy in a key window of early placental development. We have also developed vascular models of preeclampsia to simulate the vascular dysfunction characteristics of the disease within the maternal systemic vasculature. These models utilise maternal serum collected from pregnancies complicated by preeclampsia to induce vasoconstriction of human arteries, collected from pregnant patients, allowing us the ability to test whether we can reverse this feature of preeclampsia with candidate therapies.

Pregnant individuals are systematically and repeatedly excluded from clinical trials for new therapies due to fear of harm to the growing fetus. We are expanding our pipeline to not only assess candidate therapies for pregnancy complications, but to also produce robust preclinical data to demonstrate the safety/effectiveness of therapies for other underlying conditions one might have in pregnancy. These findings would guide clinicians and encourage industry to include pregnant cohorts in their clinical trials - improving equity of access to therapies in pregnancy.

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Placental Development: More Lessons from Transgenic Mice

David Simmons[1]

1. University Of Queensland, St Lucia, QLD, Australia

With the advancing sophistication of innovative human organoid models gathering pace and the well-characterised structural differences between human and mouse placental architecture, there is legitimate debate around the ongoing relevance of rodent models in placental research. Herein I posit much can still be gleaned about conserved aspects of placental formation and function using transgenic mouse models, with the caveat that context is paramount. Two critical aspects of rodent models still offer significant advantages for placental research; the ability to characterise the impact of genetic manipulations in vivo and the tractability of a system that retains embryonic connections, allowing fetal growth and development as a readout of placental manipulations and function. I will present some of our work highlighting the utility of transgenic mouse models to

investigate placental transport, the connection between placental and heart development, and the lineages of placental progenitors.

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Developmental origins of adult breast cancer risk

Wendy V Ingman[1, 2, 3]

1. Robinson Research Institute, University of Adelaide, Adelaide, SA

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

3. Basil Hetzel Institute, The Queen Elizabeth Hospital, Woodville, SA

The breast is a unique organ that undergoes the majority of its development postnatally. A rudimentary mammary gland ductal tree exists in both males and females at birth. Extensive development occurs during puberty in girls under the influence of ovarian hormones estrogen and progesterone.

There is growing evidence from epidemiological and animal studies that timing of puberty and the diet consumed during puberty have lasting consequences for adult breast cancer risk. Aligned with the developmental origins of adult health and disease paradigm, puberty can therefore be considered a key developmental stage that affects the risk of breast cancer during adulthood.

While specific components of the diet during puberty can increase breast cancer risk, a restricted diet may also affect healthy breast development. A high fat diet leads to sustained increased risk of cancer that cannot be reversed by a healthy diet during adulthood. On the other hand, increased adipose tissue deposition through increased consumption of a healthy diet reduces adult breast density and the associated risk of cancer.

Interventions that emphasise good nutrition for adolescent girls, rather than thinness, have potential to promote healthy breast development and reduced lifetime breast cancer risk.

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A new paradigm for targeting androgen action in advanced prostate cancer

Mitchell Lawrence[1, 2, 3], **Georgia Cuffe**[1], **Shivakumar KeerthiKumar**[2, 3], **Ashlee Clark**[1], **Melbourne Urological Research Alliance (MURAL)**[1], **Luke Selth**[4], **Megan Crumbaker**[5], **Renae Taylor**[2, 3, 6], **Gail Risbridger**[7, 1, 2], **Anthony Joshua**[5]

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

2. Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

3. Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC, Australia

4. Flinders University, Adelaide, SA, Australia

5. The Kinghorn Cancer Centre, St Vincent's Hospital, Sydney, NSW, Australia

6. Department of Physiology, Monash Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, VIC, Australia

7. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC 3010, Australia

Testicular androgens regulate the prostate gland throughout life. Androgens trigger morphogenesis during foetal development, maturation in puberty, and differentiation in adulthood. The onset of prostate cancer causes a switch in androgen receptor (AR) signalling that stimulates proliferation of prostate epithelium. Thus, since the 1940s, the standard-of-care for advanced prostate cancer has been to block AR activity. This temporarily controls tumour growth, but there are drawbacks. Tumours eventually develop diverse mechanisms of resistance, and patients endure mounting side-effects, including decreased sexual function. This underscores the need for new treatments.

A new strategy may reverse the decades-old paradigm of ongoing blockade of AR signalling. With Bipolar Androgen Therapy (BAT), patients oscillate between low (castrate) and high (supraphysiologic) testosterone levels. This involves simple, monthly injections of an FDA-approved dose of testosterone, and it may relieve patients of the side-effects of androgen suppression. Using high doses of testosterone to treat prostate cancer may seem counterintuitive; however, clinical trials show promising results.

Some tumours respond to BAT, while others do not. Therefore, our team is using patient-derived models of prostate cancer to investigate which tumours are most responsive to BAT. Our contemporary cohort spans diverse forms of aggressive prostate cancer from patients who progressed on current treatments, including potent AR signalling inhibitors, chemotherapy and radioligand therapy. Our data shows that BAT-sensitive patient-derived models have high levels of AR, but low levels of ligand-independent AR variants. Moreover, we have demonstrated that aggressive pathologies of prostate are sensitive to BAT. Collectively, these studies are refining the subgroups of prostate cancer that are most likely to benefit from BAT and informing the design of combination therapies to increase response rates to BAT.

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Endometrial stem cells: implications in reproductive pathologies and ageing

Shafiq Syed[1], **Muhammad Jamaluddin**[1], **Florence Bartlett**[1], **Poonam Rani**[1], **Varshini Venkata**[1], **Pradeep Tanwar**[1]

1. Global Centre for Gynaecological Diseases, University of Newcastle, Callaghan, NSW, Australia

The endometrial lining, constituting the internal layer of the uterus, is central to reproductive health due to its essential role in supporting mammalian life. One of the most notable features of the endometrium is its remarkable ability to regenerate, a

mechanism vital for reproductive success and overall health. However, the foundations of its renewal and the negative effects of its malfunctions, especially in relation to aging and reproductive pathologies in females, continue to be areas of significant interest and scrutiny. The multifaceted intricacies guiding these endometrial functions and the potential pitfalls when disrupted have been the cornerstone of my investigations. Our body of work illuminates the intricate endometrial environment, mapping from stem cell behaviour to the changes brought on by ageing. Integrating our knowledge of stem cell dynamics, notably guided by Wnt signalling pathways, with findings on age-related alterations and their ensuing consequences, we provide an encompassing overview. This integrated knowledge aims to refine and improve methods for diagnosing, preventing, and treating endometrial cancers and associated reproductive issues.

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Development of innovative strategies to preserve oocyte number and quality during cancer treatment

Karla Hutt[1]

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

Irreversible damage to the ovary and depletion of the ovarian reserve are devastating side effects of many traditional cytotoxic cancer treatments, often leaving young female cancer survivors infertile and at risk of premature menopause. Compounding this issue, our recent studies suggest that new precision drugs, like parp inhibitors and immune checkpoint inhibitors, which promise to deliver better patient outcomes with fewer side effects, may also damage the ovary and compromise fertility. Globally, ~1.4 million women under the age of 45 years are diagnosed with cancer per year and over 80% will survive. Therefore, improving quality of life by mitigating off-target treatment effects has become a critical patient-driven priority. Existing fertility preservation methods are not suitable for all patients, and no broadly effective pharmacological options exist to protect the ovaries of young girls and women receiving cancer treatment. This is due to poor understanding of how different treatments damage the reproductive tract, therefore, a lack of viable pharmacological targets. To begin to address these fundamental knowledge gaps, we are utilising preclinical naive and tumour bearing mice to examine the impact of existing and new cancer treatments on female reproductive health. We are using these models to decipher the precise cellular and molecular mechanisms that contribute to loss of oocyte number and quality during cancer treatment, and then using this knowledge to investigate innovative adjuvant therapies to protect the ovary from damage. Our long-term goal is to expand and improve fertility preservation options available to women, and thereby optimise the health and well-being of female cancer survivors world-wide.

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Testosterone treatment in eugonadal men discloses a differential association between decreased adiposity and changes in testis volume compared to placebo.

Warrick J Inder[1, 2], **Kristy P Robledo**[3], **Carolyn A Allan**[4], **Karen Bracken**[3], **Ann J Conway**[5], **Mathis Grossmann**[6, 7], **Alicia Jenkins**[8], **David Jesudason**[9, 10], **Robert McLachlan**[4], **Bronwyn GA Stuckey**[11, 12], **Bu B Yeap**[12, 13], **David J Handelsman**[5], **Gary A Wittert**[9, 14]

1. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, QLD, Australia

2. Medical School, The University of Queensland, Herston, QLD, Australia

3. NHMRC Clinical Trials Centre, University of Sydney, Camperdown, NSW, Australia

4. Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, VIC, Australia

5. ANZAC Research Institute, Concord Hospital, University of Sydney, Concord, NSW, Australia

6. Endocrinology, Austin Hospital, Heidelberg, VIC, Australia

7. Medicine, University of Melbourne, Parkville, VIC, Australia

8. Baker Heart and Diabetes Institute, Melbourne, VIC, Australia

9. School of Medicine, University of Adelaide, Adelaide, SA, Australia

10. Endocrinology, The Queen Elizabeth Hospital, Woodville South, SA, Australia

11. Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

12. Medical School, University of Western Australia, Perth, WA, Australia

13. Endocrinology and Diabetes, Fiona Stanley Hospital, Murdoch, WA, Australia

14. Freemasons Centre for Male Health and Wellbeing, South Australian Health Medical Research Institute, Adelaide, SA, Australia

Reduced testis volume in eugonadal men treated with testosterone is well-recognised. This sub-study of the Testosterone for the Prevention of Type 2 Diabetes (T4DM) Trial investigated i) baseline determinants of testis volume, ii) changes in testis volume over 2 years following randomisation to testosterone or placebo, and iii) the effect of changes in fat mass (DXA), BMI, and waist circumference (WC) on testis volume.

T4DM enrolled men aged 50-74 years, with impaired glucose tolerance or early type 2 diabetes, WC ≥95cm, serum testosterone ≤14 nmol/L and without pathological hypogonadism. All were enrolled in Weight-Watchers (WW) and randomised to 2 years treatment with testosterone undecanoate (1000 mg IM 3 monthly) or matching placebo. At one study centre, a single investigator (WJI) measured testis volume (Prader orchidometer) at baseline and final visits while blinded to treatment assignment.

The sub-study participants (n=123, 63 testosterone 60 placebo) were similar in age to the whole T4DM cohort but had higher weight (p=0.036), waist circumference (p=0.005), and BMI-graded obesity (p=0.004). Baseline testis volume was 19.4±4 ml and was inversely correlated with serum FSH (p=0.001) and LH (p=0.004), but not adiposity measures.

At 2 years, testis volume of testosterone-treated men was reduced by 5.9 ml (95% CI 4.6-7.2) more than placebo recipients ($p<0.001$), while the changes in BMI (Testosterone -1.7±2.3 kg/m^2 v Placebo -1.7±2.7 kg/m^2), fat mass (Testosterone -4.3±5.7 kg v Placebo -2.1±7.3 kg) and WC (Testosterone -6.8±5.7 cm v Placebo -4.8±7.3 cm) were not significantly different. However, reduced adiposity was associated with lower testis volume in testosterone-treated men, but with higher testis volume in placebo-treated men (treatment x fat mass interaction p-int=0.012; treatment x WC, p-int=0.01).

Two years of testosterone treatment in men with dysglycaemia reduced testis volume and was associated with decreased adiposity. However, in placebo-treated men, reduced adiposity was associated with increased testis volume.

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Effects of obesity, depression, age, and testosterone treatment on erectile function and sexual desire in older men with dysglycaemia.

Gary Wittert[1], Kristy Robledo[2], Mathis Grossmann[3], Bu Yeap[4], Bronwyn Stuckey[5], Warrick Inder[6], Ann Conway[7], David Jesudason[8], Carolyn Allan[10, 9], Rob McLachlan[10], Alicia Jenkins[11], Karen Bracken[12], David Handelsman[13]

1. Freemasons Centre for Male Health and Wellbeing, University of Adelaide, Adelaide , SA, Australia

2. NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

3. Department of Medicine, Austin Health, University of Melbourne, Melbourne, Victoria, Australia

4. Medical School, University of Western Australia, Perth, WA, Australia

5. Keogh Institute for Medical Research, University of Western Australia, Perth, WA, Australia

6. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

7. Andrology, Concord Hospital, Sydney, NSW, Australia

8. Endocrinology, The Queen Elizabeth Hospital, Adelaide, South Australia, Australia

9. Monash University,, Clayton, Victoria, Australia

10. Andrology, Hudson Institute of Medical Research, Clayton, Victoria, Australia

11. Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia

12. Sydney Musculoskeletal Health , The University of Sydney , Sydney, NSW, Australia

13. Anzac Research Institute, University of Sydney, Sydney, NSW, Australia

In men (aged 50 - 74 yrs., waist circumference (WC) ≥95cm, prediabetes or newly diagnosed T2D, and serum testosterone (T) ≤14 nmol/L), enrolled in Weight-Watchers, 2 years T-undecanoate treatment (TTr) (n=504) reduced WC and T2D risk and improved erectile function (EF) and sexual desire (SD) vs placebo (n=503) (1).

In a secondary analysis, we determined the effects of (i) baseline factors, (ii) TTr with adjustment for WC, blood pressure (BP), serum glucose, and mood over time, on EF and SD, and (iii) predictors of clinically significant effects.

Measurements: EF and SD (International Index of Erectile Function 15 questionnaire), serum trough T (LCMS/MS), glucose, WC, BP, (weeks 0, 30, 54, 78, 102), and mood (Center for Epidemiologic Studies (CES-D) questionnaire), (weeks 0, 54, 102). Clinically significant (CS) increases in SD and EF: ≥ 2 and ≥4 points respectively (2). Analyses: Linear mixed effects models, and logistic regression for CS effects.

At baseline, there were inverse associations of SD and EF with age ($P<0.001$), EF with WC ($P=0.04$), and SD with CES-D scores ($P=0.03$). TTr increased mean EF and SD scores, independent of baseline serum T ($P<0.001$); CS responses were more likely in those with low baseline EF and SD scores ($P<0.001$). TTr increased mean SD scores (Pint=0.014) and CS SD (P=0.011) more in older men; and maintained mean but not CS SD in those with decreasing mood over time (Pint=0.03). TTr did not affect mood. Independent of TTr decreasing WC over time improved mean EF ($P=0.04$) and SD scores ($P=0.04$), without CS effects. Increasing depression symptoms predicted a deterioration in EF ($p=0.006$).

TTr, visceral obesity, and mood have independent and interacting effects on EF and SD. Only TTr had CS benefits, which were greater in men with lower baseline EF and SD scores, and for SD greater in older men.

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Fertility, pregnancy and early parenting experiences of doctors

Lisa M Raven[1, 2, 3], Nada Hamad[2, 4], Ann I McCormack[1, 2, 3]

1. Department of Endocrinology, St Vincent's Hospital, Darlinghurst, NSW, Australia

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

3. Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia

4. Department of Haematology, St Vincent's Hospital Sydney, Darlinghurst, New South Wales, Australia

International studies have demonstrated unique trends in early parenting and fertility in medical doctors, however there is limited Australian data.

Aims: To describe the experiences of medical professionals regarding fertility, pregnancy and early parenting experiences.

Methods: An online survey was distributed by specialty colleges and societies to medical doctors, with completion between August 2022 and August 2023.

Results: Four-hundred and twenty-three people completed the survey, 89% were female with a median age of 36 years (interquartile range [IQR] 33-43) and the majority were in heterosexual relationships. Most responders had completed specialist training and the two largest groups of specialties represented were physicians (including 20 endocrinologists) and emergency

medicine doctors. Most (71%) of the respondents had children, with the median number of children 2 (IQR 1-2). Pregnancy loss had been experienced by 31% (n=127) of respondents; of those who experienced pregnancy loss at less than 12 weeks gestation, most did not have any time off work. Of those who experienced pregnancy loss, most would have liked more time off work, and their pregnancy loss was not acknowledged by their work or colleagues. One-hundred and twenty-five (31%) of couples had undergone testing for infertility and 20% (n=80) had used assisted reproductive technologies. The median age of first-time mothers was 32 (IQR 30-35), and almost half of responders had their first child when they were undertaking accredited specialist registrar training. The majority of respondents (60%, n=209) reported delaying having children due to their medical training. Most parents (70%, n=156) reduced their work schedule during the first 5 years of parenthood.

Conclusion: The age of first pregnancy and rates of pregnancy loss were higher in this cohort than the national averages. Doctors delay pregnancy and have high rates of infertility. Pregnancy loss is common and is rarely acknowledged by workplaces or colleagues in medicine.

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Preventing osteoporosis in spinal cord injury (POPSCI) trial: early zoledronic acid infusion in acute spinal cord injury

Shejil Kumar[1, 2, 3], Jean Doyle[1], Maria-Liza Nery[1], Roxana Heriseanu[4], Gerard Weber[4], Lianne Nier[4, 5], Roderick Clifton-Bligh[1, 6, 7], Christian Girgis[1, 6, 3]

1. Endocrinology Department, Royal North Shore Hospital, Sydney

2. Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia

3. Endocrinology Department, Westmead Hospital, Sydney

4. Department of Rehabilitation Medicine, Royal Rehab Hospital, Sydney

5. Department of Rehabilitation Medicine, Royal North Shore Hospital, Sydney

6. Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia

7. Cancer Genetics Laboratory, Kolling Institute of Medical Research, Sydney

Background: Traumatic spinal cord injury (SCI) is a significant cause of disability, exacerbated by accelerated bone loss and increased fracture risk predominantly around the knee. Few studies have explored efficacy of IV zoledronic acid (ZOL) in patients with SCI with limited follow-up <24-months.

Methods: We conducted a prospective study investigating use of early ZOL after acute SCI. The study included two cohorts; an 'intervention' cohort (n = 11) admitted to Royal North Shore or Royal Rehab Hospitals, Sydney between 2018-2022 who sustained traumatic SCI in the past 8-12 weeks and received a single dose of 4mg ZOL after baseline studies, and a 'historical control' cohort (n = 9) who sustained traumatic SCI within the last 1-5 years and received standard care. All participants underwent baseline and 6-monthly bloods (including bone turnover markers (CTx and P1NP)) and 12-monthly DXA BMD scans of lumbar spine, left hip and knee.

Results: Baseline comparisons between both cohorts are summarised in Table 1. The 'intervention' cohort had higher baseline CTx, P1NP and sclerostin concentrations consistent with higher bone turnover in acute SCI. 'Historical controls' had lower baseline left hip and femoral metaphyseal BMD consistent with longer exposure to skeletal unloading. Majority experienced an acute phase reaction after ZOL (9/11 – 82%). In the 'intervention' cohort, CTx and P1NP fell by mean 50% at 12-months and plateaued to 36-months. Left hip BMD fell gradually by mean 13% by 36-months. Left femoral metaphyseal BMD declined by mean 22% respectively at 12-months and plateaued to 36-months. No fractures occurred.

Conclusions: Early ZOL prevented a rise in bone turnover markers in acute SCI however patients still experienced rapid decline in distal femoral BMD. Further studies assessing knee BMD response to antiresorptives and longer follow-up in SCI cohorts are required to optimise fracture risk reduction.

	Intervention – early phase SCI (n=11)	Control – late phase SCI (n=9)	p-value
Age (mean ± SD, years)	39.8 ± 15.8	36.7 ± 16.2	0.666
Sex (% male)	91%	67%	0.285
Weight (mean ± SD, kg)	78.2 ± 14.9	77.5 ± 13.4	0.910
BMI (mean ± SD, kg/m^2)	24.0 ± 4.7	24.7 ± 5.5	0.774
Alcohol (% current)	91%	89%	1.000
Smoking (% current)	9%	11%	0.544
ASIA severity scale (% A or B)	55%	78%	0.370
Time since injury (mean ± SD, days)	63 ± 15	1007 ± 549	<0.001
Vitamin D (mean ± SD, nmol/L)	78.5. ± 24.8	79.6 ± 31.3	0.937
Creatinine (mean ± SD, umol/L)	60.8 ± 6.6	65.9 ± 20.2	0.488
Corrected calcium (mean ± SD, mmol/L)	2.46 ± 0.22 (n = 8)	2.38 ± 0.08	0.295
Phosphate (mean ± SD, mmol/L)	1.5 ± 0.2	1.2 ± 0.1 (n = 8)	<0.001
Alkaline phosphatase (mean ± SD, mmol/L)	101.4 ± 35.5	79.4 ± 19.2	0.114
Testosterone (mean ± SD, nmol/L)	12.6 ± 6.7 (n = 10)	20.3 ± 4.9 (n = 5)	0.042
Luteinising hormone (mean ± SD, IU/mL)	3.8 ± 1.5	3.7 ± 1.1	0.851
C-terminal telopeptide of type 1 collagen (mean ± SD, pg/mL)	1497.8 ± 692.2	627.1 ± 319.0	0.003
Procollagen type 1 N-propeptide (mean ± SD, mcg/L)	148.2 ± 78.1	74.7 ± 33.9	0.017
Sclerostin (mean ± SD, ng/L)	270.4 ± 73.1 (n = 10)	164.2 ± 71.6 (n = 5)	0.019
Lumbar spine BMD (mean ± SD, g/cm^2)	1.10 ± 0.18 (n = 10)	1.04 ± 0.16 (n = 9)	0.431
Lumbar spine T-score (mean ± SD)	+0.1 ± 1.6 (n = 10)	-0.5 ± 1.4 (n = 9)	0.432
Left femoral neck BMD (mean ± SD, g/cm^2)	0.94 ± 0.20 (n = 10)	0.70 ± 0.16 (n = 9)	0.016
Left femoral neck T-score (mean ± SD)	0.0 ± 1.4 (n = 10)	-1.7 ± 1.2 (n = 9)	0.016
Left total hip BMD (mean ± SD, g/cm^2)	1.02 ± 0.18 (n = 10)	0.77 ± 0.16 (n = 9)	0.006
Left total hip T-score (mean ± SD)	0.0 ± 1.2 (n = 10)	-1.6 ± 1.3 (n = 9)	0.010
Left femoral metaphysis BMD (mean ± SD, g/cm^2)	1.19 ± 0.21 (n = 9)	0.73 ± 0.19 (n = 8)	<0.001

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Cardiovascular risk in PCOS is associated with diabetes and hypertension: A case control linked data study in Western Australian women

Kharis Burns[1, 2], Alexander Stuckey[4, 3], Kevin Trentino[2], Gerald Watts[5, 2], Frank Sanfilippo[6, 7], Bronwyn Stuckey[8, 2, 9]

1. Department of Endocrinology and Diabetes, Royal Perth Hospital, Perth, WA, Australia

2. Medical School, University of Western Australia, Crawley, WA, Australia

3. School of Agriculture and Environment, University of Western Australia, Crawley, WA, Australia

4. Pink Lake Analytics, Nedlands, WA, Australia

5. Lipid Disorders Clinic, Department of Cardiology, Royal Perth Hospital, Perth, WA, Australia

6. School of Population and Global Health, University of Western Australia, Crawley, Western Australia, Australia

7. Pharmacy Department, Royal Perth Hospital, Perth, Western Australia, Australia

8. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

9. Keogh Institute for Medical Research, Nedlands, WA, Australia

Increased cardiovascular (CV) risk in polycystic ovary syndrome (PCOS) is assumed, based on associated risk factors including insulin resistance. Previous research is conflicting owing to heterogeneity in study design and definitions of both PCOS and CV disease (CVD). This retrospective cohort study examined CV outcomes using a uniformly diagnosed cohort of women with PCOS vs. controls using linked health data.

NIH-defined PCOS patients were identified from endocrinology clinics in Western Australia. Control women were identified from the WA electoral role and matched, 1(case):4(controls), by age, sex, postcode and Indigenous status. Clinical data for the cases were linked with administrative data from the Western Australian (WA) Data Linkage System.

CV outcomes were identified from linked hospital admissions, and included coronary heart disease, heart failure, atrial fibrillation/arrhythmia, cardiac arrest, cerebrovascular disease, and peripheral vascular disease. Events were examined collectively, based on a composite of these outcomes, and as individual outcomes. Multivariable stratified Cox proportional hazards regression models with time-dependent covariates were used to examine the effect of PCOS on CV outcomes.

Overall, 1035 PCOS-affected women and 4148 controls were followed for an average of 33.0 (SD 9.6) years. PCOS cases had higher incidence of diabetes, hypertension, COPD and fatty liver; these covariates were included as time dependent variables in models. Unadjusted hazard ratios showed significantly higher risk of both overall CVD (ICD-9_CM codes 390-459, ICD-10-AM I00-I99) (HR 1.33, CI 1.14-1.56) and the composite CV endpoint (unadjusted HR 1.55, CI 1.07-2.24). However, following adjustment for the time dependent variables the HR for composite CV outcome in cases vs. controls was 1.17 (95% CI 0.81-1.68).

The difference in findings following adjustment for diabetes and hypertension, implies that PCOS per se is not the cause of differences in CV outcome. Instead, this suggests traditional CV risk factors drive CV risk in women with PCOS.

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Prevalence and characteristics of low-renin hypertension in an Australian primary care population

Sonali Shah[1, 2], Renata Libianto[1, 2], Morag J Young[2], Peter J Fuller[1, 2], Jun Yang[1, 2]

1. Department of Endocrinology, Monash Health, Clayton, VIC, Australia

2. Centre of Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, VIC , Australia

Publish consent withheld

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X Linked Hypophosphatemia and Burosumab treatment in adults

Malu Alvarez[1], Maria-Liza Nery[1], Rory Clifton-Bligh[1, 2]

1. Department of Diabetes, Endocrinology, and Metabolism, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Northern Clinical School, Sydney Medical School, University of Sydney, Camperdown, NSW, Australia

X-linked hypophosphataemic (XLH) rickets is caused by a genetic mutation of the PHEX gene which results in increased levels of fibroblast growth factor 23 (FGF23). Excess FGF23 supresses renal reabsorption of phosphate and the renal synthesis of 1,25 dihydroxy vitamin D, resulting in low serum phosphate levels. This dysregulation of phosphate homeostasis disrupts the mineralisation and ossification of bone, causing abnormal bone development and short stature1. Individuals may experience bone and joint pain, muscular dysfunction, impaired ambulation, dental complications, pseudofractures, early osteoarthritis, enthesopathy and hearing difficulties, in varying severity2,3

Conventional treatment involves supplementation of phosphate and vitamin D in an attempt to normalise serum levels, but this does not target the underlying dysregulation of phosphate homeostasis. Conventional treatment is poorly tolerated and there is limited evidence of the benefits of conventional treatment in adults4.

Burosumab is a new drug treatment for XLH that has achieved favourable results in clinical trials.5,6 It is a monoclonal antibody that binds to and inhibits FGF23, thereby increasing phosphate and 1,25 dihydroxy vitamin D serum levels, leading to improvements in rickets and in growth in children improvement in fracture healing and decreased pain and stiffness in adults.

This presentation will focus on the nursing aspects of caring for adult patients with XLH requiring Burosumab treatment: first presentation to adult clinics, assessment tools and diagnostic tests required, and the safety and administration of Burosumab.

1. Carpenter et al. 2011
2. Chesher et al. 2018
3. Skrinar et al. 2019
4. Carpenter et al. 2014
5. Carpenter et al. 2018
6. Insogna et al. 2018

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Rare Genetic Bone Disorders in Children, Diagnosis and Management

Craig Munns[1]

1. The University of Queensland, South Brisbane, QUEENSLAND, Australia

Over 400,000 children are living with a rare disease, the majority of which are genetic in origin. The explosion of genetic technology over the last decade has allowed us to identify the underlying genetic disorder in an increasing number of these children, although approximately 50% of children with a suspected Mendelian condition remain undiagnosed. Obtaining a genetic diagnosis allows for accurate genetic counselling, disease-targeted surveillance or therapy, and increasingly advanced therapeutic trials.

Interdisciplinary care is essential to optimal management of children with rare genetic bone disorders, with the importance of psychological impact of living with a rare disease being increasingly recognised.

Over the past 12 months, Commonwealth approval has been obtained for three transformative therapies in children with genetic bone disorders – Burosumab (Crysvita©) for X-linked hypophosphataemia, Asfotase Alfa (Strensiq©) for hypophosphataemia and Vosoritide (Voxzogo©) in achondroplasia. All three of the targeted therapies are now used across Australia and have the potential to be transformative to the children who have the underlying disorder and families. New models of care are needed to deliver these therapies.

The ultimate goal is cure. For the first time, gene therapy has the potential to offer this to children with rare genetic bone disorders. We have seen this with Zolgensma in children with spinal muscular atrophy and there is promising discovery data that gene therapy may be on the horizon for children with rare genetic bone disorders.

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Accounting for the Variability in Responses to Physiologic Stressors in Hypothalamic Amenorrhea

Janet Hall[1]

1. National Institute of Environmental Health Sciences, NIH, Durham, NORTH CAROLINA, United States

Hypothalamic amenorrhea (HA), a reversible form of hypogonadotropic hypogonadism, has a population prevalence of <5% but is markedly increased in the setting of metabolic imbalance due to exercise and/or caloric restriction and stress. Interestingly, there is considerable inter-individual variability in the reproductive system response to apparently similar levels of diet, exercise and/or stress. We have now determined that women with HA have an increased burden of variants in genes association with Kallmann Syndrome or normosmic hypogonadotropic hypogonadism that may confer increased susceptibility to these physiologic stressors. We have also shown that the burden of variants in genes associated with the cross-talk between metabolic sensing and GnRH control through kisspeptin, GABAergic, glutaminergic, thyroid and orexin pathways is increased in HA vs control women. In normal women, moderate energy restriction for as little as 5 days is associated with sleep disruption for at least a full month augmenting the negative effect of the luteal phase on sleep. We have further shown that this degree of energy restriction results in adaptation of the thyroid axis as well as changes in appetite, leptin and orexin designed to conserve energy and increase appetite. Interestingly, the use of fatty acids as an energy source is not immediately reversed by consumption of a normocaloric snack. Finally, we have demonstrated that in women in whom LH pulse frequency is decreased in response to moderate energy restriction, individual responses to reduced energy availability prioritize either reproductive or metabolic adaptations.

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The importance of the (pro)renin receptor in preeclampsia: Too much of a good thing?

Kirsty Pringle[1, 2]

1. Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, Australia

2. School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia

Preeclampsia affects 5-7% of all pregnancies and is responsible for more than 7,000 maternal and 500,000 fetal deaths worldwide each year. Preeclampsia is associated with maternal hypertension, vascular injury and kidney and liver dysfunction and can lead to haemolysis, and seizures. It also increases the risk of fetal growth restriction and preterm birth by over 4-fold. A better understanding of the pathophysiology of the disease, and the development of new effective therapies is therefore essential. It is broadly accepted that poor placentation resulting in maternal endothelial and vascular dysfunction causes preeclampsia however, the mechanisms and molecular pathways underlying this pathophysiology are largely unknown.

This presentation will focus on a newly identified molecule, the prorenin receptor (PRR), and its role in both placental development and in causing the clinical symptoms of preeclampsia. We have shown, in a placental specific PRR knockout mice model, that PRR is essential for the establishment of a healthy and functional placenta. We have also demonstrated that high levels of sPRR causes endothelial dysfunction *in vitro* and that excess maternal circulating sPRR levels cause a preeclampsia-like phenotype (i.e., maternal hypertension and vascular dysfunction) in pregnant rats. Subsequent experiments have demonstrated that blocking the PRR with a specific peptide antagonist, PRO20, can prevent the endothelial dysfunction induced by preeclamptic serum. We therefore propose that not only is soluble PRR the 'missing link' in the pathogenesis of preeclampsia, but that targeting soluble PRR is a novel therapeutic strategy to treat this disorder.

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Therapeutic development for pregnancy – a crisis that can no longer be ignored

Natalie Hannan[1]

1. University of Melbourne; Mercy Hospital for Women; The Northern Hospital, Heidelberg, VIC, Australia

Major obstetric complications including preterm birth, preeclampsia, fetal growth restriction and stillbirth are the leading cause of perinatal morbidity and mortality globally. Death from obstetric complications disproportionately affects pregnancies in low and middle-income countries and communities. Critically these complications lack appropriate effective treatments.

Pregnant and breastfeeding women are 'therapeutic orphans'. They are systematically excluded from therapeutic research, development, and clinical trials. Predominantly this is due to fear of harm to the neonate. Furthermore, the majority of medications used in pregnancy are used 'off-label' due to lack of appropriate understanding of any potential harmful effects in pregnancy, or the passage of drugs to the neonate and especially whether there are teratogenic consequences. While our risk aversion is understandable, and there have been efforts to improve inclusion of pregnant populations in clinical trials, progress remains stagnant. We can no longer ignore this crisis; appropriate inclusion of pregnant women in research, development and clinical trials of therapeutic agents is urgently needed.

Our team has a dedicated focus towards advancing our knowledge of therapeutic actions during embryo implantation, placentation, and maternal vascular adaptation in pregnancy, as well as focusing on treating the underlying pathophysiology of major obstetric complications. We have carefully developed a novel preclinical pipeline for sophisticated therapeutic development, utilising primary human tissue assays and mouse models to test innovative therapeutic strategies, to examine new drugs and innovative delivery methods.

Importantly the novel strategies we are developing are focused on clinical translation and offer exciting possibilities to enhance equity of access for therapeutic development for pregnancy and vitally the future treatment of obstetric diseases.

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Functional characterization of enhancer RNAs expressed from oncogenic androgen receptor-bound enhancers in advanced therapy-resistant prostate cancer

Rayzel Fernandes[1], Moray Campbell[2], Charlotte Bevan[1]

1. Department of Surgery and Cancer, Imperial Centre for Translational and Experimental Medicine, Imperial College London, Hammersmith Hospital, London, UK

2. Cedars-Sinai Medical Center, California, USA

Prostate cancer (PCa) which has relapsed after first line androgen deprivation therapy (ADT), known as castration resistant prostate cancer (CRPC), is incurable and frequently lethal. Resistance is also observed with second generation antiandrogens, such as enzalutamide and apalutamide. PCa progression and the acquisition of therapy resistance is associated with changes in binding of the androgen receptor (AR, the key therapy target in PCa) to different collections of cis-regulatory enhancer elements. Recently, enhancers have been found to be transcribed, producing non-coding enhancer RNAs (eRNAs), which are increasingly being recognized for their role in contributing to enhancer function. While eRNAs have been shown to be transcribed from several critical AR-bound active enhancers, their role in AR-regulated gene expression and progression to CRPC remains largely unknown.

In this project, we aim to identify potentially oncogenic roles for such eRNAs in selective disruption of AR enhancer interactions thereby altering target gene expression in CRPC. To this end, we performed Global Run On (GRO) sequencing (GRO-Seq) to capture nascent RNA transcriptomes from isogenic pairs of therapy-sensitive and -resistant PCa cell lines (C42Parental, C42EnzR, V16D, MR49FEnzR), with and without DHT stimulation. GRO-seq datasets identified differentially expressed de-novo novel (unannotated) non-coding RNA transcripts in resistant versus sensitive cell lines. Integration of these data with publicly available genomic enhancer annotations, ChIP-seq, and ChIA-PET datasets was used to identify transcripts that are linked to AR regulated enhancers and thus potentially represent resistance associated eRNAs. Several of the differentially expressed eRNAs encoding enhancers are associated with genes linked to PCa progression, including transcription factors such as Grainy Head Like Transcription Factor 2 (GRHL2) as well as transcriptional regulators such as the corepressor REST corepressor 1 (RCOR1). Significantly, differentially expressed non-coding RNA transcripts also included previously characterized long non-coding (lncRNA) associated with PCa, (e.g. SCHLAP1, PCAT1) and novel lncRNA, several of which are transcribed from regions encoding super-enhancers. Ongoing studies are testing the functional implications of these novel enhancer associated RNAs on AR signalling and downstream effects in CRPC.

In conclusion, we have identified several eRNAs and lncRNAs that are dysregulated in CRPC and are exploring their role in enhancer functioning and potential utility as therapeutic targets in this disease.

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Evolution of Thyroid Cancer Management: Advancements in Risk Assessment, Functional Imaging, and Targeted Therapies

Matti Gild[1]

1. Royal North Shore Hospital, Paddington, NSW, Australia

A paradigm shift in thyroid cancer has ushered in a new era of precision medicine, fundamentally altering the approach to both low and high-risk disease. In this transformative landscape, risk assessment of thyroid nodules has evolved beyond traditional methods. Novel techniques, such as deep machine learning and genetic profiling, are now instrumental in evaluating the potential malignancy of thyroid nodules. This enhanced risk assessment has profound implications for patient management. One of the most notable changes is the management of low-risk thyroid cancers. In the past, such cases often led to immediate surgical intervention. However, contemporary guidelines emphasise the importance of active surveillance. This approach recognises that not all low-risk thyroid cancers require aggressive treatment, minimising unnecessary surgeries and their associated risks. Additionally, the integration of functional imaging techniques, such as I^{131}, ^{18}F-FDG PET/CT, and ^{68}Ga-DOTATATE PET/CT, has significantly improved the diagnosis and prognostication of differentiated thyroid cancer (DTC) and medullary thyroid cancer (MTC). These imaging modalities provide valuable insights into the extent and behaviour of thyroid tumours, guiding treatment decisions. For advanced thyroid cancers, the advent of tyrosine kinase inhibitors has revolutionised the management of metastatic disease. These targeted therapies have demonstrated remarkable efficacy in controlling disease progression and improving the quality of life for patients with advanced thyroid cancer. Furthermore, neoadjuvant therapy, although still under investigation, has shown promise in small series, offering a potential avenue to enhance treatment outcomes.

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The Mineralocorticoid System – More Than a Regulator of Blood Pressure in the Pathophysiology of Metabolic Syndrome?

Moe Thuzar[2, 1]

1. Department of Endocrinology & Diabetes, Princess Alexandra Hospital, Brisbane, QLD, Australia

2. 1. Endocrine Hypertension Research Centre, Faculty of Medicine, The University of Queensland, Brisbane, QLD, Australia

Metabolic Syndrome is a group of conditions that increase the individual's cardiovascular risk, and the components include obesity, hypertension, impaired glucose metabolism and dyslipidaemia. The clustering of the conditions within same individuals suggests a common pathophysiological process. The mineralocorticoid system is traditionally viewed as a regulator of blood pressure. This talk will discuss the novel role of the mineralocorticoid system in the pathophysiology of metabolic syndrome.

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Progesterone receptor isoform balance and chromatin structure are critical drivers of progesterone response in the normal breast and breast cancer

Tram Doan[1], Mariah Tahan[1], Barbara Guild[1], Christine Clarke[1], Dinny Graham[1, 2]

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

2. Westmead Breast Cancer Institute, Westmead Hospital, Westmead, NSW, Australia

The ovarian hormone progesterone is a critical regulator of normal epithelial development in the breast. Acting through its nuclear receptors PRA and PRB, which form functionally distinct hetero- and homodimers, progesterone regulates the transcription of a suite of developmental and proliferative genes to mediate the formation of new branching ductal structures in the developing breast during pregnancy and the luteal phase of each menstrual cycle. In normal tissues, PRA and PRB are equivalently expressed and are assumed to act primarily as a heterodimer. However, in breast cancer, PRA commonly becomes predominant, and over-expression of this isoform is associated with a poorer outcome. Progesterone treatment of primary normal breast organoids increases DNA replication licensing and cell cycle activity, to increase progenitor cell numbers. Single cell profiling of normal breast organoids after treatment confirmed this effect and suggested direct stimulation of the progenitor compartment. We hypothesized that in the malignant setting, changes to PR isoform level and chromatin organisation combine to result in aberrant transcriptional regulation, leading to deleterious activation of stem and proliferation pathways in breast cancer. Modulating the balance between PRA and PRB in breast cancer cell models, we used ChIP-seq and ATAC-seq to investigate the relative influence of the two isoforms on progesterone regulation of transcriptional markers of progenitor activity. We found that predominant or exclusive expression of PRA resulted in a marked expansion of the progesterone regulated cistrome. Moreover, PRA bound substantially more frequently and with lower fidelity to non-canonical PR binding sites to mediate abnormal transcriptional responses and regulation of a number of cancer stem cell markers. Taken together our data suggest that breast cancer cells exploit normal progesterone regulatory processes to stimulate expansion of lesions in the breast, corroborating the role of progesterone as a promoter of breast cancer progression.

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The glucocorticoid receptor: a diverse cell signalling regulator modulated by many steroids and drugs

Timothy Cole[1]

1. Department of Biochemistry & Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

The adrenal gland synthesizes a group of well characterised steroid hormones with powerful physiological regulatory effects across the human body. One of these is cortisol that is a strong agonist to both the glucocorticoid and mineralocorticoid receptors. Both proteins are well characterised nuclear receptors that act primarily as ligand-modulated transcriptional regulators of the genome in a vast array of cell types within the body. The glucocorticoid receptor (GR) also elicits rapid non-genomic effects via monomer interactions with many proteins in the cytosol. Functional roles of GR signalling include regulation of embryo development, integrated metabolism, immune suppression, neuronal modulation and a range of other anti-stress responses. Gene-targeted mouse models have defined tissue and cell-type-specific functional roles across the body including the brain, metabolic tissues and the immune system. Synthetic steroid ligands and non-steroidal drugs have been developed to treat a wide range of clinical diseases and conditions. These will be described and discussed. Selective glucocorticoid receptor modulators represent an expanding list of potential drugs for clinical use. Newer novel prodrugs to selectively target the GR are also under development. Newer concepts of signalling 'unknowns' for the GR include detection of multimeric receptor complexes in the nucleus, the potential formation of heterodimers with the MR and perhaps other steroid hormone receptors. Finally, the integration of genomic and non-genomic GR signalling responses within a cell for a final cellular change remains a challenge to understand. Current understanding of these mechanisms and their future clinical impact in vivo with be discussed.

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SMAD4 promotes somatic-germline contact during oocyte growth

Sofia Granados Aparici[2, 1], **Qin Yang**[1, 3], **Hugh Clarke**[1, 3]

1. Research Institute, McGill University Health Centre, Montréal, Quebec, Canada

2. Universitat de València/INCLIVA, Valencia, Spain

3. Departments of Obstetrics and Gynecology and Biology, Division of Experimental Medicine, McGill University , Montréal, Quebec, Canada

Development of the oocyte requires physical contact with the surrounding granulosa cells of the follicle. Contact is achieved through transzonal projections (TZPs), specialized filopodia that emanate from granulosa cells. Transforming growth factor (TGFβ) family ligands produced by the oocyte increase the number of TZPs, but how they do so is unknown. Using an inducible Cre recombinase strategy together with expression of green fluorescent protein to verify Cre activity in individual granulosa cells, we examined the effect of depleting the canonical TGFβ mediator SMAD4. We observed a 20-50% decrease in the total number of TZPs within SMAD4-depleted granulosa cell-oocyte complexes, and a 50% decrease in the number of newly generated TZPs when these granulosa cells were reaggregated with oocytes. TZPs of SMAD4-depleted cells were also longer than controls and more frequently oriented towards the oocyte and the transmembrane proteins, N-cadherin and Notch2, were reduced by 50% in these cells. SMAD4 may thus modulate a network of cell adhesion proteins that stabilize the attachment of TZPs to the oocyte, thereby amplifying signaling between the two cell types.

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Practice-changing recommendations from the 2023 Pituitary Society prolactinoma guidelines

Sunita De Sousa[2, 1]

1. The University of Adelaide, Adelaide, SA, Australia

2. Royal Adelaide Hospital, Adelaide, SA, Australia

The latest Pituitary Society prolactinoma guidelines released in September include many new recommendations based on evidence published since the pre-existing 2006 Pituitary Society prolactinoma guidelines and the 2011 Endocrine Society hyperprolactinaemia guidelines. Some recommendations reflect contemporary practice, whilst others are relatively novel concepts for endocrinologists to consider in their care of patients with hyperprolactinaeemia. The most striking recommendation is for surgery to be offered upfront as an alternative to dopamine agonists to patients with microprolactinomas and selected macroprolactinomas. This talk will critically appraise the apparent paradigm shift in the surgical management of prolactinomas from traditionally selecting cases that are likely to respond poorly to dopamine agonists to now selecting cases that will respond well to surgery.

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What's Hot in Diabetes in 2023?

Shane Hamblin[1]

1. Western Health, St Albans, VIC, Australia

This talk will focus on the newer treatment options and cardiovascular/renal outcome trials for type 2 diabetes and the latest technology options for type 1 diabetes. The various diabetes treatment guidelines will be discussed and contrasted with PBS subsidised options. If you are an endocrinologist devoted to the pituitary, thyroid, adrenal, bone or gonad, this is the talk for you. If you already slave away in multiple diabetes clinics, there will be some practical tips to take back to the coalface.

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Sustainability in Endocrinology: Net Zero Leads Program, NSLHD Planetary Health

Lyndal Tacon[3, 1, 2], Connor Aitken[3, 1], Nicola Groskops[2]

1. Faculty of Medicine and Health, The University of Sydney, NSW, Australia

2. Planetary Health, Northern Sydney Local Health District (NSLHD), NSW Health, NSW, Australia

3. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

Climate change is widely recognised as the most pressing global challenge of our time. In parallel, the rising prevalence of diabetes and obesity continues to massively impact human health[1]. The most direct link between climate change and an increased incidence of type 2 diabetes can be drawn between overconsumption and under-supply of food resulting from adverse growing conditions[1-3]. Concurrently, increasing evidence indicates that individuals living with diabetes are directly impacted by climate change-induced events such as temperature extremes, which drive increased risk of hospitalizations and mortality[4]. Healthcare is a significant contributor to greenhouse gas emissions. The Australian healthcare sector produces approximately 7% of the nation's CO2 emissions; 70% of the healthcare contribution is from hospital activities, including 19% from pharmaceutical use[5]. Delivering environmentally sustainable health services is critical for all sectors. The novel Net Zero Clinical Leads Program, as part of the NSLHD Planetary Health Framework 2021-2023, offers clinicians the opportunity to embed sustainable practices in local healthcare. Areas particularly relevant to Endocrinologists include reducing pharmaceutical waste, reducing single-use plastics, reducing low value care whilst utilising Telehealth to reduce transport related carbon emissions. The DISPENSE study (DISposable insulin PENS: Environmental concerns) has evaluated attitudes to insulin pen prescription with the goal of implementing an evidence-based program to drive environmentally sustainable practice.

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Artificial intelligence - baby steps to solving some clinical issues in endocrinology

Christopher Gilfillan[1]

1. Eastern Health, Box Hill, VIC, Australia

Important work by others has demonstrated that the interindividual variation in glycaemic responses to food are at least as variable as the differences between foods. The standard dietary advice based on glycaemic index must therefore be called into question and the importance of and individual approach to dietary advice is emphasized.

The development of a device that can monitor food intake and provide advice based on individual glycaemic responses to that food has become the focus of my research in collaboration with the Monash Faculty of IT and the Monash Institute of Medical Engineering. This work uses a smart device to obtain an image of food and then correlates this with the glycaemia response to the consumption of that food. The AI will categorize the images and predicts glycaemic AUC based on individual data. We have collected data from 36 individuals and over 3000 food item images with over 40,000 glucose/time observations. The next step is to train the AI on these data and then apply to the same 36 individuals to validate the accuracy of predictions of AUC glucose.

It is anticipated that an individual user will be able to use their smart device to image food and receive a traffic light waning of its suitability with respect to glycaemic impact prior to consumption. Such an approach may be able to improve control in subject with diabetes but may also prevent progression in those at risk of the diagnosis.

Advances in image processing will have impact in many other areas of medicine. One example is in screening for diabetic retinopathy. In a real-world study of the technology, we imaged the retinas of 236 participants. The AUC of the ROC was 0.92, the sensitivity 96.9% and the specificity 87.7%. Simultaneous grading by an ophthalmologist identified only one false negative. With the declining capacity of non- ophthalmologists to screen for retinopathy this technology is a significant advance.

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The role of ACE2 in protecting against placental oxidative stress induced by hypoxia/reoxygenation

India Brooker[1, 2], Sarah Delforce[1, 2], Joshua Fisher[2, 3], Jessie Sutherland[1, 2], Saije Endacott[1, 2], Eugenie Lumbers[1, 2], Kirsty Pringle[1, 2]

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

2. Mothers and Babies Research Program, Hunter Medical Research Institute, New Lambton Heights, New South Wales, Australia

3. School of Medicine and Public Health, University of Newcastle, Callaghan, New South Wales, Australia

Fetal growth restriction (FGR) is a leading cause of infant morbidity and mortality characterised by deficient placentation. Consequently, the placenta experiences intermittent hypoxia and reoxygenation (H/R), which induces a state of oxidative

stress. FGR placentae also have reduced antioxidant capacity, including reductions in the critical antioxidant enzyme of the renin-angiotensin system, angiotensin-converting enzyme 2 (ACE2). This study aimed to model placental H/R *in vitro* and investigate the role ACE2 plays in the placental pathophysiology of FGR.

Healthy term human placental villous explants (n=7) were cultured in normoxic (8% O_2, 6hrs) or acute H/R (1hr in 1% O_2, 5hrs in 8% O_2) conditions. During culture, explants were treated with diminazene aceturate (DIZE) or recombinant human (rh)ACE2 to activate or replace placental ACE2, respectively. Subsequently, ACE2 expression, oxidative stress markers (nicotinamide adenine dinucleotide phosphate oxidase (NOX4 and NOX5)), and antioxidant capacity (catalase and superoxide dismutase) were assessed.

Exposure to acute H/R increased placental *ACE2* mRNA expression (*P*=0.041), an unexpected outcome as physiologically, FGR placentae exhibit a decrease in *ACE2*. Despite enhanced antioxidant potential with elevated ACE2, the mRNA expression of oxidative stress markers *NOX4* and *5* were significantly increased by H/R (*P*=0.013 and 0.008, respectively), with *NOX5* significantly correlated to *ACE2* (*P*=0.049, *r*=0.756). Moreover, placental superoxide dismutase activity was decreased (*P*=0.002), while catalase activity was increased (*P*=0.03) with acute H/R. Treatment with DIZE or rhACE2 were unable to ameliorate the acute H/R-induced upregulation of *NOX4/5* expression or alter superoxide dismutase and catalase activity compared to vehicle controls.

We have for the first time shown that under acute H/R conditions, placental ACE2 expression is upregulated but is unable to overcome oxidative stress induced by enhanced NOX4 and 5 expression. Further research exploring the effects of prolonged H/R, which is likely to exhaust ACE2 and more closely reflect the FGR placenta, are warranted.

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Parallels between folic acid deficiency and excess in placental endocrine function: implications for gestational diabetes mellitus

Jessica Williamson[1], Melanie D Smith[1], Anya L Arthurs[1], Dylan McCullough [1], Shalem Leemaqz[1], Claire T Roberts[1], Tanja Jankovic-Karasoulos[1]

1. Pregnancy Health and Beyond Laboratory, Flinders Health and Medical Research Institute, Flinders University, Adelaide, South Australia, Australia

Folic acid (FA) food fortification paired with periconceptional FA supplementation leads to folate excess during pregnancy, which is increasingly associated with gestational diabetes mellitus (GDM) risk. Though the mechanisms remain unknown, we recently showed hormones that regulate insulin resistance and glucose homeostasis (placental growth hormone (GH2); placental lactogen (hPL)) are altered in pregnancies post-FA fortification. We hypothesized that FA acts directly on the placenta to promote trophoblast proliferation (placental growth) and/or function (hormone secretion). To address this hypothesis, early and mid-gestation placental villus explants (N=45; 6-16 weeks' gestation) were treated with FA at 0 nM (acute deficiency), 10 nM (physiological deficiency), 40 nM (adequate), 200 nM (elevated) or 2000 nM (excess). Hormone secretion into culture media was measured 72 h post-treatment by ELISA. FA regulated GH2 and hPL *in vitro.* hPL secretion displayed a U-shaped response. Relative to the physiological norm (40 nM), 0 nM and 2000 nM FA increased hPL secretion by 40% (p = 0.009) and 25% (p = 0.08), respectively. GH2 concentration was also 25% higher in 2000 nM FA compared to 40 nM (p = 0.03). Real-time proliferation of placental cell lines revealed a similar biphasic relationship, where proliferation decreased with 0 nM and 2000 nM relative to 40 nM in JEG-3 and BeWo cytotrophoblasts, but not extravillous trophoblast HTR-8/SVneo. This research demonstrates FA affects placental endocrine function *in vitro* in early and mid-gestation human placentae. Interestingly, FA also affected proliferation of cells from syncytiotrophoblast lineage (hormone secreting trophoblasts) only. The effects of excess FA parallel those of acute deficiency, suggesting FA uptake and/or action is dysregulated in high-FA conditions, resulting in a deficient-like state. In the context of widespread FA fortification and supplementation in Australia, determining the effects of high FA intake on pregnancy health is essential.

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Vasoconstriction pathways are altered with insulin treatment in pregnancy: implications for understanding vascular dysfunction in gestational diabetes

Bianca R Fato[1, 2, 3, 4], Natasha de Alwis[1, 2, 3, 4], Sally Beard[1, 2, 3, 4], Natalie K Binder[1, 2, 3, 4], Tu'uhevaha Kaitu'u-Lino[1, 2, 4], Natalie Hannan[1, 2, 3, 4]

1. University of Melbourne, Parkville, VICTORIA, Australia

2. Department of Obstetrics and Gynaecology , University of Melbourne, Parkville, Victoria, Australia

3. Therapeutics Discovery and Vascular Function Group, Heidelberg, Victoria, Australia

4. Mercy Hospital for Women, Heidelberg, Victoria, Australia

Gestational diabetes mellitus is a significant complication of pregnancy that is associated with maternal vascular dysfunction. Endothelin-1 (ET-1) is a potent vasoconstrictor that contributes to endothelial dysfunction and vasoconstriction, however its regulation in gestational diabetes, and to a large extent in pregnancy, is unclear.

Circulating ET-1 was measured (ELISA) in plasma from patients with gestational diabetes (n=16), and gestation-matched controls (n=19). Human arteries were dissected from omental fat biopsies collected at caesarean section (n=28); gene expression of ET-1 and its receptors, ET_A and ET_B, were assessed (qPCR). Using wire myography, we investigated ET-1 artery constriction from patients with gestational diabetes, compared to gestation-matched controls (n=7). Diabetic cases were stratified by clinical management; diet (n=5) or insulin treatment (n=6). In further studies, arteries were pre-incubated with ET_A and/or ET_B antagonists (BQ123 and BQ788; n=7). We explored the effect of insulin by exposing healthy omental arteries (n=7) to insulin (10mU/mL) or vehicle. Additionally, healthy omental arteries were pre-treated with placental conditioned media containing high (25mM) or standard (5mM) glucose to model hyperglycaemia.

Circulating ET-1 levels and expression of ET-1, ET_A, and ET_B in omental arteries was not different in vasculature complicated by gestational diabetes. Interestingly, arteries treated with insulin during pregnancy demonstrated reduced ET-1 constriction compared to control, and diabetic diet managed pregnancies. *Ex vivo* short-term incubation of arteries with insulin showed reduced ET-1 constriction, suggesting insulin alters vasoactivity in pregnancy. Antagonising ET_B receptors did not reduce arteriole ET-1 constriction in pregnancies treated with insulin, substantiating insulin treatment during pregnancy prevents vasoconstriction. Lastly, in models of placental hyperglycaemia no changes were detected in ET-1 driven constriction.

Insulin treatment during pregnancy reduces harmful ET-1 induced vasoconstriction. These data suggest that insulin modulates vasoconstrictor pathways and improves vascular function in gestational diabetes. Further investigation is needed to define the role of ET-1 in pregnancy.

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Metabolic profiles are dysregulated in pregnant women before and after the diagnosis of preeclampsia.

Lucy Bartho[1], Daniel McKeating [2], Anthony Perkins[2], Sue Walker[1], Teresa MacDonald[1], Natasha Pritchard[1], Natalie Hannan[1], Stephen Tong[1], Tu'uhevaha Kaitu'u-Lino[1]

1. Department of Obstetrics and Gynaecology, University of Melbourne and Mercy Hospital for Women, Melbourne, Victoria, Australia

2. Griffith University, Southport, QLD, Australia

Introduction

Metabolomics is the study of small molecules (metabolites), within cells, tissues and biofluids.

Maternal metabolites are important for optimal maternal health and fetal development throughout pregnancy. Changes in metabolites may hold potential for predicting those at risk for developing preeclampsia. The aim of this study was to assess metabolic profiles in the maternal circulation before and after development of preeclampsia.

Methods

Maternal plasma samples were collected from two independent cohorts: 1) Established preeclampsia cohort – collected from 50 patients diagnosed with early-onset preeclampsia (<34 weeks' gestation), and 25 gestation-matched controls. 2) Preeclampsia prediction cohort, collected at 36 weeks' gestation; 17 patients who later developed preeclampsia, and 72 randomly selected controls. Metabolomics was performed by Metabolomics Australia on the Agilent 6545 QTOF Mass Spectrometer. 174 metabolites were quantified in the established preeclampsia cohort, and 631 metabolites in the preeclampsia prediction cohort. MetaboAnalyst 5.0 was used for data pre-processing and statistical analysis.

Results

In the established preeclampsia cohort, 77 metabolites were significantly dysregulated in plasma from women with early-onset preeclampsia, compared to gestation-matched controls ($P<0.05$). Pathway analysis revealed aminoacyl t-RNA biosynthesis, arginine biosynthesis and D-glutamine and D-glutamate metabolism were significantly altered in patients with preeclampsia. In the preeclampsia prediction cohort, 16 metabolites were significantly dysregulated in plasma from women who later developed preeclampsia. Pathway analysis revealed amino acid and medium chained fatty acid metabolites were altered in patients who developed late-onset preeclampsia.

Conclusion

This study identified altered metabolites in maternal plasma collected from women with established early-onset preeclampsia, and those who were later diagnosed with preeclampsia. Metabolomics holds significant potential as a non-invasive assessment of maternal and fetal health. More studies are required to further investigate metabolic profiles in preeclampsia to determine if altered metabolites provide a therapeutic window for preeclampsia.

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The soluble (pro)renin receptor (s(P)RR), a new therapeutic target for the treatment of maternal endothelial dysfunction in preeclampsia

Lachlan G Schofield[1, 2], Sarah J Delforce[1, 2], Saije K Endacott[1, 2], Eugenie R Lumbers[1, 2], Kirsty G Pringle[1, 2]

1. Hunter Medical Research Institute (HMRI), Newcastle, NSW, Australia

2. University of Newcastle, Newcastle, New South Wales, Australia

Preeclampsia affects 3-5% of all pregnancies and is characterised by new-onset hypertension in conjunction with gross endothelial dysfunction. In preeclamptic women, plasma levels of soluble (pro)renin receptor (s(P)RR) are elevated compared with normotensive pregnancy. We have recently shown that recombinant s(P)RR produces both endothelial dysfunction *in vitro*, and renal arterial dysfunction and hypertension in pregnant rats. Hence, this study aimed to assess the effects of PRO20, an s(P)RR antagonist, in ameliorating s(P)RR-induced endothelial dysfunction.

Human uterine microvascular endothelial cells (HUtMECs, n=4) were incubated for 24h with either; vehicle, 100nM recombinant s(P)RR, or 10% pooled patient serum (from normotensive or preeclamptic pregnancy, n=7) with or without 10nM PRO20. Markers of endothelial dysfunction were assessed via immunoblot, qPCR, and ELISA.

HUtMECs treated with recombinant s(P)RR displayed increased expression of endothelial dysfunction makers including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and endothelin-1 mRNA expression (P=0.003, P=0.001, P=0.009 respectively), along with elevated endothelin-1 protein secretion (P<0.001) compared with controls. The s(P)RR-induced increase in ICAM-1 mRNA (P=0.020) and endothelin-1 mRNA and protein levels (P=0.019 and P=0.032) were mitigated by treatment with PRO20. However, VCAM-1 mRNA levels were unchanged with PRO20 treatment compared with controls.

HUtMECs cultured with preeclamptic patient serum produced similar increases in the expression of endothelial dysfunction markers VCAM-1, ICAM-1, and endothelin 1 mRNA levels (P=0.004, P=0.004, and P=0.001, respectively) and endothelin-1 protein levels (P<0.001) compared with normotensive controls. Treatment with PRO20 was unable to mitigate the preeclamptic serum-induced increase in of VCAM-1, ICAM-1, or endothelin-1 mRNA but it did restore endothelin-1 protein levels (P=0.003).

Our data shows that marked endothelial dysfunction induced by recombinant s(P)RR *in vitro* can be resolved by inhibiting s(P)RR using PRO20. Future studies assessing the efficacy of PRO20 treatment *in vivo* are necessary to explore whether inhibiting s(P)RR could be a potential therapeutic for preeclampsia.

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Successful ultrasound diagnosis of pregnancy in sheep by application of a deep learning model

Madison Golledge[1], Gordon Refshauge[2], Jessica Rickard[1], Yang Song[3], Simon de Graaf[1]

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

2. NSW DPI, Cowra, NSW, Australia

3. UNSW, Sydney, NSW, Australia

Point-of-care diagnostic imaging is crucial in various veterinary and agricultural settings. In sheep, pregnancy diagnosis by ultrasound is routinely undertaken to determine pregnancy status, fetal number, age and even health. However, diagnostic accuracy remains challenging, even for highly experienced scanners. Deep learning holds potential to address such challenges and improve accuracy and efficiency of ultrasound imagery interpretation. This project aimed to evaluate the ability of a deep learning model to identify pregnant and non-pregnant ewes from ultrasound imagery.

Transcutaneous ultrasound (OviScan 6, BCF Australia, Mitcham VIC) imagery was recorded from 937 ewes, 44 days post 5-week joining. From these recordings, images were created every 1/12 second and labelled with a diagnosis class (pregnant or non-pregnant), determined by a trained scanner. To achieve class balance, 1,838 images from 62 pregnant ewes and 1,826 images from 48 non-pregnant ewes were randomly split between training (70%), validation (20%), and testing (10%) datasets. A binary classification Convolutional Neural Network (1) was trained on the training and validation datasets, using binary cross-entropy loss. The architecture included two convolutional layers and a fully connected layer, using a batch size of 32. The trained model was then evaluated on the testing dataset, generating a confusion matrix for precision and recall assessment. Grad-CAM (2) was used to visualise pixels of importance. Testing accuracy was 98.92%. Accuracy was 98.19% for pregnant ewes (1.00 precision; 0.99 F1-score; 0.98 recall) and 100% for empty ewes (0.97 precision; 0.99 F1-score; 1.00 recall).

This study shows deep learning is highly accurate at identifying pregnant and non-pregnant ewes, giving confidence for future detailed classifications including fetal number, age, health, and placentome types. This model demonstrated its potential to diagnose pregnancy quickly and accurately for on-farm sheep management, as well as to classify ultrasounds of other species and humans for research, treatment, and animal management.

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2. Selvaraju, RR, Cogswell M, Das A, Vedantam R, Parikh D, Batra D. Grad-CAM: Visual Explanations from Deep Networks via Gradient-based Localization. In: Proceedings of the IEEE International Conference on Computer Vision; 2017.

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Career journeys: Navigating work-life balance, job security and funding as an ECR

Lisa Raven[1]

1. St Vincent's Hospital, Darlinghurst, NSW, Australia

The ESA and SRB Early Career Committees present a workshop discussing the possible paths and challenges for early career members. Dr Lisa Raven will present on her experiences and tips on making the big decision, as well as striving for work-life balance and financial stability. There will be a panel discussion and opportunities to ask questions.

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Where did I come from? A career in reproductive biology

Jo James[1]

1. University of Auckland, Auckland , New Zealand

A/Prof Jo James leads a research team that seeks to combine in vitro experimental work, in vivo and ex vivo imaging, and in silico modelling techniques to understand how placental function is impaired in fetal growth restriction. The overall aim of this work is to identify novel, mechanism-based, approaches to predict, detect and treat this condition. She has >70 publications, and her team's work has attracted >$13 million NZD in funding, including support by the Marsden Fund, HRC, CureKids and

Wellcome Leap. Following her PhD she spent 15 years on fixed-term contracts, and the success of her career across this time has been built on a combination of self-supported soft funding, strong mentorship, leaning into teaching responsibilities, and grabbing opportunities when they arose. In this talk, she will highlight key lessons learnt across this journey, and reflect on how on earth she managed to make it to where she is now.

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Performance of genetic testing for phaeochromocytoma & paraganglioma: 15-year experience at a large tertiary referral centre.

Simon Ryder[1, 2], Emma Duncan[3], David Pattison[1]

1. Royal Brisbane & Womens Hospital, Queensland Health, Brisbane, QLD, Australia

2. Logan Hospital, Meadowbrook, QLD, Australia

3. King's College, London, United Kingdom

Phaeochromocytomas and paragangliomas (PPGL) are highly heritable tumours and germline testing is recommended for all individuals with PPGL. However, traditional clinical approaches may preclude optimal PPGL management. We assessed the use of genetic testing in PPGL management in a tertiary centre with 15 years of access to germline testing.

This retrospective cohort study included 110 probands diagnosed with a first presentation of PPGL between 2005-19. The primary outcome was the proportion of individuals referred for genetic testing within 12 months of diagnosis. Univariate and multivariate analyses were performed, examining genetic testing rates over time and factors potentially influencing the use of genetic testing.

48/110 (44%) individuals diagnosed with PPGL did not undergo genetic testing or counselling. Genetic testing rates improved between 2014-19, compared with two prior five-year tertiles (p=0.009). On univariate analysis, factors associated with genetic testing including endocrinologist involvement, younger age, a positive family history, a personal history of a tumour associated with a hereditary PPGL syndrome, bilateral PC, and presence of synchronous PPGL. On logistic regression, individuals with endocrinologist involvement were more likely to undertake genetic testing (OR=10.9, 95% CI [3.4, 43.7]) and increasing age was associated with a lower likelihood of genetic testing (p=0.008). Of 54 individuals who underwent germline testing, 15 (28%) were found to have a pathogenic variant. Pathogenic variants were more likely in extra-adrenal (1/4, 25%) or head and neck PGL (11/23, 48%) than in individuals with PC (3/27, 11%, p=0.006). Although there was a suggestive trend, there was no significant difference in the detection of pathogenic variants between older individuals (>50 years) (5/27, 19%) and younger individuals (<50 years) (10/27, 37%, p=0.13).

This study supports the use of genetic testing in all individuals with PPGL but gaps exist in clinical practice. Broader education is needed to optimise use of personalised medicine approaches.

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2. Lenders JW, Duh QY, Eisenhofer G, et al. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2014;99(6):1915-1942.
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Pituitary Neuroendocrine Tumours without distinct lineage differentiation express stem cell marker SOX2

Nele F Lenders[1, 3, 2], Tanya Thompson[1, 3], Jeanie Chui[4], Julia Lowe[4], Warrick J Inder[5, 6], Peter E Earls[4], Ann I McCormack[1, 3, 2]

1. Department of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia.

2. St Vincent's Clinical School, University of New South Wales, Sydney, NSW, Australia.

3. Garvan Institute of Medical Research, Sydney, NSW, Australia.

4. Department of Anatomical Pathology and Cytopathology, St Vincent's Pathology, Sydney, NSW, Australia

5. University of Queensland, Brisbane, QLD

6. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, QLD, Australia

Context: The recent WHO 2022 Classification of pituitary neuroendocrine tumours (PitNETs) identified a novel group of tumours, 'plurihormonal tumours without distinct lineage differentiation'. By definition, these express multiple combinations of lineage commitment transcription factors, in a monomorphous population of cells. The biology of these tumours has not yet been well characterised.

Objectives: To determine the expression of stem cell markers (SOX2, Nestin, CD133) within PitNETs without distinct lineage differentiation, immature PIT-1 lineage and acidophil stem cell tumours, compared with committed cell lineage tumours.

Methods: Retrospective evaluation of surgically resected PitNETs from St Vincent's Hospital, Sydney. Patients were selected to cover a range of PitNET types, based on transcription factor and hormone immunohistochemistry. Clinical data was collected from patient files. Radiology reports were reviewed for size and invasion. Samples were analysed by immunohistochemistry and RT-qPCR for SF-1, PIT-1, T-PIT, SOX2, Nestin and CD133. Stem cell markers were compared between tumours without distinct lineage differentiation and those with classically "mature" types.

Results: On immunohistochemistry, SOX2 was positive in a higher proportion of tumours without distinct lineage differentiation compared with those meeting WHO lineage criteria, 7/10 (70%) v 10/42 (23.4%), P=0.005. CD133 was positive in 2/10 tumours without distinct lineage differentiation but 0/41 meeting lineage criteria, P=0.003. On RT-qPCR, there was no significant

difference in relative expression of stem cell markers (SOX2, CD133, Nestin) between tumours with and without distinct cell lineage.

Conclusions: Our study is the first to biologically characterise plurihormonal tumours without distinct lineage differentiation. PitNETs without distinct lineage differentiation exhibit a higher expression of the stem cell marker SOX2 compared with other lineage-differentiated tumours, suggesting possible involvement of stem cells in their development. By expanding our knowledge of pituitary tumourigenesis, we aim to develop scope for targeted therapies that may be used to treat tumours in early stages.

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Pregnancy outcomes following bariatric surgery in Queensland, Australia: A data-linkage report

Jade Eccles-Smith[1, 2], Alison Griffin[3], H David McIntyre[4, 5], Marloes Nitert Dekker[6], Helen L Barrett[7, 8]

1. Department of Obstetric Medicine, The Royal Brisbane and Women's Hospital, Herston

2. Mater Research Institute-The University of Queensland, Brisbane

3. QIMR Berghofer, Herston

4. Mater Research, South Brisbane

5. Obstetric Medicine, Mater Health, South Brisbane

6. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane

7. Royal Hospital for Women, Randwick

8. Faculty of Medicine, University of New South Wales, Sydney

In Australia, bariatric procedures doubled between 2005 and 2015 with 80% of surgeries performed on women of child bearing age [1]. Pregnancy following bariatric surgery is associated with mixed maternal and fetal outcomes. Limited data are available regarding pregnancy outcomes after laparoscopic sleeve gastrectomy.

A statewide hospital and perinatal data register linked cohort matched study was performed. In total, 2,018 births in 1,677 women with prior maternal bariatric surgery were registered in the Queensland Hospital Admitted Patient Data Collection and matched with deliveries during 2013-2018 in the Perinatal Data Collection. The first singleton pregnancy following bariatric surgery for each woman was used for analysis. Women were excluded if BMI was missing or if bariatric surgery procedures had been reversed, revised or ambiguously coded. A total of n=1282 cases and n=12820 controls were analyzed matched on BMI, smoking, age and parity. Continuous variables were analyzed using paired t-tests and categorical variables were analyzed using Pearson's Chi-square or Fisher's exact test.

Of 1282 women with a singleton delivery after bariatric surgery, 93% had undergone laparoscopic sleeve gastrectomy. In women with previous bariatric surgery, there was more assisted reproductive technology use (10.7% vs 8.0%, p<0.001) and preterm birth (<37 weeks) (10.5% vs 7.8%; p=0.007). Offspring had lower absolute birthweight (3223g ± 605g vs 3418g ± 595g; p<0.001), lower percent of large for gestational age (LGA) (8.6% vs 14.1%; p<0.001) and higher percent of SGA infants (10.7% vs 7.3%; p<0.001) than offspring born to matched women. Percent of GDM was lower in women with previous bariatric surgery (15% vs 20%; p<0.001).

Glycaemic and metabolic shifts caused by pre-pregnancy bariatric surgery modify obesity associated pregnancy and neonatal outcomes. Our results suggest that pregnancy outcomes following maternal bariatric surgery differ from matched controls in a cohort of women with primarily gastric sleeve surgery.

1. 1. AIHW, Weight loss surgery in Australia 2014–15: Australian hospital statistics. 2017.

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The effect of cyproterone and spironolactone on breast development in transgender women: a randomised controlled trial

Lachlan M Angus[2, 1], Shalem Y Leemaqz[3], Jeffrey D Zajac[2, 1], Ada S Cheung[2, 1]

1. Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia

2. Department of Medicine (Austin Health), University of Melbourne, Heidelberg, Victoria, Australia

3. College of Medicine and Public Health, Flinders University, Bedford Park, South Australia, Australia

Objective: Transgender women commonly use cyproterone or spironolactone as anti-androgens with estradiol to assist with feminisation. However, the optimal anti-androgen is unclear. We aimed to assess the effect of these anti-androgens on breast development and hypothesised that cyproterone would result in greater breast development than spironolactone due to greater androgen receptor antagonism and suppression of serum total testosterone.

Design: Double-blind, randomised controlled trial

Methods: Transgender women newly commencing estradiol were randomised to spironolactone 100mg daily or cyproterone 12.5mg daily for six months. The primary outcome was measurement of breast development via breast chest distance with secondary outcomes of estimated breast volume using the BreastIdea Volume Estimator application and serum total testosterone.

Results: Fifty-five participants were included in per protocol analysis (cyproterone group n=28, spironolactone group n=27). Baseline age, body mass index, breast indices, serum estradiol and serum total testosterone were comparable. At six months, the mean (standard deviation) breast chest distance was 9.2cm (3.0) in the cyproterone group versus 8.3cm (2.7) in the spironolactone group (p=0.27). The mean (SD) estimated breast volume was 190.25 mL (158.60) in the cyproterone group and

157.84mL (112.03) in the spironolactone group (p=0.39) with significant inter-individual variation (range 20.27 – 787.77 mL). The mean (SD) serum total testosterone was 1.48 nmol/L (3.45) in the cyproterone group and 4.29 nmol/L (5.44) in the spironolactone group (p=0.04). Serum estradiol levels were comparable. Use of cyproterone was associated with mild hyperprolactinaemia and spironolactone with an increase in serum urea and creatinine.

Conclusions: Choice of anti-androgen should be individualised based on clinician and patient preference, with consideration of associated side effects. Further research is needed to optimise breast development in transgender women.

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RANKL inhibition creates a pro-osteoclastic environment, leading to an overshoot in serum TRAP and accelerated bone resorption following treatment withdrawal.

Albert Kim[1], Ariel Castro-Martinez[1], Vicoria Taylor[1], Suraj Dhakal[1], James Smith[1], Jacqueline Center[1, 2], Christian Girgis[3, 4], Peter Croucher[1, 2], Michelle McDonald[1, 2, 4]

1. Skeletal Diseases Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

2. St Vincent's Clinical School, School of Medicine and Health, UNSW Sydney, Kensington, NSW, Australia

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

4. Faculty of Medicine & Health, University of Sydney, Sydney, NSW, Australia

Denosumab withdrawal triggers rapid bone mineral density (BMD) loss via accelerated bone resorption. Development of optimal sequential therapy is hindered by poor understanding of the cellular mechanisms and sub-optimal serum turnover marker assessment. We compared temporal changes in RANKL, serum TRAP5b and osteoclast precursors to CTX, P1NP and BMD, to define alternative tools to guide sequential treatment.

Seven week female C57BL/6 mice received 2-weeks of saline or thrice-weekly OPG:Fc (10mg/kg) to inhibit RANKL, then withdrawn from therapy (OPG-W). Following longitudinal BMD and serum measurement, mice were harvested at weeks 2, 8, 11 and 13 for RANKL, TRAP5b, P1NP and CTX. Week 8, marrow-flushed, long-bone samples were assessed for RANKL mRNA. Week 6 bone marrow samples were analysed for osteoclast precursors ($NK1.1^-$ $Ter119^-$ $CD3^-$ $Ly6G^-$, $B220^-$, $CD11b^{lo}$, $CD117^{int}$, $CD115^+$).

Following OPG:Fc withdrawal, BMD increased 24% at week 8 in OPG-W ($p<0.01$), declining at week 10 and normalised to vehicle at week 13. At week 8, serum TRAP, CTX and P1NP were all suppressed in OPG-W ($p<0.001$). At week 11, serum TRAP was elevated in OPG-W ($p=0.01$), P1NP and CTX remained equivalent to vehicle. At week 13, serum TRAP, P1NP and CTX were all greater in OPG-W ($p<0.01$).

Serum RANKL levels at week 2 were elevated with OPG:Fc ($p<0.001$), peaking 13-fold higher at week 8 ($p<0.0001$), returning to vehicle at week 11. Prior to the overshoot in serum TRAP levels in OPG-W (at week 11), bone RANKL mRNA was elevated at week 8 ($p<0.01$) and osteoclast precursors at week 6 ($p<0.05$).

Rebound BMD decline preceded the increase in clinical turnover markers (P1NP, CTX). Elevated serum TRAP occurs earlier, prior to bone loss, and may better guide sequential therapy. Increased serum and bone RANKL levels and an accumulation of osteoclast precursors were detected prior to the overshoot in TRAP.

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Anti-Müllerian hormone regulates organ-size in the ovary.

Michael Pankhurst[1]

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

Anti-Müllerian hormone is a TGFβ-superfamily member that inhibits primordial follicle activation, induces early preantral follicle atresia, promotes late preantral/early antral growth and inhibits FSH-responses and aromatase expression in antral follicle granulosa cells. Of these diverse actions, the induction of preantral follicle atresia has the greatest effect on the number of developing follicles in the ovary but the reason for this role has not yet been determined. Our research suggests that without substantial amounts of preantral follicle atresia, the ovary would not be able to contain the large number of antral follicles growing rapidly in the follicular phase. AMH released from large follicles appears to be a key regulator, preventing excessive numbers of antral follicles from developing to the antral stage. We also show that most proteolytic cleavage of the inactive precursor form of the protein (proAMH) occurs in the theca and stroma. The AMH in follicular fluid is predominantly the inactive form, with small quantities of cleaved, active form ($AMH_{N,C}$) that performs the growth-promoting functions of AMH. When the AMH leaves follicular fluid, the majority is converted to the $AMH_{N,C}$ where in high concentrations it performs the inhibitory functions, including suppression of primordial follicle activation and induction of preantral follicle atresia. In this way, AMH can have two divergent functions in the same organ.

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Reproductive function of men conceived with intra-cytoplasmic sperm injection

Sarah Catford[1]

1. Alfred Hospital, Melbourne, VIC, Australia

Since its introduction for male factor infertility, the use of intra-cytoplasmic sperm injection (ICSI) has increased. Given the strong genetic basis of male infertility, the risk of transmitting infertility to future generations is concerning. Furthermore, offspring health is affected by paternal health and sperm quality, irrespective of genetic causes of infertility. Concerns regarding the use of ICSI include the heritability of infertility, the effects of poor-quality spermatozoa on offspring health, and the potential for the technique itself to induce epigenetic changes with long-term health effects. Given the prevalence of male infertility and

the widespread use of ICSI, understanding the possible adverse health effects is an important clinical and public health issue. Additionally, the mechanisms responsible for potential adverse health outcomes remain unclear and efforts to separate the effects of paternal infertility from treatment factors are necessary. This session will present current literature on the reproductive health of men conceived using ICSI with an emphasis on Australian data. The results of an Australian study evaluating the reproductive health of a cohort of young men conceived with ICSI will be presented. The findings of this study, the largest to date globally, contribute to a limited pool of knowledge on the reproductive function of men conceived with ICSI and the collective understanding of the overall health of adults conceived with ICSI.

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Gonadal inhibins: Gatekeepers of female fertility and metabolic health

Kelly Walton[1]

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

Gonadal hormones inhibin A and inhibin B (a/b_A or a/b_B heterodimers) are classically known for their abilities to constrain follicle stimulating hormone (FSH) production from pituitary gonadotroph cells. It is largely accepted that inhibins downregulate *FSHB* transcription by disabling receptor activation by related activins ($b_{A/B}/b_{A/B}$ dimers). Beyond FSH regulation, roles for inhibins in female and male fertility have been poorly understood owing to a lack of appropriate physiological models. Here, armed with new mouse models of dysregulated inhibins we have uncovered novel roles for inhibins in gonadal function and fertility. Our, and supporting works by our collaboration at McGill University (Montreal), have shown that maintenance of inhibin physiological activity is important not only for FSH regulation, but also for embryo and foetal survival during pregnancy in female mice. Additionally, together we have uncovered evidence to support that partial inactivation of inhibins actually enhances fertility in female mice. More recently we have discovered that persistent inhibin inactivity in female mice is associated with the development of polycystic ovaries. Serendipitously, using these inhibin inactivated mouse models we have also identified roles for inhibins in the regulation of adiposity in females. Significantly, as these phenotypes pertain only to female mice, we are unveiling new sex-specific activities for the inhibins. Ultimately these findings signify inhibins as not only gatekeepers of female reproductive health, but also metabolic health.

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The Queensland Family Cohort Study: discovering the mechanisms where stress and inflammation collide

Vicki Clifton[1]

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

Glucocorticoids derived from the mother and acting via the placenta are essential for fetal development. However, pregnancy complications that result in excess glucocorticoid exposure can be detrimental to the fetus. The glucocorticoid-regulated mechanisms that confer different outcomes for the fetus may be due to different patterns of placental glucocorticoid receptor (GR) isoform expression and our team discovered that there are multiple placental GR protein isoforms which are conserved across species and vary in relation to sex, fetal growth and maternal complications.

Some GR isoform patterns appear protective against an adverse outcome for the fetus while others appear detrimental including GRα-D1. Placental GRα-D1 expression is increased in the presence of several pregnancy complications and linked to enhanced transcription of pro-inflammatory cytokines. Specifically, GRα-D1 was increased in placentae of women with a mental illness, women with asthma and pregnancies complicated with a small-for gestational age fetus. Furthermore, the increased expression of GRα-D1 was associated with increased expression of inflammatory cytokines and transcription factors in the placentae of complicated pregnancies. Using trophoblast cells *in vitro* nuclear localisation of GRα-D1 was increased following an inflammatory challenge and its expression was not altered significantly by concomitant hydrocortisone treatment. Placental *TNFα, IL-6* and *ICAM 1* mRNA were upregulated when GRα-D1 was localised to the nucleus and were not significantly altered in the presence of hydrocortisone. These findings show for the first time that a placental pro-inflammatory response cannot be inhibited by glucocorticoids when GRα-D1 is expressed. We propose this is a new mechanism that may explain why inflammation persists in the presence of high concentrations of glucocorticoids and stress.

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Of "omics" and organoids: understanding the dynamic marsupial histotroph

Jennifer C Hutchison[1], **Angus Sutherland**[1], **Esther Diez Santa Gertrudis**[1], **David L Potter**[1], **Andrew Pask**[1]

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

Background:

Australian marsupials are facing a biodiversity crisis, requiring quick and decisive action to prevent further extinction. Assisted Reproduction Technologies are a promising frontier in species conservation: however, while embryo culture methodologies have been developed for eutherian species, marsupial embryo culture has not yet been optimized. Given marsupial reliance on histotrophic nutrition for the majority of gestation, close examination of uterine fluid contents is necessary for optimizing embryo culture. Using a dasyurid animal model, this research identifies key uterine morphological details, metabolites of interest within the uterine fluid relevant to embryo culture medium composition, and has developed endometrial epithelial organoids as an *in vitro* model of the highly secretory marsupial uterus.

Methods:

Uteri from fat-tailed dunnarts *(Sminthopsis crassicaudata)* were collected at pertinent stages of the estrus cycle and across gestation. Micro-CT defined the internal structure of the uterus, and cellular detail was resolved using routine histological

techniques. Uterine fluid was collected and metabolites detected by mass spectrometry. Following enzymatic digestion of uterine tissue, organoids were grown from the endometrial epithelium using previously established techniques, and immunohistochemically compared to the donor tissue in to assess cell population purity (E-Cadherin, vimentin), proliferation (PCNA), secretion (PAS), and morphology (H&E).

Results:

The dunnart uterus is highly glandular with a folded luminal surface, and contains a dynamic fluid microenvironment, with several metabolites appearing to be differentially abundant across the estrus cycle. Endometrial epithelial cells can be retrieved and cultured as organoids in previously defined medium. Organoids contain proliferating epithelial cells ($PCNA^+$, $E\text{-}Cadherin^+$), and exclude stromal cells ($Vimentin^-$). Similarly to the endometrial glands, organoids accumulate PAS^+ secretions.

Conclusion: We have derived the first marsupial endometrial epithelial organoids, from the highly glandular dasyurid uterus. Examination of the uterine fluid and morphology, combined with this *in vitro* model, provides key insights into the marsupial uterine microenvironment.

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Elucidating the functional role of *VEZT* in endometriosis lesion formation

Jacqueline F Donoghue[1], Brodie Bilston[1], Michelle Dang[1], Leonie Cann[1], Georgina Prewer[1], Andrew Kueh[2], Marco Herold[2], Martin Healey[3], Peter AW Rogers[1]

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

2. Blood Cells and Blood Cancer Division, Walter and Eliza Hall Institute, Parkville, Victoria, Australia

3. Gynaecology Endometriosis and Pelvic Pain Unit, Royal Women's Hospital, Parkville, Victoria, Australia

INTRODUCTION: Endometriosis is a common gynaecological disease where endometrial tissue implants in locations ectopic to the uterus. *VEZT* encodes a transmembrane adherens junction protein known as Vezatin that plays essential roles in pre-implantation embryo development and increased *VEZT* expression is associated with an increased risk of endometriosis. The aim of this study was to evaluate the functional outcomes of overexpressing *VEZT* in a unique mouse model of endometriosis.

METHODS: *VEZT* was overexpressed in human endometrial stromal cell lines followed by RNA-seq and validation of findings qPCR. Immunohistochemistry was used to localise vezatin protein in human endometrium, ectopic lesions and mouse tissues. Following *VEZT* induction in VEZT-Cre mice, minced donor uterine tissue was injected intraperitoneally into recipient mice. VEZT-Cre and C57/BL6 wildtype (WT) mice received allografts and xenografts recovered at 4 and 8 weeks.

RESULTS: *VEZT* (vezatin) was significantly increased in secretory phase endometrium and localised to endometrial epithelial cells, decidualised stromal cells, spiral arterioles, inflammatory cells. Vezatin was also upregulated in endometriotic lesion stromal cells, epithelium and vasculature. *VEZT* overexpression in stromal cells significantly upregulated genes involved with the innate immune system, interferon pathways and cytokines responsible for macrophage recruitment and phenotypic switching. *VEZT* overexpression in VEZT-Cre mice uterine tissue significantly increased expression of *ESR2, CTGF, TGFb* and *TNFa* while significantly reducing *PGR* expression compared to WT mice. VEZT-Cre mice grafted with uterine fragments for 8 weeks, developed large cystic lesions throughout the peritoneum including the liver, pancreas, ovary, bowel, peritoneum and fat pads while WT lesions regressed.

CONCLUSION: This is the first study of its kind to elucidate several functional roles of the endometriosis risk gene *VEZT* in endometrial stromal cells, endometriosis lesions and a unique conditional *VEZT* knock-in mouse model of endometriosis. These findings demonstrate *VEZT* has the potential as a valuable endometriosis biomarker and therapeutic target.

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Gamma/delta T cells comprise a major element of the uterine immune response to seminal fluid in mice

Sarah Robertson[1], Kerrie L. Foyle[1], Hon Yeung (Dexter) Chan[1], Ha Tran[1], John E. Schjenken[2]

1. University of Adelaide, Adelaide, SA, Australia

2. The University of Newcastle, Newcastle, NSW, Australia

Seminal fluid induces major changes in the female reproductive tract to initiate maternal immune tolerance for embryo implantation and placental development. The full extent of immune cell changes and their contribution to endometrial receptivity is not fully defined. RNA-sequencing has revealed that T cell recruitment is amongst the top biological pathways induced in the endometrium by seminal fluid in mice[1]. Genes associated with gamma/delta (γδ) T cells were dominant amongst the differentially regulated genes, including γδ TCR genes *Trdc* and *Tcrg-C1* (2.0- and 2.5-fold increase) and γδ T cell function genes *Il7r* and *Blk* (2.5- and 2.8-fold increase). Therefore, we sought to test the hypothesis that seminal fluid contact elicits changes in uterine γδ T cell populations. To assess this, we utilised flow cytometry to quantify and phenotype γδ T cells in the endometrium and uterine-draining lymph nodes on day 3.5 post-coitum (pc) after mating with intact (INT), vasectomised (VAS), seminal vesicle-deficient (SVX), and SVX/VAS BALB/c males, as well as virgin estrous females (n=11-12/group). The γδ T cell population in the uterus was dramatically expanded after INT or VAS mating by 8.3-and 10-fold compared to estrous females, with 22.4- and 21.9-fold more γδ T cells expressing proliferation marker Ki67. Similar increases in γδ T cells were seen in draining lymph nodes. The increase did not occur after mating with SVX or SVX/VAS males, consistent with the gene expression changes that depended on seminal plasma. These findings demonstrate that expansion of a resident γδ T cell population is a major but hitherto unidentified element of the uterine immune response regulated by seminal fluid. Given the central roles of γδ T cells in epithelial homeostasis in other mucosal surfaces, this response may modulate receptivity to embryo implantation - but future studies are required to define its exact contribution to reproductive success.

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Sperm participate in modulating the cervical epithelial cell immune response to seminal fluid

David J Sharkey[1], Hannah E Lyons[1], Sarah A Robertson[1]

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

Seminal fluid introduced into the female reproductive tract at coitus induces an inflammation-like response within the cervical tissues of women. The response involves induction of cytokines and chemokines to drive leukocyte recruitment and activation, that in turn promote embryo implantation and establish immune tolerance towards male allo-antigens. Soluble factors in seminal plasma are important mediators of seminal fluid signalling. Recently we showed in mice that sperm facilitate the female response – but whether sperm also contribute in women is unclear. In this study, we tested the hypothesis that human sperm interact with Ect1 cervical epithelial cells to induce cytokine production *in vitro*. Nine normozoospermic men (according to WHO VI guidelines) of reproductive age men provided semen samples for the study. Ect1 cells were incubated with 10% (v/v) whole semen, 10% seminal plasma, 10% washed sperm, or washed sperm at fixed concentrations of 1, 5, and 10M/ml, then cytokine secretion was assessed in 24hr supernatants by multiplex microbead assay. Electron microscopy was utilised to visualize physical interactions between sperm and Ect1 cells. Washed sperm - as well as whole semen and seminal plasma – acted to increase Ect1 cell CSF2, CXCL8, IL1B and IL6 cytokine production (n=9, all $P < 0.01$), with sperm-mediated induction occurring in a dose-dependent manner for CSF2, CXCL8 and IL1B (n=9, all $P < 0.02$). Electron microscopy showed evidence of sperm head attachment to microvilli on the Ect1 cell surface, and in some instances, full engulfment by Ect1 cells. This data shows that human sperm interact closely with cervical epithelial cells and act to modulate their cytokine production. Understanding the mechanisms that mediate this interaction will progress understanding of biological processes by which maternal immune tolerance is generated, and may aid in the development of interventions that improve immune receptivity to embryo implantation.

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Examining the potential for a zinc-based IUD to provide non-hormonal contraception with fewer side effects

Kirsten Shankie-Williams[1], Maddison Holley[1], Laura Lindsay[1], Margot Day[1], Samson Dowland[1]

1. University of Sydney, Sydney, NSW, Australia

There is a global crisis of unintended pregnancy: nearly half of all pregnancies each year are unplanned, with severe consequences for women, society and public health. A major contributor is a lack of suitable contraceptive options, with many current options causing severe side effects leading to early discontinuation of use. There are urgent calls for the development of more non-hormonal contraceptive options, with the only current non-hormonal long-acting reversible contraceptive (LARC) being the copper intrauterine device (IUD).

We have used a rat IUD model to investigate the potential of a zinc-based IUD in comparison to the current copper standard. This model demonstrated that both copper and zinc IUDs were 100% effective at preventing pregnancy, and that zinc IUDs provided long-term protection. This was a reversible effect, as once the zinc IUDs were removed the rats rapidly returned to fertility.

Embryo culture experiments revealed that the zinc IUD specifically impacted early embryo development, unlike the copper IUD. Histological analysis of the endometrium revealed damage and inflammation in uterine horns containing a copper IUD, but only minor epithelial changes in those containing a zinc IUD. Immunofluorescent analysis of leukocytic infiltration, which is associated with inflammatory and painful side effects, revealed further differences. This work indicates zinc impacts the early embryo, rather than the endometrium, which may lead to a reduced side effect profile for the zinc IUD.

This work establishes the basis for development of a zinc-based IUD, demonstrating the fundamental requirements of long-acting efficacy and reversibility. Initial findings indicate that histological and immunological signs of side-effects are reduced in this zinc IUD model. Further development of this zinc-based intrauterine contraceptive could provide women with a new non-hormonal LARC option that is desperately needed and help address the “neglected crisis of unintended pregnancy” (UNFPA 2022).

1. United Nations Population Fund (UNFPA) State of World Population report, Seeing the Unseen: The case for action in the neglected crisis of unintended pregnancy. 2022

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Phosphoproteomic analysis of equine endometrial organoids exposed to embryo secretions

Stephanie Waugh[1], Edwina F Lawson[1], Luke Carroll[2], Karthik Kamath[2], Zamira Gibb[1], Aleona Swegen[1]

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

2. Australian Proteome Analysis Facility, Macquarie University, Macquarie Park, NSW, Australia

The equine embryo remains mobile and without physical attachment to the endometrium for a prolonged period in early pregnancy. Embryo-maternal signalling is critical during this time but is poorly understood, while a definitive mechanism of maternal recognition of pregnancy remains elusive. In this study we used an endometrial organoid system to examine the

maternal response to embryo secretions by analysing the endometrial phosphoproteome following exposure to embryo-conditioned medium.

Mare endometrial organoids were derived from isolated endometrial glands from uteri obtained immediately post-mortem. Embryos were recovered by uterine lavage from Standardbred mares at day 8-9 post-ovulation and cultured in protein-free medium for 12 h to generate embryo-conditioned medium. Organoids were primed with estradiol and progesterone and co-incubated for 30 min with embryo-conditioned (n=9) or control (n=3) media (diluted 1:100). Organoids were processed to remove media and secretome components. Proteins were extracted from cell pellets with urea-based extraction buffer, digested with trypsin, TMT labelled and enriched for phosphopeptides. Phosphopeptides were detected via LC-MS/MS.

We detected a total of 4,218 phosphorylation sites mapping to 795 protein IDs. Abundances of 27 phosphopeptides were altered between control media-exposed and embryo-conditioned media-exposed organoids (16 increased and 11 decreased). Notable findings include altered phosphorylation of PGRMC1 (progesterone receptor membrane component 1) and MGLL (monoglyceride lipase). PGRMC1 forms part of a non-classical system of progesterone action, with changes in PGRMC1 phosphorylation suggesting an alternative route of progesterone receptor activation is involved in equine embryo-maternal signalling. MGLL is involved in prostaglandin synthesis and may be important for the inhibition of prostaglandin synthesis during maternal recognition of pregnancy.

In summary, this study describes the immediate response of mare endometrial organoids to compounds released by the embryo and identifies novel pathways likely to be implicated in signalling between the embryo and maternal system in the horse.

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Immunobiological Properties of Human Fascia Lata (HFL) vs Mesh: Implications for Pelvic Reconstructive Surgery

Saeedeh Darzi[1, 2], David Hennes[2], Kallyanashis Paul[1, 2], Caroline Gargett[1, 2], Anna Rosamilia[2], Jerome Werkmeister[1], Shayanti Mukherjee[1, 2]

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

2. Obstetrics and Gynaecology, Monash University, CLAYTON, VIC, Australia

Human Fascial Lata (HFL) is utilized in urogynecology and urology for incontinence procedures and, more recently, for sacrocolpopexy or graft-augmented vaginal repair when synthetic meshes aren't suitable (1,2). However, the durability of HFL grafts compared to synthetic mesh for sacrocolpopexy, known for its low recurrence rate, remains unclear. Moreover, understanding HFL's morphological, cellular, matrix, and immunological properties post-implantation is limited.

This research assesses HFL's morphometric features and in vivo response using a murine abdominal incision model. A direct comparison with synthetic polypropylene mesh is made to determine long-term implications and graft durability. The study also explores the molecular mechanisms driving HFL's integration for pelvic organ prolapse augmentation.

HFL tissue was collected from women undergoing sacrocolpopexy or pubovaginal sling insertion (n=26). Mice were implanted with HFL or synthetic mesh (n=8 mice/gp/time-point) via abdominal incision. Assessment occurred at 7 and 90-day intervals, involving histological stains, gene expression analysis, and immunofluorescence for macrophage response.

HFL is mainly collagenous connective tissue with few cells. Both grafts integrated well without erosions. At 7 days, HFL exhibited strong tissue integration with host cell infiltration, while polypropylene mesh displayed loose integration with inflammation and giant cell formation. Ongoing gene expression analysis suggests varied immune responses and integration mechanisms.

HFL outperforms polypropylene mesh in tissue integration and durability, making it a promising surgical graft for pelvic reconstruction. Advanced techniques offer insights into HFL's in vivo response, illuminating tissue integration and foreign body mechanisms. With its sturdy structural proteins, HFL emerges as an ideal implant for augmentative pelvic reconstructive procedures.

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Characterization of the role for Dynamin 2 in the regulation of human endometrial receptivity

Wei Zhou[1, 2], Poppy Downing[1, 2], Michaela Sacco[1, 2], Leiani L Santos[1, 2], Wan T Teh[1, 3, 4, 5], Evdokia Dimitriadis[1, 2]

1. Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, VIC, Australia

2. Gynaecology Research Centre, Royal Women's Hospital, Melbourne, VIC, Australia

3. The Royal Women's Hospital, Parkville, VIC, Australia, Melbourne, Victoria, Australia

4. Melbourne IVF, Melbourne, Victoria

5. Epworth HealthCare, Melbourne, Victoria, Australia

Abnormal endometrial receptivity is a major cause of infertility and implantation failure. In each menstrual cycle, the endometrium remodels to accept an implanting blastocyst only in the window of 'receptivity'. During this window, the endometrial luminal epithelium becomes adhesive to an implanting blastocyst. Endometrial glands produce and secrete factors apically into the uterine cavity to prepare the initial blastocyst attachment and implantation. However, we have a profound lack of knowledge on how these processes are regulated. The dynamin (DNM) family comprises three canonical isoforms of GTPase generally responsible for the regulation of membrane trafficking events such as exocytosis and intracellular trafficking.

This study aimed to characterise the role of DNM in regulating endometrial receptivity. Our qPCR data showed that among three isoforms examined, only *DNM2* was significantly upregulated ($P<0.05$) in the fertile receptive endometrium compared to the non-receptive, proliferative endometrium. Further characterization of DNM2 confirmed a significant increase ($P<0.05$) of immunostaining intensity in the fertile receptive endometrium, compared to proliferative endometrium. In fertile receptive endometrium, DNM2 was localized in the apical and basal surfaces of the glandular and luminal epithelium, respectively. Endometrial organoids were further used to mimic endometrial glands *in vitro* since DNM2 showed a conserved apical membrane localization. DNM2 expression in fertile endometrium-derived organoids was significantly increased ($P<0.05$) by progesterone stimulation, compared to estrogen treatment only, as confirmed by qPCR and immunoblotting. Such an increase was not recorded in infertile endometrium-derived organoids. Using Ishikawa cells (receptive endometrial cell line) to mimic endometrial luminal epithelium *in vitro*, it was identified that knockdown of *DNM2* significantly reduced ($P<0.01$) Ishikawa cell adhesive capacity to trophoblast progenitor spheroids (blastocyst surrogates). Furthermore, *DNM2* knockdown in Ishikawa cells significantly affected the expression of receptivity markers including *SPP1* and *STAT3*. Collectively the data encourages further investigation of the mechanisms underpinning DNM2 regulation on endometrial receptivity.

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Importin α4 is critical for establishing normal sperm morphology in mice

Yoichi Miyamoto[1], Yuki Okada[2], Kate L. Loveland[3], Masahiro Oka[1]

1. Center for Drug Design Research, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan

2. Institute of Quantitative Biosciences, The University of Tokyo, Tokyo, Japan

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

Aims: Nuclear-cytoplasmic transport is a fundamental process in eukaryotic cells. Importin α proteins play a central role in the nuclear transport processes, and there are 6 subtypes in mouse and 7 in human. Although some subtypes show high expression in specific male germ cells, it is still unclear what function each of these subtypes performs in male reproduction.

Methods: We established a knock-out (KO) mouse of Importin α4 protein, which is encoded by the *Kpna4* gene. The *Kpna4 KO* mouse was subfertile and yielded smaller litter sizes than those of wild-type (*WT*) males. To understand the molecular function of Importin α4 protein in spermatogenesis, we performed an in vitro fertilization assay, sperm motility assays, transmission electron microscopy and a comprehensive proteomics analysis.

Results: Sperm from the *Kpna4 KO* mouse had significantly reduced quality and motility, and the acrosome reaction was also impaired. Transmission electron microscopy revealed striking defects in sperm from the *KO* mice, including abnormal head morphology and multiple axoneme structures. A comprehensive proteomics analysis of testis from the KO mice followed by the ChIP-Atlas enrichment analysis which searches for proteins significantly bound around multiple query genes indicated that genes perturbed by loss of Importin α4 are regulated by Taf7l and Tbpl1 transcription factors, both of which are necessary for spermatogenesis. In addition, genes encoding those proteins identified by the proteomics analysis were characterized by active histone marks such as H3K4me3 and H3K27ac in the testes and sperm-related cells.

Conclusion: Our findings indicate that Importin α4 is critical for establishing normal sperm morphology in mice, and genetic loss of Importin α4 could disrupt the chromatin status in testicular cells, resulting in reduced expression of genes critical for sperm formation.

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MROH9 is essential for sperm function and male fertility in mice and men

Jessica EM Dunleavy[1], Joseph Nguyen[1], Anne E O'Connor[1], D. Jo Merriner[1], Joris A Veltman[2], Donald F Conrad[3], Frank Tüttelmann[4], Brendan J Houston[1], Moira K O'Bryan[1]

1. School of BioSciences and Bio21 Institute, Faculty of Science, The University of Melbourne, Parkville, VIC, Australia

2. Biosciences Institute, Newcastle University, Newcastle-upon-Tyne, United Kingdom

3. Oregon National Primate Research Center, Oregon Health & Science University, Portland, Oregon, United States

4. Institute of Reproductive Genetics, University of Münster, Münster, Germany

Publish consent withheld

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An investigation into the effect of aging on DNA integrity in stallion spermatozoa

Róisín Griffin[1], Natasha Harrison[1], Narantsatsral Sandagdorj[1], Kaitlin Mcintosh[1], Laura A Dennis[2], Ceilidh Jenkins[1], Aleona Swegen[1], Zamira Gibb[1]

1. College of Engineering, Science and Environment, The University of Newcastle, Callaghan, NSW, Australia

2. School of Agriculture, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

The progeny of younger stallions record significantly higher racing speeds (Sharman et.al.,2022) and more race wins (Brazil et.al.,2016), compared those sired by older stallions. We hypothesize that this may be due to an age-associated increase in sperm harbouring DNA damage, as has been demonstrated in other species. This study aimed to investigate the relationship between stallion age and sperm DNA damage.

Post-breeding urethral semen samples were collected from commercial Thoroughbred stallions (n=219). Samples were diluted (2:1,Equiplus®:semen), and fractionated (using Equipure™) to isolate the high-quality sperm populations. Sperm concentration and motility were assessed on-site, before aliquots were fixed and snap-frozen for morphological and DNA damage

assessments. Pregnancy scan results, pertaining to each breeding, were collated. For this study, stallions aged ≤9 years were categorised as “young”, while stallions aged ≥16 years were categorised as “aged”.

Sperm count and motility parameters did not differ significantly between age cohorts ($P \geq 0.05$). Spermatozoa from older stallions had higher levels of morphological abnormalities (37.9±2.65% vs 19.9±1.23% for aged and young stallions, respectively;$P \leq 0.001$)—the most prominent of which was head defects (12.7±1.50% for aged vs 6.5±0.84% for young stallions;$P \leq 0.001$). In line with these findings, DNA strand breaks were higher in spermatozoa from older stallions (sperm chromatin dispersion assay:15.4±2.08% vs 9.9±1.30% for aged and young stallions, respectively;$P \leq 0.001$). This may be attributed to poor chromatin packaging, as older stallions had a concurrent deficiency of sperm protamines compared to young stallions (7.7±1.36 vs 4.2±0.41 chromomycin A_3 positive cells;$P \leq 0.05$). Interestingly, the increased DNA damage recorded by older stallions did not influence pregnancy outcomes ($P \geq 0.05$). In conclusion, we have confirmed our hypothesis that sperm DNA damage increases with age in stallions. Further research is required to determine whether age-related DNA damage compromises the genome of the resulting progeny, and how this might contribute to losses in racing performance and potentially, reduced offspring health.

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H3K79 methyltransferase DOT1L is required for the long-term maintenance of spermatogonial stem cells

Yuki Okada[1], Yoshinori Makino[1]

1. The University of Tokyo, Bunkyo, TOKYO, Japan

Publish consent withheld

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Axonemal dynein light chain domain containing 1 (AXDND1) plays essential roles in sperm tail formation and controls germ cell content during the first wave of spermatogenesis

Brendan Houston[1], Jo Merriner[1], Anne O'Connor[1], Joseph Nguyen[1], Don Conrad[2], Jessica Dunleavy[1], Moira O'Bryan[1]

1. The University of Melbourne, Parkville, VIC, Australia

2. Oregon National Primate Research Center, Hillsboro, Oregon, USA

Male infertility is a common condition affecting at least 7% of men worldwide and is often genetic in origin. Using exome sequencing, we have identified an infertility-causing mutation in *AXDND1* in a man with azoospermia. This ‘stop-gain’ mutation affects the axonemal dynein domain and causes a complete loss of AXDND1 function. *Axdnd1* is highly testis enriched in mammals, and in male germs is largely expressed in spermatocytes and spermatids. We generated *Axdnd1* knockout mice with a premature stop codon in exon 3 to further explore the role of AXDND1 in male fertility. *Axdnd1* knockout mice were infertile and presented with a multifaceted phenotype that worsened with age. At 7 weeks of age, just after the first wave of spermatogenesis and epididymal maturation, spermatogenesis was intact and knockout males interestingly generated 30% additional sperm compared to wild type. However, sperm collected from the cauda epididymides of knockout males were completely immotile and morphologically abnormal. Electron microscopy revealed the sperm axoneme to be severely disrupted, with key accessory structures such as outer dense fibres missing. By 10 weeks of age there was a significant loss of germ cells was observed, in concert with an increase in the immune cell population in the intertubular space of *Axdnd1* knockout testes. The loss of germ cells worsened with age and by 6 months only 20% of tubules presented with intact spermatogenesis. This translated to a 99.3% reduction in epididymal sperm count compared to wild type, and the presence of precociously sloughed germ cells and immune cells in the cauda epididymis. Although classified as an axonemal dynein protein that plays roles in axoneme function, our data suggest AXDND1 primarily plays roles in cytoplasmic dynein function in male germ cells. Specifically, we hypothesise AXDND1 is required for cargo transport during spermatogenesis, including into the developing sperm tail.

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Dynamic changes of the sperm proteome during epididymal maturation in the Eastern Grey Kangaroo

Sarah Kunkel[1], Stephen Johnston[1], Taylor Pini[2]

1. School of Environment, University of Queensland, St. Lucia, QLD, Australia

2. School of Veterinary Science, University of Queensland, Gatton, QLD, Australia

While advanced reproductive technologies (ARTs) are widely used in domestic animals, successful implementation of ARTs to conserve wildlife species remains challenging. In macropods, crucial aspects of fundamental reproductive biology, including changes induced by epididymal maturation, remain unknown, limiting the development of ARTs. In this context we performed a proteomic analysis of sperm from the caput, corpus, and cauda epididymis of Eastern Grey Kangaroos (n = 6) to profile changes during epididymal maturation. Samples prepared by FASP digestion were analysed by LC-MS/MS with SWATH acquisition. 3,889 proteins were identified overall. Proteins which were most abundant in sperm across all epididymal regions included ATP synthase F1 subunit alpha (ATP5F1A) and A-kinase anchoring protein (AKAP4). The top proteins in corpus and caput sperm were involved in protein binding, while the most abundant proteins in cauda sperm were involved in ATP metabolic

processes and generation of precursor metabolites. Comparing corpus to caput sperm, 266 proteins differed significantly (104 increased, 162 decreased). Comparing corpus to cauda sperm, 502 proteins differed significantly (193 increased, 309 decreased). Proteins with differing abundance between regions largely had catalytic and binding activities. Proteins with higher abundance in caput and corpus sperm included lysosomal proteins (CTSC, NAAA) and chaperones (CALR, TXNDC5). From corpus to cauda, there was a clear increase in proteins involved with locomotion (CFAP47, RSPH6A, FER). Proteins involved in glycolysis (ENO1, GAPDH), mitochondrial ATP synthesis (ATP5F1A, CKB) and microtubule function (DNAH1, TEKT1) increased in abundance towards the cauda. These proteomic alterations likely underlie the significant sperm structural remodeling unique to marsupial epididymal transit (e.g. formation of midpiece fibre network, post-testicular acrosome formation). These results also support the necessity of completing epididymal maturation for development of full motility potential, including both energy production and cytoskeletal maturation.

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Phosphoproteomic analysis of human sperm capacitation reveals roles for novel kinases in regulating sperm motility and acrosomal exocytosis

Nathan Burke[1, 2, 3], David A Skerrett-Byrne[1, 2], Amanda L Anderson[1, 2], Shaun D Roman[4], John E Schjenken[1, 3], Brett Nixon[1, 2, 5], Elizabeth G Bromfield[1, 2, 5, 3]

1. The Priority Research Centre for Reproductive Biology, The University of Newcastle, Callaghan, New South Wales 2308, Australia

2. Infertility and Reproduction Research Program, Hunter Medical Research Institute, New Lambton Heights, New South Wales, Australia

3. School of BioSciences, Faculty of Science, Bio21 Institute, The University of Melbourne, VIC, Australia

4. NSW Health Pathology, NSW Health, Newcastle, NSW, Australia

5. These authors contributed equally to this work,

Extrinsic microenvironments provide crucial stimuli to transcriptionally and translationally silent human spermatozoa priming them for fertilisation. Residency in the female reproductive tract is one such microenvironment that establishes fertilisation competency by promoting capacitation associated phosphorylation signalling cascades. With some kinases considered synonymous with successful sperm capacitation (e.g. protein kinase A), protein phosphorylation forms a dynamic and essential component of sperm maturation. Despite the essential nature of phosphorylation to mammalian fertilisation, a comprehensive analysis of the phosphoproteomic landscape of capacitating human spermatozoa has yet to be reported.

To characterise the cellular signalling events underpinning sperm capacitation we performed phosphopeptide enrichment and high-resolution tandem mass spectrometry to quantify protein phosphorylation in populations of non-capacitated human spermatozoa as well as those subjected to capacitation stimuli *in vitro*. This strategy successfully identified 2,350 site-specific phosphorylation events mapped across 902 unique sperm proteins. In congruence with previous findings indicating the importance of tyrosine phosphorylation to fertilisation, a 2-fold increase (representing a 104% gain) in tyrosine phosphorylated sites was observed following capacitation, compared to a modest 5% gain in the phosphorylation of serine residues under the same conditions. Capacitation significantly upregulated phosphorylation in 124 proteins (1.5-fold change, $p<0.05$) and stimulated phosphorylation of a further 40 proteins. Of this subset of capacitation-sensitive phosphoproteins, 44% had a previously characterised role in sperm function, including A-kinase anchoring protein 4 (AKAP4) and heat shock protein A2 (HSPA2), which are critical contributors to motility and sperm-egg binding. Mapping of phospho-residues to upstream kinases revealed a suite of novel sperm kinases with previously unappreciated functions. Pharmacological inhibition of p21 activated kinase 1 (PAK1) and polo-like kinase 1 (PLK1) during capacitation hampered sperm progressive-motility, while AKT serine/threonine kinase 1 (AKT1) inhibition suppressed acrosomal exocytosis. These findings permit a new understanding of key kinases that act as functional regulators of human spermatozoa.

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Super resolution analysis of meiotic recombination in murine spermatocytes

Lynette Mirembe[1], Sarah Hilet[1], Ashley Rozario[1], Donna Whelan[1], Cathryn A Hogarth[2]

1. Department of Rural Clinical Sciences, La Trobe Rural Health School, La Trobe University, Bendigo, Victoria, Australia

2. Department of Rural Clinical Sciences, La Trobe Rural Health School, La Trobe University, Wodonga, Victoria, Australia

Meiosis is the key step in sexual reproduction driving genetic variability. Genetically unique haploid gametes arise because of meiotic recombination, the pairing of homologous chromosomes and switching of their genetic material. Errors in recombination result in chromosomal abnormalities that can lead to germ cell loss or unhealthy gametes. Advances in microscopy and immunofluorescence imaging have been key in unravelling the molecular events that take place during recombination but molecular-level understanding of the precise distribution of key meiotic proteins during recombination remains poor. This study employed *d*STORM super-resolution microscopy (SRM), which uses the single molecule localisation approach, to map key proteins at 10 to 20 nm spatial resolution, a 10-fold resolution increase in comparison to conventional fluorescence imaging. To capture high resolution snapshots of meiosis, SYCP3, γH2AX, MLH1 and RAD51 were indirectly immunolabeled in primary spermatocytes and SRM was performed on a customised inverted microscope equipped with high power continuous wave lasers and a sensitive sCMOS camera. SRM of SYCP3 revealed the double-helix structure of the lateral elements of the synaptonemal complex. Imaging of γH2AX and RAD51 identified significant cluster-like colocalization with the XY chromosomes during the pachytene stage, which are often unable to be detected using conventional fluorescence imaging. Single foci of MLH1 signal were also detected unilaterally within sister chromatids. This study is the first description of SRM mapping of meiotic recombination. Ultimately, the ability to visualise meiosis and recombination in high resolution will allow for an improved understanding of the effects of mutations and toxins on this essential process in germ cell development.

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Management of high fracture risk in post-menopausal osteoporosis

Frances Milat[1]

1. Monash Health, Clayton, VIC, Australia

For many years, osteoporosis management guidelines have recommended anti-resorptive therapies as first line treatments, particularly with bisphosphonates. However, recent randomised trials in patients with severe osteoporosis have demonstrated that anabolic agents reduce fractures to a greater extent than antiresorptive medications. Consequently, international osteoporosis guidelines have proposed an individualised approach based on fracture risk stratification to include patients at 'very high risk of fracture', who should be managed with anabolic agents as first-line therapy. This talk will discuss stratification of fracture risk, examine the efficacy of antiresorptive and anabolic therapies and will also give practical guidance in the management of post-menopausal osteoporosis.

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Recommendations from the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome

Helena Teede[1], **Anju Joham**[2], **Chau Thien Tay**[3]

1. Monash Health/Monash University, Clayton, Vic, Australia

2. Monash Health, Clayton, VIC, Australia

3. Monash University, Clayton, VIC, Australia

STUDY QUESTION: What is the recommended assessment and management of those with polycystic ovary syndrome (PCOS), based on the best available evidence, clinical expertise, and consumer preference?

SUMMARY ANSWER: International evidence-based guidelines addressprioritized questions and outcomes and include 254 recommendations and practice points, to promote consistent, evidence-based care and improve the experience and health outcomes in PCOS.

WHAT IS KNOWN ALREADY: The 2018 International PCOS Guideline was independently evaluated as high quality and integrated multidisciplinary and consumer perspectives from six continents; it is now used in 196 countries and is widely cited. It was based on best available, but generally very low to low quality, evidence. It applied robust methodological processes and addressed shared priorities. The guideline transitioned from consensus based to evidence-based diagnostic criteria and enhanced accuracy of diagnosis, whilst promoting consistency of care. However, diagnosis is still delayed, the needs of those with PCOS are not being adequately met, evidence quality was low and evidence-practice gaps persist.

STUDY DESIGN, SIZE, DURATION: The 2023 International Evidence-based Guideline update reengaged the 2018 network across professional societies (including ESA) and consumer organisations with multidisciplinary experts and women with PCOS directly involved at all stages. Extensive evidence synthesis was completed. Appraisal of Guidelines for Research and Evaluation-II (AGREEII)-compliant processes were followed. The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework was applied across evidence quality, feasibility, acceptability, cost, implementation and ultimately recommendation strength and diversity and inclusion were considered throughout.

PARTICIPANTS/ MATERIALS, SETTING, METHODS: This summary should be read in conjunction with the full Guideline for detailed participants and methods. Governance included a six-continent international advisory and management committee five guideline development groups, and paediatric, consumer, and translation committees. Extensive consumer engagement and guideline experts informed the update scope and priorities. Engaged international society-nominated panels included paediatrics, endocrinology, gynaecology, primary care, reproductive endocrinology, obstetrics, psychiatry, psychology, dietetics, exercise physiology, obesity care, public health and other experts, alongside consumers, project management, evidence synthesis, statisticians and translation experts. Thirty-nine professional and consumer organizations covering 71 countries engaged in the process. Twenty meetings and five face-to-face forums over 12 months addressed 58 prioritized clinical questions involving 52 systematic and 3 narrative reviews. Evidence-based recommendations were developed and approved via consensus across five guideline panels, modified based on international feedback and peer review, independently reviewed for methodological rigour, and approved by the Australian Government National Health and Medical Research Council (NHMRC).

MAIN RESULTS AND THE ROLE OF CHANCE: The evidence in the assessment and management of PCOS has generally improved in the past five years, but remains of low to moderate quality. The technical evidence report and analyses (~6000 pages) underpins 77 evidence-based and 54 consensus recommendations, with 123 practice points. Key updates include: i) further refinement of individual diagnostic criteria, a simplified diagnostic algorithm and inclusion of anti-Müllerian hormone (AMH) levels as an alternative to ultrasound in adults only; ii) strengthening recognition of broader features of PCOS including metabolic risk factors, cardiovascular disease, sleep apnea, very high prevalence of psychological features, and high risk status for adverse outcomes during pregnancy; iii) emphasising the poorly recognised, diverse burden of disease and the need for greater healthcare professional education, evidence-based patient information, improved models of care and shared decision making to improve patient experience, alongside greater research; iv) maintained emphasis on healthy lifestyle, emotional wellbeing and quality of life, with awareness and consideration of weight stigma; and v) emphasizing evidence-based medical therapy and cheaper and safer fertility management.

LIMITATIONS, REASONS FOR CAUTION: Overall, recommendations are strengthened and evidence is improved, but remain generally low to moderate quality. Significantly greater research is now needed in this neglected, yet common condition. Regional health system variation was considered and acknowledged, with a further process for guideline and translation resource adaptation provided.

WIDER IMPLICATIONS OF THE FINDINGS: The 2023 International Guideline for the Assessment and Management of PCOS provides clinicians and patients with clear advice on best practice, based on the best available evidence, expert

multidisciplinary input and consumer preferences. Research recommendations have been generated and a comprehensive multifaceted dissemination and translation programme supports the Guideline with an integrated evaluation program.

STUDY FUNDING/COMPETING INTEREST(S): This effort was primarily funded by the Australian Government via the National Health Medical Research Council (NHMRC) (APP1171592), supported by a partnership with American Society for Reproductive Medicine, The Endocrine Society, European Society for Human Reproduction and Embryology, and the Society for Endocrinology. The Commonwealth Government of Australia also supported Guideline translation through the Medical Research Future Fund (MRFCRI000266). HJT and AM are funded by NHMRC fellowships. JT is funded by a Royal Australasian College of Physicians (RACP) fellowship. Guideline development group members were volunteers. Travel expenses were covered by the sponsoring organisations. Conflicts ofinterest were strictly managed according to NHMRC policy and are available with the full guideline, technical evidence report, peer review and responses. The guideline was peer reviewed by special interest groups across our 39 partner and collaborating organisations, including ESA and was independently methodologically assessed against AGREEII criteria and was approved by all members of the guideline development groups and by the NHMRC.

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Menopausal hormone therapy and cardiovascular risk

Bronwyn Stuckey[1]

1. Keogh Institute for Medical Research, Nedlands, WA, Australia

Over the last 70 years, oestrogen therapy for the management of menopausal symptoms has undergone a metamorphosis from perceived cardiovascular protection to perceived cardiovascular risk. The former perception is based on the very convincing evidence from the Nurses Health Study cohorts and the epidemiological data surrounding early menopause. The latter, and later, perception is based on the very disquieting results from the randomised controlled studies of Heart and Estrogen/Progestin Replacement Study (HERS) and the Women's Health Initiative study (WHI). The reality is probably more nuanced than the conclusions presented by any of these studies. When face to face with a patient, the clinician must negotiate the appropriate decision pathway around the interaction between cardiovascular risk, cardiovascular disease, menopause, and oestrogen +/- progestogen containing hormone therapy. This presentation will attempt to do just that.

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Managing the Menopause – What's New

Christina Jang[1]

1. Royal Brisbane and Women's Hospital, Herston, QLD, Australia

This talk will discuss two topics in the management of the menopause.

Hot flushes affect up to 70% of menopausal women and can significantly impair their quality of life. Menopausal Hormone therapy (MHT) is the most effective therapy for the management of vasomotor symptoms associated with the menopause. MHT however is contraindicated for some women including those with a history of oestrogen-dependent malignancy. The KNDy (Kisspeptin-Neurokinin B-Dynorphin) neurons of the hypothalamus which express Neurokinin B are now understood to play a major role in the aetiology of hot flushes. These neurons innervate the thermoregulatory centre and undergo hypertrophy in postmenopausal women as a result of declining oestrogen. The Neurokinin 3 receptor antagonists are a novel, non-hormonal treatment for hot flushes. Early studies have shown them to be effective in relieving both severity and frequency of hot flushes. They represent an exciting future therapeutic option for women for whom MHT is contraindicated.

Progestogens are an essential component of MHT for women with an intact uterus to prevent endometrial hyperplasia and malignancy. They vary in their clinical structure an interact with other steroid receptors to varying degrees, thereby causing unwanted side effects. The use of micronized progesterone and dydrogesterone has become widespread in recent years with evidence suggesting lower rates of breast cancer compared to synthetic progestogens. Whether standard doses of micronized progesterone provides adequate endometrial protection, particularly at higher doses of oestrogen, has recently been a contentious topic of discussion. The safety and side effect profile of the different progestogens will be reviewed.

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PUMA blockade protects oocytes from chemotherapy-induced depletion

Lauren R Alesi[1], Roseanne Rosario[2], Amy L Winship[1], Jessica M Stringer[1], Richard A Anderson[2], Karla J Hutt[1]

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

2. MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom

Irreversible ovarian damage and permanent depletion of oocytes are devastating side effects of chemotherapy, often leaving female cancer survivors infertile and at risk of premature menopause. Unfortunately, current fertility preservation options have significant drawbacks, with no strategies available to protect both fertility and long-term endocrine function[1]. PUMA, an apoptotic protein, triggers oocyte death following exposure to DNA-damaging insults, like chemotherapy[2]. In fact, genetic loss of PUMA preserves fertility post-chemotherapy without compromising offspring health[3]. Excitingly, a small-molecule PUMA inhibitor (PUMAi) is now available, making PUMA blockade for fertility preservation a real therapeutic possibility.

To assess whether PUMAi can prevent oocyte apoptosis post-chemotherapy, postnatal day 7 C57BL6/J mouse ovaries (n=6/group) were cultured *ex vivo* for 24 hours with the cyclophosphamide metabolite 4-HC (8μM) ± PUMAi (200μM). Similarly, human ovarian cortical pieces (n=5 patients) were cultured *in vitro* with 4-HC (2μM) ± PUMAi (200μM). Lastly, adult mice

(n=6/group) received 10mg/kg PUMAi 2h before and 20h after 150mg/kg cyclophosphamide in vivo. This regimen was based on a previous study where PUMAi prevented intestinal stem cell apoptosis post-chemotherapy[4].

Whilst 4-HC alone depleted primordial follicles by 87% ($p<0.0001$) in mice *ex vivo*, PUMAi rescued primordial follicles by 40% ($p<0.01$). Although 4-HC alone decreased the proportion of healthy primordial follicles ($p<0.0001$) in human ovarian tissue *in vitro*, PUMAi treatment restored this ($p<0.05$). In mice *in vivo*, cyclophosphamide alone reduced primordial follicles by 75% ($p<0.01$). Remarkably however, PUMAi rescued 50% of primordial follicles post-cyclophosphamide ($p<0.05$). This is extremely promising, as partial protection of just 12% of follicles in genetic PUMA knockout models sustained female fertility[3,5].

These data demonstrate that PUMA blockade is a promising avenue for fertility preservation prior to chemotherapy treatment. Further studies are already underway to ensure that PUMAi does not impact the anti-tumour efficacy of chemotherapy and determine whether oocyte quality is also preserved post-treatment.

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Non-invasive metabolic imaging using a lab-on-a-chip device detects differeces in metabolic profile of early embryos

Erick Vargas-ordaz[1], Hope Newman[2], Sally Catt[2], Reza Nosrati[1], Victor Cadarso[1], Adrian Neild[1], Fabrizzio Horta[2]

1. Mechanical Engineering and Aerospace , Monash University, Melbourne, VIC, Australia

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

Background: Selecting the most suitable embryos for implantation and subsequent healthy live birth is crucial to the success rate of assisted reproduction and offspring health. A promising alternative is the non-invasive imaging of live embryos to establish metabolic activity performance. However, metabolic imaging has been only achieved using highly complex advanced microscopy methods that are costly and challenging, limiting the potential for deployment within fertility clinics. Thus, we aimed to develop an affordable and scalable optofluidic device capable of non-invasively obtaining high-resolution 3D images of the metabolic activity in live mouse embryos.

Method: Optofluidic devices were manufactured by cast-moulding using a negative photoresist (MicroChemicals GmbH-Germany) following a standard UV-photolithography process. The microstructures fabricated of PDSM integrated Light Sheet Fluorescence Microscopy into a microfluidic system, including on-chip micro-lenses to generate a light sheet at the center of a microchannel. Super-ovulated F1 (CBA/C57Bl6) mice were used to produce 2-cell embryos and embryo culture experiments. Blastocyst formation rates and embryo quality (immunocytochemistry) were compared between study groups. Furthermore, inhibition of metabolic activity (FK866 inhibitor) during embryo culture was also assessed. A convolutional neural network (CNN; ResNet 34) model using metabolic images was also trained.

Results: The results indicated no significant difference between the imaged and non-imaged embryos for embryo development as well as embryo quality at the blastocyst stage ($p>0.05$). Embryos with inhibited metabolic activity by FK866 showed a decrease in blastocyst formation as well as a reduction in metabolic activity measured by non-invasive metabolic imaging compared to controls (Control: 0.75 arbitrary units [AU]; Inhibitor: 0.5 AU; $p<0.0001$). Metabolic images predicted blastocysts formation with an AUC of 0.92.

Conclusion: This study reports an optofluidic device capable of non-invasive metabolic imaging of live embryos using a similar concept as previously reported using FLIM technology and hyperspectral microscopy.

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GM-CSF treatment of frozen/thawed bovine sperm improves sperm function and quality.

Annie Whitty[1, 2], Kylie Dunning[1, 3], Karen Kind[4, 2], Nicole Mcpherson[1, 5], Mark Nottle[1]

1. The University of Adelaide, School of Biomedicine, & Robinson Research Institute, Adelaide Medical School, The University of Adelaide, Adelaide, SOUTH AUSTRALIA, Australia

2. The Davies Livestock Research Centre, The University of Adelaide, Adelaide, SA, Australia

3. Australian Research Council Centre of Excellence for Nanoscale BioPhotonics, Institute for Photonics and Advanced Sensing, Adelaide, South Australia, Australia

4. School of Animal and Veterinary Sciences, The University of Adelaide, Adelaide, South Australia, Australia

5. Freemason Centre for Male Health and Wellbeing, Adelaide, South Australia, Australia

Sperm cryopreservation is essential for cattle *in vitro* embryo production (IVP). However, many sperm are rendered unviable post-thaw [1]. Granulocyte macrophage colony stimulating factor (GM-CSF) is present in seminal plasma and the female reproductive tract and its receptors are found on bovine sperm [2-4]. Whether GM-CSF can improve the health of post-thaw

bovine sperm is unknown. The present study aimed to determine if *in vitro* addition of GM-CSF to *frozen/thawed* bovine sperm could improve sperm function, fertilisation and embryo development outcomes following IVP.

Thawed bovine sperm (N=4 bulls/4reps) were treated with 0, 0.1, 1, 2 or 10 ng/ml of recombinant bovine GM-CSF at 38.5C for 45 min and assessed for motility on CASA and quality parameters: glucose uptake, mitochondrial ROS, intracellular Ca^{+}, and mitochondrial membrane potential (MMP) by flow cytometry. Capacitation as measured by chlortetracycline staining, and DNA integrity (HALO sperm) were examined following 5 h. IVP was performed on *in vitro* matured oocytes (N=200/5rep/group), fertilised with post-thaw bovine sperm treated with 2 ng/ml GM-CSF. Presumptive zygotes were cultured in 6% CO2; 7% O2; N_2 balance, and day 8 blastocyst development examined. Cell numbers were determined by differential staining.

GM-CSF (2 and 10 ng/ml) increased progressive and rapid motility (10%), and glucose uptake (13%), while 1, 2, and 10 ng increased capacitation (17%) of post-thaw treated sperm ($P<0.05$), but had no effect on mitochondrial ROS, intracellular Ca^{+}, MMP or DNA integrity. Oocytes fertilised with treated sperm had increased fertilisation rates (69.6 vs 81.7, P=0.006), hatching blastocyst rates (5.6% vs 7.8%, P= 0.048), and blastocyst inner cell mass numbers (35.3 vs 41.8, P=0.026) compared with control.

Our data suggest that GM-CSF treatment of frozen/thawed bovine sperm increases sperm function and improves IVP embryo quality and could be a useful addition to cattle IVP protocols to increase embryo yield and quality.

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Seminal fluid histocompatibility antigens contribute to T cell priming after mating in mice

Ha M. Tran[1], Hon Yeung (Dexter) Chan[1], David L. Adelson[2], Sarah A. Robertson[1]

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

2. Department of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, SA, Australia

At conception, the female reproductive tract is exposed to factors in seminal fluid that prime the female T cell response to initiate maternal immune tolerance towards the ensuing genetically disparate fetus. Amongst the most antigenic proteins in seminal fluid are male major histocompatibility (MHC) antigens. Interaction between these antigens and T cells is thought to affect the quality of the immune response to impact subsequent pregnancy development, but this has not been formally evaluated. Our study tests the hypothesis that disparate MHC and minor histocompatibility (MiHC) antigens both stimulate the T cell repertoire after mating, leading to improved reproductive efficiency. Using C57BL/6 female mice, we assessed fetal and placental development at 17.5 days post-coitum (dpc) following mating with males of three genotypes: BALB/c (MHC and MiHC disparate), BALB/b (MHC matched and MiHC disparate), or C57BL/6 (MHC and MiHC matched). To comprehensively analyse the T cell repertoire, we sequenced T cell receptor (TCR) gDNA from uterine-draining lymph nodes (udLNs) at 3.5 dpc from females in each mating group, with unmated C56BL/6 females as a comparison. We found that pregnancies sired by Balb/c males had a 16% increase in fetal to placental weight ratio indicative of greater placental efficiency compared to pregnancies sired by Balb/b or B6 males (n=14-16/group). This was accompanied by ~2.5-fold increased udLNs and splenic weight in females mated to Balb/c males (n=12-15/group). Genomic sequencing revealed that mice mated with Balb/c males also exhibited decreased T cell receptor diversity and increased clonal proliferation (n=3-5/group). These findings demonstrate that disparate seminal fluid histocompatibility antigens, especially MHC, contribute to the activation and expansion of a paternal antigen-specific T cell repertoire to support placental development and function. Further research is underway to investigate the underlying mechanisms and define these interactions at a higher resolution with single-cell RNA-seq.

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PRC2: a novel regulator of ovarian folliculogenesis

Ellen G Jarred[1, 2], Gabrielle Pulsoni[1, 2], Dagmar Wilhelm[3], Patrick S Western[1, 2]

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. Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia

The development of unique cell types in multicellular organisms is achieved through careful coordination of gene expression, involving signalling, transcription factors and epigenetic modifications. Tight regulation of the epigenome is therefore critical for normal cell function and epigenetic modifications are often disrupted in disease, including cancer. This has led to substantial

development of drugs to specifically target epigenetic modifiers for cancer treatment. Despite substantial influence of epigenetic modifications on cell identity and function, very little is understood about the epigenetic regulation of ovarian development or how dysregulation of epigenetic modifications contributes to ovarian dysfunction in women. Polycomb Repressive Complex 2 (PRC2) is a widely conserved epigenetic modifier which catalyses the repressive modification Histone 3 Lysine 27 trimethylation (H3K27me3). While PRC2 regulates cell function and identity in many developmental contexts, whether PRC2 regulates somatic cell development and function in the ovary is unknown. Using a combination of genetic and pharmacological mouse models and human granulosa tumour cells (KGN cells), we investigated how reduced PRC2 function impacts ovarian function. We demonstrate that PRC2 is essential for normal granulosa cell proliferation, follicular development and steroidogenic enzyme expression in mouse ovaries. Further, the PRC2 inhibitor MAK683 substantially reduced both H3K27me3 and proliferation of KGN cells, suggesting PRC2 may also regulate proliferation in human granulosa cells and could be a useful target for treatment of specific ovarian cancers. These findings provide the first evidence that PRC2 is an essential regulator of follicle development and female endocrine regulation. Our work generates important insights into epigenetic regulation of ovarian development, with potential implications for understanding disorders of female reproductive health for which abnormal granulosa cell function and steroid production have roles, such as granulosa cell tumours, primary ovarian insufficiency and infertility, and how emerging PRC2 inhibiting drugs may impact both healthy ovarian tissue and ovarian cancer cells.

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The nuclear transport factor, Importin 5 (*Ipo5*) is essential for maintenance of the adult spermatogonial pool

Penny A F Whiley[1, 2], Michael M C Luu[1], Daniel Garama[2], Mark Baker[3], Robin M Hobbs[1, 2], Kate L Loveland[1, 2]

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

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

3. Priority Research Centre for Reproductive Science, University of Newcastle, Newcastle, NSW, Australia

Importins selectively transport proteins into the nucleus to effect gene transcription changes and can also sequester proteins to specific intracellular domains [1]. Importin 5 (IPO5) has been implicated in mediating BMP and WNT signalling [2, 3]. Its dynamic expression and subcellular localisation in maturing germ cells suggests it mediates developmental switches at key points in spermatogenesis [1, 4]. Germline-specific *Ipo5* deletion after E15.5 and at PND3 both lead to germ cell loss in the first round of spermatogenesis. Its importance in adult spermatogenesis is unknown, and we hypothesised IPO5 is vital for spermatogonial survival.

To assess IPO5 function in spermatogonia *in vivo*, we developed an inducible *Ipo5* knockout model (*Ipo5*$^{TAM\text{-}KO}$). Control and *Ipo5*$^{TAM\text{-}KO}$ adult males were injected with Tamoxifen for 2 consecutive days. Testes were harvested 2-, 4-, and 6-weeks post injection (n=3-5/timepoint). Testis tissue was analysed using immunofluorescence and real-time PCR. Additionally, IPO5 binding partners were identified in cultured adult mouse undifferentiated spermatogonia using immunoprecipitation and mass-spectrometry (n=4).

Testis weights of *Ipo5*$^{TAM\text{-}KO}$ mice were unaffected at 2-weeks, but progressively reduced at 4- and 6-weeks post-injection, consistent with spermatogenic failure. *Ipo5* deletion efficiency was variable, so tubules were classified by IPO5 expression: normal, partial or none. Absence of IPO5 in tubules was accompanied by progressive reduction of PLZF^{+} (undifferentiated spermatogonia) and GILZ^{+} cells (spermatogonia and spermatocytes) at 2- and 4-weeks, which resulted in Sertoli cell-only tubules at 6-weeks ($p<0.0001$). Amongst the >60 IPO5 binding partners identified were ribosome biogenesis proteins, histones, and SALL4, essential for differentiation and long-term maintenance of undifferentiated spermatogonia [5].

These findings demonstrate IPO5 is required for maintenance of the adult spermatogonial pool, consistent with emerging knowledge of its roles in normal/pathological states. Ongoing investigations will identify critical molecules and pathways involved in male fertility, shedding light on the intricate roles of IPO5 in spermatogonial biology.

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Sizing Up FGR: Integrating insights into pathophysiology from the cell to the organ scale

Jo James[1]

1. University of Auckland, Auckland , New Zealand

Fetal growth restriction (FGR, babies <3rd growth centile) is a significant clinical issue with no effective treatment other than preterm delivery. The pathophysiology of FGR is established in early pregnancy, but is clinically silent until at least mid-gestation, making this disorder particularly challenging to predict or treat. Indeed, over half of FGR cases remain undetected prior to delivery, and this is a key reason that FGR is the largest risk factor for stillbirth. At its core, FGR results from poor placental exchange function due to a heterogenous combination of factors including impaired trophoblast growth and function, reduced placental villous and vascular branching, and inadequate remodelling of the uterine vasculature. In turn, all these

factors combine to impact both the haemodynamics of blood flow in the intervillous space and the resulting placental capacity to transfer nutrients and oxygen to the fetus. In line with the heterogenous pathophysiology of FGR, A/Prof James' team takes a wide approach to better understand the dynamic events that lead to this disorder across pregnancy, combining stem cell and primary tissue models, in vivo and ex vivo imaging, and in silico tools to integrate physiology across multiple scales and better understand the relationships between distinct pathological components. This talk will cover a spectrum of work focused on understanding the pathophysiology of FGR from the cell to the system level, with a view to improving our ability to better predict, detect, and treat this disorder in the future.

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Glucagon stimulation test: clinical and biochemical predictors of adult growth hormone deficiency

Nayomi Perera[1], John M Wentworth[1], Christopher J Yates[1, 2], Angeline JJ Shen[1], Cherie Chiang[1]

1. Diabetes and Endocrinology, Royal Melbourne Hospital, Parkville, VIC, Australia

2. Department of General Medicine , Melbourne University , Parkville, Victoria, Australia

To access growth hormone (GH) replacement on the Australian Pharmaceutical Benefits Scheme, adults with acquired pituitary insufficiency must undergo stimulation testing to confirm GH deficiency (GHD), however US Endocrine Society Guidelines recommend that the presence of deficiencies in three or more pituitary axes strongly suggests the presence of GHD, and in this context provocative testing is optional [1,2]. The commonly performed glucagon stimulation test (GST) takes up to five hours and requires admission to an ambulatory treatment ward. We aimed to identify clinical and biochemical predictors of GHD that could be used to simplify or shorten GST duration [3].

A prospective observational study of GST performed at Royal Melbourne Hospital from January 2019 to June 2023 was conducted and approved by local ethics committee.

Of the 94 GST performed, , n=74 (79%) were consistent with GHD . Abnormal GST was associated with deficiency of ≥2 pituitary hormone axes ($p<0.0005$), resected macroadenomas >1cm ($p<0.05$) and prior cranial irradiation ($p=0.02$). All 27 individuals with undetectable baseline fasting GH had GHD, while six patients had fasting GH above the stimulation threshold of 3.0mg/l, thereby had normal GH axis and did not require GST. Receiver operator curve analysis of the ability of fasting GH to predict GHD returned an area under the curve of 0.902 (95% CI 0.8230 – 0.9818, $p<0.0001$). The combination of ≥ 2 pituitary hormone deficiencies and fasting GH <0.5ug/L had a positive predictive value of 98% for diagnosis of GHD.

Measurement of fasting GH has potential to identify individuals with GH sufficiency, thereby obviating the need for prolonged, costly stimulation testing. The combination of fasting GH and pituitary hormone deficiencies predicted GHD in our centre, work has commenced to verify this finding in an independent cohort as well as in the paediatric setting

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Tall Cell Papillary Thyroid Cancer: The Westmead and Royal North Shore experience

Louis Britten-Jones[1], Spinder Samra[2], David Goltsman[2], Gideon Sandler[2], Matti Gild[1, 3], Christian Girgis[1, 2]

1. University of Sydney, Darlinghurst, NSW, Australia

2. Westmead Hospital, Sydney, Sydney, NSW, Australia

3. Royal North Shore Hospital, Sydney, NSW, Australia

Aims

This multicentre retrospective cohort study aimed to further develop understanding of tall cell subtype papillary thyroid cancer (tcPTC) by analysing presentation, treatment, and outcomes, as compared to classical papillary thyroid cancer (cPTC). Longitudinal analysis was undertaken to establish risk factors associated with early recurrence and to determine the significance of tall cell histology as an independent prognostic factor.

Methods

Clinicopathological and treatment data was collected for tcPTC and cPTC patients treated at Westmead Hospital between 2013 and 2023 and tcPTC patients treated at Royal North Shore Hospital between 2018 and 2023. Thyroglobulin and thyroglobulin antibody levels were collected to classify biochemical response. Further neck surgeries were used as a surrogate marker of structural recurrence.

Results

Presentation and treatment were analysed for 416 patients (n=51 for tcPTC, n=365 for cPTC). On univariate analysis, tcPTC was found to present at an older age (53.6 years v 46.4 years, $p<0.01$), with greater rates of positive surgical margins (31.37% v 16.44%, $p<0.05$), and greater rates of microscopic (47.06% v 22.74%, $p<0.001$) and gross extrathyroidal extension (15.69% v 6.30%, $p<0.05$). Radioactive iodine (RAI) therapy was more frequently given to tcPTC patients (66.67% v 41.09%, $p<0.001$). Longitudinal analysis was conducted for 236 patients (n=24 for tcPTC, n=212 for cPTC). Multivariate analysis found no

difference in the odds of developing early recurrence between the tcPTC cohort and the cPTC cohort (OR=0.65, p>0.1). Positive surgical margins (OR=2.84, p<0.005) and lymphovascular invasion (OR=2.73, p<0.005) were independently associated with early recurrence.

Conclusions

tcPTC displays more aggressive features than cPTC at time of diagnosis and is treated more aggressively. Positive surgical margins and lymphovascular invasion were found to be independent predictors of early recurrence, but tall cell histology itself was not. This indicates that more aggressive treatment may not be warranted for all patients with tcPTC.

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Comparison of M-TIRADS to Established Thyroid Classification Systems – A Real-World Retrospective Analysis of Current Thyroid Nodule Ultrasound Scoring Systems.

Scott McNeil[1, 2], Ilona Lavender[3], Dee Nandurkar[3], Peter Coombs[3], Eldho Paul[4], Jennifer Wong[1, 2]

1. Monash Health Endocrinology and Diabetes Unit, Melbourne, Victoria, Australia

2. Department of Medicine / School of Clinical Sciences at Monash Health, Monash University, Melbourne, Victoria, Australia

3. Ultrasound, Monash Imaging, Monash Health, Melbourne, Victoria, Australia

4. Monash Centre for Health Research and Implementation, Monash University, Melbourne, Victoria, Australia

Aim:

To compare and validate the M-TIRADS to established thyroid classification systems on their diagnostic accuracy of malignancy in thyroid nodules.

Methods:

We conducted a retrospective analysis on 1795 patients presenting for fine needle aspiration (FNA) of thyroid nodules to a single centre between 2012 and 2022. Sonographic images were classified and scored with the American Thyroid Association, Korean, American College of Radiology and Monash Thyroid Imaging, Reporting, and Data Systems (K-TIRADS, ACR-TIRADS and M-TIRADS), with M-TIRADS scoring for increased vascularity and size >4cm. 2211 thyroid nodules were biopsied with cytopathological results reported using the Bethesda system. Outcomes of these systems were then compared with Bethesda results.

Results:

Overall, there were 2211 specimens. 2070 (93%) were classified as benign and 141 (7%) samples were malignant. ATA diagnosed 128 and K-TIRADS diagnosed 127 of 141 malignant nodules. ACR-TIRADS diagnosed 95 and M-TIRADS 101 of 141 malignant nodules. 1532 nodules deemed intermediate-high risk in the ATA guidelines and 1529 in K-TIRADS were benign. 1621 nodules were unnecessarily biopsied based on ATA guidelines, and 1611 for K-TIRADS. 1227 nodules recommended for biopsy by ACR-TIRADS and 1368 by M-TIRADS were benign.

The sensitivity of M-TIRADs was 71.8% (95% Confidence Interval (CI), 63.7 – 79.1), specificity 27.8% (95%CI, 25.8 – 30.2). The sensitivity of ACR-TIRADs was 67.6% (95%CI, 59.2 – 75.2), specificity 35.3% (95%CI, 33.1 – 37.6). The sensitivity of ATA was 90.8% (95%CI, 84.9 – 95.0), and specificity was 10.0% (95%CI, 8.7 - 11.5). The sensitivity of K-TIRADs was 90.1% (95%CI, 84.0 - 94.5), specificity 10.6% (95%CI, 9.2 – 12.1).

Conclusion:

M-TIRADS diagnostic performance was similar to ACR-TIRADS and hence addition of vascularity and size did not achieve higher sensitivities or specificities. Overall, each of the thyroid classification systems demonstrated strengths and weaknesses. There remain significant limitations in utilising ultrasound characteristics for risk stratification.

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Efficacy of Radionuclide Therapy (I-131 MIBG OR Lu-177-DOTATATE) in the Treatment of Inoperable or Metastatic Phaeochromocytoma and Paraganglioma.

Amanda Seabrook[1], Edward Hsiao[2], David Chan[2], Bruce Robinson[3], Elizabeth Bernard[2], Roderick Clifton-Bligh[3]

1. Familial Cancer Service, Royal North Shore Hospital, Sydney, NSW, Australia

2. Nuclear Medicine Department, Royal North Shore Hospital, Sydney, NSW, Australia

3. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

Context: Inoperable or metastatic phaeochromocytoma (PC) and paraganglioma (PGL) present a management challenge with high variability in response to systemic therapies. The aim of this study was to assess efficacy and toxicity from two therapeutic radiopharmaceutical agents; Iodine-131 metaiodobenzylguanine (I-131 MIBG) and Lutetium-177-DOTA-octreotate (Lu-177-DOTATATE).

Methodology: This retrospective audit assessed efficacy of radionuclide therapy utilising three variables to assess response; clinical (symptoms and anti-hypertensive medication dose changes), functional (catecholamine levels) and radiological (tumour size).

Results: 16 treatment naïve patients were included in this study: nine patients were diagnosed with PC and seven PGL. Eight patients received I-131 MIBG and eight Lu-177-DOTATATE. There was a significant fall in the mean systolic blood pressure following administration of radionuclide therapy; 133mmHg [IQR 124.5-145.5] to 127mmHg [IQR 112.5-130.8] (p = 0.05). Disease control rate was high for all variables (composite clinical 92%; functional 100%; radiological 100%). Overall response rate was greatest clinically with patients predominantly showing stable disease radiologically (composite clinical 83%; functional 45%; radiological 8%). Median PFS (defined as progression in any clinical, functional or radiological variable) was 17 months with no difference in those receiving I-131 MIBG (16 months, range 5-24 m.) compared with Lu-177-DOTATATE (27 months, range 3-40 m.)(95% CI 0.16-2.3, p = 0.46)

Conclusion: Patients diagnosed with inoperable and metastatic PC/PGLs have few therapeutic options. We have shown utility of I-131 MIBG and Lu-177-DOTATATE in symptomatic management, blood pressure control and hormone secretion, and stabilisation of tumour growth.

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Characterising the microenvironment of pituitary neuroendocrine tumours

Lydia S Lamb[2, 1], Nele F Lenders[2, 1, 3], Peter Earls[4], Julia Low[1, 4], Hao-Wen Sim[2, 1, 5, 6], Ann I McCormack[2, 1, 3]

1. St Vincent's Clinical School, University of New South Wales, Sydney, NSW, Australia

2. The Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

3. Department of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia

4. Department of Anatomical Pathology, Sydpath, St Vincent's Hospital, Sydney, NSW, Australia

5. Department of Medical Oncology, St Vincent's Hospital, Sydney, NSW, Australia

6. NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

Publish consent withheld

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Immune cell representations following immune checkpoint blockade in patients experiencing thyroid irAEs

Christopher A Muir[1, 2], Thomas Ashhurst[3], Venessa HM Tsang[4, 2], Alexander M Menzies[5, 2, 6], Roderick J Clifton-Bligh[4, 2, 7], Georgina Long[2, 6], Richard Scolyer[2], Mainthan Palendira[3]

1. St. Vincent's Hospital, Darlinghurst, NSW, Australia

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

3. Charles Perkins Centre, University of Sydney, Camperdown, NSW, Australia

4. Endocrinology, Royal North Shore Hospital, St. Leonard's, NSW, Australia

5. Medical Oncology, Royal North Shore Hospital, St. Leonard's, NSW, Australia

6. Medical Oncology, Melanoma Institute Australia, Wollstonecraft, NSW, Australia

7. Cancer Genetics, Kolling Institute of Medical Research, St. Leonard's, NSW, Australia

Background: Thyroid immune related adverse events (irAEs) are a frequent side effect of immune checkpoint inhibitor (ICI)-treatment and often result in permanent hypothyroidism requiring lifelong thyroid hormone replacement. Despite the frequency of thyroid-irAEs and their prognostic importance, pathogenic mechanisms driving onset remain incompletely characterized.

Aims: To compare immune cell subpopulations in ICI-treated patients with thyroid-irAEs to those without thyroid-irAEs.

Methods: Using mass cytometric analysis, we studied immune cell populations in peripheral blood samples from patients receiving ICI-treatment for melanoma. Samples were collected at baseline (prior to ICI-treatment) and at the time of a thyroid-irAE (or at a matched timepoint for control group patients) to define the composition of circulating immune cells and associated ICI-treatment induced changes.

Results: Ten patients were enrolled in the study (7 'thyroid-irAE' and 3 'control' patients). Initial unsupervised FlowSOM clustering identified 40 meta-clusters representing cell populations within the major innate and adaptive immune compartments. Differences in circulating immune cells were present at baseline between the two groups. As a percentage of total cells, B-lymphocytes were higher in thyroid-irAE group patients (median 9%, range 7-13) compared with control patients (median 4%, range 4-6; p=0.02). Conversely, NK-cells were lower in thyroid-irAE group patients (median 22%, range 8-24) compared with control group patients (median 38%, range 27-40; p=0.02). As a percentage of total cells at baseline, $CD4^+$ T-cells, $CD8^+$ T-cells, monocytes, and gdT-cells were not different between groups. Furthermore, thyroid-irAE patients experienced significant ICI-treatment related alterations in the composition of circulating cells, whereas no ICI-treatment related changes were observed in any cell lineage in control group (no thyroid-irAE) patients.

Conclusions: Significant differences in the composition of immune cells exist between ICI-treated patients with thyroid-irAEs and those without. Further study is required to determine whether differences have relevance as biomarkers of thyroid irAE susceptibility.

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Multi-substrate metabolic tracing reveals dependency on fatty acid metabolism in human prostate cancer

Gio Fidelito[1], David P De Souza[2], Birunthi Niranjan[3], Shivakumar Keerthikumar[4], Matthew J Watt[1], Renea A Taylor[4, 3]

1. Department of Physiology, University of Melbourne, Melbourne, Victoria, Australia

2. Metabolomics Australia, Bio21 Institute, University of Melbourne, Melbourne, Victoria, Australia

3. Monash Biomedicine Discovery Institute, Department of Physiology, Monash University, Melbourne, Victoria, Australia

4. Peter MacCallum Cancer Center, Melbourne, Victoria, Australia

Cancer cells undergo metabolic reprogramming to meet increased bioenergetic demands. Studies in cells and mice have highlighted the importance of oxidative metabolism and lipogenesis in prostate cancer, however, the broader metabolic landscape of human prostate cancer remains unclear. The aim of this study was to study metabolic substrate utilisation in patient-derived xenografts (PDXs) representing different stages of prostate cancer disease progression. The methods included

radiometric (^{14}C) and stable (^{13}C) isotope tracing assays in precision-cut PDX slices derived from 35 individual tumours, including nine benign, 13 localised, and 13 metastatic prostate tissues. Glucose, glutamine, and fatty acid oxidation was variably up-regulated in cancerous PDXs compared to benign PDXs, while lactate oxidation was unchanged. *De novo* lipogenesis (DNL) and storage of free fatty acids into phospholipids and triacylglycerols were increased in malignant PDXs. There was no difference in substrate utilisation between localised and metastatic PDXs and hierarchical clustering revealed marked metabolic heterogeneity across all PDXs. Mechanistically, glucose utilisation was mediated by acetyl-CoA production rather than carboxylation of pyruvate, while glutamine entered the TCA cycle through transaminase reactions before being utilised via oxidative or reductive pathways. Blocking fatty acid uptake or fatty acid oxidation with pharmacological inhibitors was sufficient to reduce cell viability in all PDX-derived organoids (PDXOs) examined, whereas blockade of DNL, or glucose or glutamine oxidation induced variable and limited therapeutic efficacy in PDXOs. These findings demonstrate that human prostate cancer, irrespective of disease stage, can effectively utilise all metabolic substrates, albeit with marked heterogeneity across tumours. We also confirm that fatty acid uptake and oxidation are targetable metabolic vulnerabilities in human prostate cancer.

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Characterisation of a novel modulator of TGF-b signalling in muscle

Hugo WG Herron-Vellacott[1], Jackson C Hawken[1], Adam Hagg[1], Ying Wang[2], Daniel J Bernard[2], Kelly L Walton[1, 3]

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

2. Department of Pharmacology and Therapeutics, University of McGill, Montreal, Quebec, Canada

3. Department of Physiology, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Several members of the transforming growth factor-b (TGF-b) family including growth and differentiation factors -8 and -11 (GDF8 and GDF11), the activins, and bone morphogenetic proteins (BMPs) play essential roles in the regulation of muscle homeostasis. We identified a novel TGF-b type III-like receptor present in skeletal muscle that binds select TGF-b ligands. Here, we examined if and how the receptor modulates TGF-b signalling. In heterologous HEK293 cells, GDF8 and GDF11-stimulated SMAD2 phosphorylation was significantly reduced (-36% and -48% respectively) upon ectopic expression of the receptor. As these ligands regulate muscle physiology, we next examined the effects of receptor over-expression in mice. An adeno-associated viral vector (AAV) expressing the receptor (AAV:receptor) or control AAV (AAV:iRFP) was administered ($1x10^9$-$5x10^9$vg) to the tibialis anterior (TA) muscles of 8-week old healthy male wild-type C57BL/6J mice. At 4-weeks post injection, TAs were harvested for histological and molecular assessment. TAs injected with AAV:receptor were on average significantly reduced in mass relative to the TAs injected with control AAV:iRFP (-8.4%, n=5, $*p<0.05$) owing to muscle fibre atrophy. Receptor over-expression suppressed the BMP-driven SMAD1/5/8 signalling pathway whilst simultaneously upregulating the SMAD2/3 pathway to trigger an atrophic response in muscle, as assessed by western blot analysis of TA muscle protein lysates. Collectively, the findings indicate that overexpression of the novel type III-like receptor in muscle may promote atrophy via modulation of TGF-b signalling. As the receptor is present in skeletal muscle, our findings suggest that it may act as physiological regulator of muscle homeostasis via interactions with select TGF-b ligands.

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How the ovarian niche maintains and promotes gametogenesis

Monica Laronda[1, 2]

1. Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA

2. Department of Pediatrics, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Alkylating chemotherapies, which are used to treat cancers and blood disorders, cause dose-dependent destruction of germ cells, and may induce fibrosis within gonadal tissues. The primary option for fertility preservation in children with ovaries is to undergo ovarian tissue cryopreservation (OTC), as eggs are not produced until after puberty. Currently, the only method to restore fertility is through autotransplantation of OTC tissue. Although ovarian tissue transplantation in these patients resulted in >140 reported live births, the rate of live birth for ovarian tissue transplantation is 20-30 % and the tissue only produces hormones for an average of 2-5 years. The low rate of successful live births and short duration of restored hormones is attributed to an immediate, increased activation of primordial follicles upon transplantation, which results in depletion (~ 80% loss) of the finite oocyte pool. The key to ovarian graft longevity lies in the balance between maintaining the bank of ovarian primordial follicles while promoting regular activation. The ovarian microenvironment is dynamic and continues to develop after birth and through puberty. It is compartmentalized into an avascular, rigid cortex that contains primordial follicles and a vascular, pliable medulla that contains growing follicles. Proteins of interest were identified after mapping the presence and relative abundance of matrisome proteins across porcine and bovine ovaries. Biochemical and physical attributes of ovarian matrisome proteins were modulated using a protein filtration technique to generate novel ovarian matrisome-derived hydrogels. These hydrogels were used to encapsulate and assay small murine follicle growth. Additionally, an assay was developed to identify the potential role of paracrine-secreted factors from human ovarian interstitial cells on follicle growth. These assays identified that a matrisome glycoprotein and interstitial cell-derived paracrine factors that are only secreted in the presence of follicles, promote follicle growth. Additional evaluation of how variations in these microenvironment properties influence quiescence versus growth are ongoing. This research will support efforts to engineer a bioprosthetic ovary that will support human folliculogenesis and increase the fertility and hormone potential of ovarian tissue transplants.

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Establishing a weight management clinic in the regional setting; a Northern Territory Perspective

Anna Wood[2, 1]

1. Menzies School of Health Research, Darwin, NT, Australia

2. Department of Endocrinology, Royal Darwin Hospital, Darwin, NT, Australia

Whilst it is well established that weight loss results in improvements in complications of obesity, there is minimal real-world data on the effectiveness of current treatments, particularly in the regional and remote context. At Royal Darwin Hospital, we established the first Weight Management Clinic in the NT designed to service both urban and remote residents of the NT. We aimed to determine whether such a service is effective and how it could be improved. A quarter of our clients are Aboriginal and Torres Strait Islander people, with 15% living remotely. This population differs from that in published efficacy trials, and therefore it is not clear that results from published studies are generalisable to our community. We determined the impact of the Weight Management Clinic on the health and wellbeing of people living with obesity attending the clinic (including efficacy, tolerability and side effects of treatments offered). We also determined patient satisfaction with the care provided through the clinic and patterns of patient attendance/non-attendance to the clinic. This has highlighted areas for improvement and resources required for the successful ongoing provision of a Weight Management Clinic.

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Understanding hepatokines secretion in NAFLD: implications for the treatment of metabolic diseases

Matthew Watt[1]

1. University of Melbourne, Clayton, VIC, Australia

Nonalcoholic fatty liver disease (NAFLD) is a common complication of obesity that encompasses a spectrum of liver disorders, including nonalcoholic fatty liver and the more progressive nonalcoholic steatohepatisis (NASH), which is histologically defined by steatosis, lobular inflammation, and hepatocyte ballooning. NAFLD is associated with insulin resistance, dyslipidemia and an increased risk of type 2 diabetes and coronary heart disease, and progression to NASH increases the risk of fibrosis, cirrhosis, hepatic decompensation, and liver-related mortality. Notably, there is a paucity of pharmacotherapies available for the treatment of NAFLD and related co-morbidities.

This presentation will outline our team's efforts to elucidate the complement of proteins secreted by the human liver in progressive NAFLD and how this information has been used to understand inter-tissue communication and identify novel pathways to improve insulin sensitivity and glycemic control. Our studies identified 3333 secreted proteins from human liver, of which 9.8% were classically secreted and 3.2% were differentially regulated in human NASH. Hexosaminidase A and Arylsulfatase A were shown to be upregulated in NASH and unexpectedly, these proteins were shown in cell culture and pre-clinical mouse studies to enhance glycemic control via liver to skeletal muscle communication and induction of distinct signalling pathways. In addition, proteins contained within small extracellular vesicles were shown to induce positive effects on blood glucose control by signaling to skeletal muscle to enhance glucose effectiveness and the pancreas to increase glucose-stimulated insulin secretion. Together, these results identify liver secreted proteins as regulators of skeletal muscle metabolism and harnessing this signalling may unlock new therapeutic options for impaired glycemic control.

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Diabetes and glycogen: how diabetes affects the quantity and quality of our "glucose batteries"

Mitchell A Sullivan[1], **Ellen Tejo**[1], **Xinle Tan**[2], **Bin Deng**[3], **Ziyi Wang**[4], **Benjamin Schulz**[2], **Zhenxia Hu**[5], **Jospehine Forbes**[1], **Robert Gilbert**[4]

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

2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

3. Department of Pharmacy, Union Hospital, Tongji Medical College,, Huazhong University of Science and Technology (HUST), Wuhan, China

4. Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia

5. Department of Pharmacy, Renmin Hospital of Wuhan University, Wuhan, China

Blood glucose control is a fundamental necessity for sustaining human life. Disruption of this intricate system, for example in diabetes which affects over 537 million individuals worldwide, can result in severe complications and increased mortality. A pivotal aspect of blood glucose regulation revolves around the storage of glucose in highly branched polymers known as glycogen, our "glucose batteries". The indispensability of healthy glycogen is underscored by its ubiquitous presence in organisms ranging from bacteria to mammals, as well as its presence across a wide range of tissues and cell types in humans.

In most tissues, an individual glycogen particle, termed β particle, will reach sizes of ~50,000 glucose molecules (~30 nm in diameter). In the liver, these β particles, via a currently unknown mechanism, form agglomerates known as α particles (~100-200 nm in diameter).

The following insights into the interplay between glycogen and diabetes have emerged: 1) Liver glycogen α particles in diabetes are molecularly fragile and contain longer chain-lengths, potentially contributing to a failure in blood glucose homeostasis; 2) abnormally large levels of glycogen accumulates in the kidneys of individuals with diabetes, the consequence of which remains unknown. The pathological potential of abnormal aggregations of glycogen is made evidence by several severe and often fatal glycogen storage disease, for example Lafora Disease and Andersen's Disease.

The discoveries regarding diabetes and glycogen structure/metabolism have sparked a number of ongoing investigations, including: 1) A search to discover what binds the smaller β particles together, to form large α particles in the liver; 2) investigations on whether common anti-diabetic drugs affect glycogen levels and structure; and 3) concerted efforts to determine the effect of abnormal kidney glycogen on kidney function and blood glucose homeostasis in preclinical models of diabetes.

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Navigating the Complex Landscape of Type 2 Diabetes Care: Leveraging Digital Health Solutions for Improved Management

Anish Menon[1]

1. Princess Alexandra Hospital, Metro South Health, Brisbane, QLD, Australia

The challenges in Type 2 diabetes care span a spectrum of factors, including patient education, self-management, access to care, and the need for continuous monitoring. Patients often grapple with understanding their condition, adhering to prescribed treatment plans, and making sustainable lifestyle changes. Healthcare providers, on the other hand, contend with resource limitations and the overwhelming demand for diabetes care services. Furthermore, the COVID-19 pandemic has exacerbated these challenges by disrupting traditional healthcare delivery methods.

Digital health solutions, such as mobile applications, wearable devices, telemedicine platforms, and data analytics tools, have emerged as transformative tools to revolutionize Type 2 diabetes care. These technologies empower patients by facilitating self-care behaviours through real-time information, self-monitoring, and remote consultations. They also support healthcare providers in delivering personalized care and optimizing treatment plans through data-driven insights.

This presentation will discuss specific digital health interventions, their potential benefits, and challenges associated with their adoption. It highlights the importance of patient engagement, data privacy, and healthcare system integration. Ultimately, embracing digital health solutions offers a promising pathway to enhance the quality of care, improve patient outcomes, and mitigate the burden of Type 2 diabetes on individuals and healthcare systems.

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Insulinoma localisation with PET/CT

Emma Boehm[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

PET/CT imaging of insulinoma is a case study in precision diagnostics. This talk will describe the clinical utility of PET imaging for insulinoma localisation in both sporadic and syndromic disease (e.g. MEN1) and how molecular imaging phenotype reflects the underlying disease biology. I will also discuss how PET imaging phenotype can be used to stratify patients as being suitable for Peptide Receptor Radionuclide Therapy for both hormone and oncologic control in patients with advanced disease.

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Re-differentiation therapy for RAI refractory thyroid cancer: the I-FIRST Trial

David Pattison[1]

1. Royal Brisbane & Women's Hospital, Herston, QLD, Australia

Radioactive iodine (RAI) refractory thyroid cancer has a poor prognosis. Research to re-differentiate thyroid cancer to restore iodine avidity is one approach being pursued to improve clinical outcomes. This presentation will explore the current knowledge in this field including the I-FIRST trial, an MRFF funded prospective multi-centre study aiming to demonstrate restoration of RAI uptake to facilitate effective I-131 treatment. The trial is actively recruiting with a rational treatment approach using short-term mutation directed tyrosine kinase inhibitor therapy (MEK-inhibitor if *NRAS* mutant or MEK/BRAF inhibitor if *BRAF-V600E* mutant), thyroxine withdrawal TSH stimulation and dosimetric I-124 PET to guide the suitability for RAI therapy.

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Emerging PET/CT imaging in primary aldosteronism – our experience with Ga68-Pentixafor PET/CT

Jimmy Shen[1]

1. Monash Health and Hudson Institute of Medical Research, Clayton, VIC, Australia

Primary aldosteronism (PA) is common and accounts for at least 10% of the hypertensive population. Once PA is confirmed, differentiating between unilateral and bilateral disease is essential to guide therapeutic management, either with unilateral adrenalectomy for aldosterone producing adenoma (APA) or mineralocorticoid receptor (MR) antagonist for bilateral PA. The process of PA subtyping relies on adrenal vein sampling (AVS) which remains the gold standard to determine lateralization. However, AVS is operator dependent, labour intensive, expensive and invasive, also posing the risk of a failed or inconclusive study. Due to the high incidence of non-functional adrenal adenomas, CT imaging has not been shown to accurately localize the source of aldosterone excess, therefore it cannot be recommended as a tool for lateralization. With the rising cases of PA detected through screening, there is an increasing need for an accurate, non-invasive and cost-effective diagnostic modality to determine PA subtype.

Adrenal PET imaging, which employs a tracer (i.e., 11C-metomidate) directed at 11 beta-hydroxylase (CYP11B) in the adrenal glands have shown good accuracy and concordance with AVS results in detecting APA. However, a wider utility of this compound is limited by the short half-life of the radionuclide isotope, high cost of production and the lack of specificity for aldosterone synthase. More recently, a highly selective PET tracer for aldosterone synthase has been developed with a clinical trial currently underway. Another PET tracer directed at the chemokine receptor CXCR4 using Ga68-Pentixafor PET/CT has shown much promise in several recent clinical studies to detect APA in patients with PA. This symposium will share our experience with Ga68-Pentixafor PET/CT scan in our current prospective study of PA patients undergoing AVS.

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Peptide Receptor Radionuclide Therapy (PRRT): Beta particle therapy and beyond

James McNeil[1, 2]

1. Nuclear Medicine PET & Bone Densitometry , Royal Adelaide Hospital, SA, Australia

2. Faculty of Health and Medical Sciences, The University of Adelaide, SA, Australia

Neuroendocrine tumours (NETs) are a heterogeneous group of uncommon hormonally active neoplasms that express somatostatin receptors. Peptide receptor radionuclide therapy (PRRT) has been successfully used to treat gastroenteropancreatic neuroendocrine tumours (GEPNET) and other somatostatin receptor expressing tumours for almost 40 years. The first patient treated with PRRT in Australia was in 1996 with ^{111}In-octreotide at the Peter MacCallum Cancer Centre [1].

The mainstay of initial therapy was with Auger electron producing Indium-111 (^{111}In), however it was quickly replaced with the beta particle emitters: initially the long range Yttrium-90 (^{90}Y) and followed by the short range ^{177}Lu-DOTA-Octreotate [1]. The short range beta particle production of Lutitium-177 (^{177}Lu) has made it favourable and now the mainstay of GEPNET treatment for metastatic or unresectable disease [2, 3]. The benefits for an individual patient, including progression free survival and quality of life, are weighed against possible complications of nephrotoxicity and bone marrow toxicity [4].

Targeted alpha therapy is currently an investigational therapy proposed to supersede beta therapy with an even shorter range to avoid collateral non-tumour tissue irradiation. In addition, the higher linear energy transfer (LET) of an alpha is more likely to result in cell death [5]. The following isotopes are under investigation: Actinium-225 (^{225}Ac), Lead-212 (^{212}Pb) and Bismuth-213 (^{213}Bi) [6].

The following presentation will discuss the history of PRRT followed by a review of current and proposed new therapies including alpha therapy, dosimetry following therapy.

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New Insights from Old Mammals: Evolution and Organisation of Mammalian Sex Chromosomes

Frank Grutzner[1]

1. University of Adelaide, Adelaide, SA, Australia

The iconic Australian egg-laying mammals have revolutionised our understanding of the evolution of mammalian sex chromosomes. Non-homology and differentiation of sex chromosomes in monotremes and therian mammals revealed the independent evolution of those sex chromosomes approximately 180 million years ago. The parallel evolution of two sex chromosome systems in mammals provides a unique opportunity to investigate fundamental aspects of sex chromosomes differentiation and regulation, including dosage compensation and meiotic sex chromosome inactivation. In addition, analysis of the monotreme autosomes that share homology to the therian X chromosome could allow insight into sex chromosomes in early mammals. Here I will discuss our research into the monotreme sex chromosome complex and share recent work that show sex chromosome like characteristics of the autosomal homolog to the therian X in platypus, which may provide a first glimpse into the sex chromosome system in early mammals.

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Mammalian life-extension by female contraception and male castration

Michael Garratt[1]

1. University of Otago, Dunedin, New Zealand

Reproduction is expected to constrain survival and cause sex-differences in ageing. Sterilization inhibits reproduction, but predictions differ for how this should influence survival depending on sex, how sex-hormones are affected and species life-history. Using data from zoo housed mammals, I will show that sterilization is associated with a consistent increase in lifespan, an effect observable in both sexes and many mammalian orders including primates, carnivores, ungulates and bats. Further meta-analysis of published data show that increases in survival metrics with sterilization are observable in laboratory and wild environments in vertebrates, after male castration and a variety of different female contraceptive methods. However, these effects occur irrespective of sex-differences in lifespan, suggesting costs of reproduction do not explain sex-specific ageing. These results highlight that the drive to reproduce is a consistent force that constrains survival across vertebrates, altering biological ageing processes that limit lifespan regardless of the environment in which an animal resides.

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Environmental influences on sex and the evolution of sex-determining mechanisms

Lisa Schwanz[1]

1. University of New South Wales, Randwick, NSW, Australia

Many organisms commit to having either male or female gonads. This commitment depends on genetics, but is often sensitive to environmental conditions. In this talk, I will discuss how environmental lability in sexual development can drive evolutionary transitions in sex-determining mechanisms from genotypic to environmental sex determination. Thus, a mechanistic understanding of sex development can inform our understanding of the evolution of sex determining mechanisms. In turn, applying an evolutionary perspective to sex development can inform how developmental mechanisms operate.

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The curious case of avian sex determination (remote presentation)

Craig Smith[1], **Andrew Major**[1], **Kirsten Morris**[2], **Mark Ford**[2], **Tim Doran**[2]

1. Monash University, Clayton, VIC, Australia

2. ACDP, CSIRO, Geelong, Victoria, Australia

A long-held paradigm of reproductive biology is that sexual development is a two-step process; chromosomal sex directs the embryonic gonads to form ovaries or testes, which then secrete sex hormones to feminise or masculinise the rest of the body. Yet, the concept has increasingly been challenged by curious data that do not fit the model. Evidence from gynandromorphic birds supports direct genetic effects upon sexual differentiation in addition to sex steroid hormones. Gynandromorphs are bilateral sex chimeras, male on one side of the body and female on the other. We examined a naturally occurring gynandromorphic chicken that was chromosomally ZZ on one side of the body and largely ZZ on the other, but with 10% ZW cells, based on karyotyping. The gonads of this bird at sexual maturity were ambiguous. The right gonad was a testis, with SOX9+ Sertoli cells, DMRT1+ germ cells and active spermatogenesis. The left gonad was atypical . Histologically, it was primarily testicular, but with a small number of peripheral follicles. The bird had very low levels of serum 17b-estradiol and high levels of testosterone. Yet the bird was female on one side of the body. Despite the low percentage of ZW cells on that side, the bird was asymmetrically female. This indicates that sexually dimorphic structures such as the wattle, spur and feathering must be at least partly independent of sex steroid effects. Given the lack of chromosome-wide dosage compensation in birds, various sexually dimorphic features may arise due to Z gene dosage differences between the sexes. In the gonads, the Z-linked gene, *DMRT1*, directs testis formation. Monoallelic *DMRT1*deletion causees ovary formation yet the somatic tissues remain “male”. This again supports the notion of cell autonomous sex in birds. Our lab is currently conducting studies to determine how *DMRT1* induces testis formation in the chicken.

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Consumption of nitrate in drinking water during pregnancy and lactation disrupts rat offspring growth and adult behaviour

Leaf Kardol[1], **Avalon Grey**[1], **Christine Jeffries-Stokes**[1], **Annette Stokes**[1], **Sarah Bourke**[1], **Caitlin Wyrwoll**[1]

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

Nitrate is a leading drinking water contaminant in Australia. Recent epidemiological studies have linked nitrate in drinking water to low birth weight, preterm birth, and increased congenital anomalies. However, there is a lack of evidence to support these associations. To address this, we established a rat model of pregnancy whereby female Wistar rats (n=6-10/group) had 0 (control), 50 or 100mg/L (Australian safety threshold for adult consumption) of sodium nitrate added to their drinking water four weeks before timed mating, until weaning. One cohort was investigated at 21 days of gestation (E21) to assess fetal and placental development. Birth outcomes of a second cohort were characterised, followed by offspring neurodevelopmental and behavioural testing. At E21, male offspring in the 50mg/L group were 5% lighter (P=0.0008) with 7% heavier placentas (P=0.016) than control. In contrast, male weights were not significantly altered in the 100mg/L group. Female placental weights showed more variability in the 100mg/L group than the control and 50mg/L groups (P=0.0047). In cohort 2, timing of birth was unaffected, but a third of births in the 100mg/L group were complicated due to substantially overgrown fetuses. At 7 and 14 days of age, female offspring were 10% and 3% heavier respectively (P=0.0021 and P=0.0227) and male offspring weight was more variable in the 50 and 100mg/L groups compared to the control (P=0.0224 and P=0.0006), although these differences disappeared by 21 days of age. Exposure to 100mg/L nitrate impaired neurodevelopment at 14 days of age, and in adulthood, increased anxiety-like behaviour in male offspring, as assessed by elevated plus maze. Maternal and offspring metabolic parameters, such as blood glucose, were marginally affected. These outcomes highlight that nitrate contamination in the drinking water of pregnant people has implications for offspring growth and neuropsychiatric outcomes in later life.

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Is maternal folate excess following Australian folic acid food fortification doing harm in pregnancy?

Tanja Jankovic-Karasoulos[1], **Melanie D Smith**[1], **Shalem Leemaqz**[1], **Jessica Williamson**[1], **Dylan McCullough**[1], **Anya L Arthurs**[1], **Gustaaf A Dekker**[3, 2], **Claire T Roberts**[1]

1. Flinders University, Bedford Park, ACT, Australia

2. Lyell McEwin Hospital, Elizabeth Vale, Adelaide, South Australia, Australia

3. The University of Adelaide, Adelaide, SA, Australia

Gestational diabetes mellitus (GDM) incidence in Australia increased from 5.6 to 19.3% over a decade[1], coinciding with the 2009 folic acid (FA) food fortification mandate. FA has been associated with increased insulin resistance and GDM[2]. Given that most pregnant women in countries with FA food fortification are exceeding the recommended daily FA intake[3], we aimed to establish how maternal folate status, placental endocrine function, maternal insulin resistance and GDM incidence have changed post-FA food fortification.

We analysed data from pregnancy cohorts recruited prior to (SCOPE; 2005-2008; N=1164) and post (STOP; 2015-2018; N=1300) FA food fortification. Circulating folate, red cell folate (RCF), insulin, glucose, prolactin (PRL), human placental lactogen (hPL) and placental growth hormone (GH2) were measured in SCOPE and STOP early pregnancy maternal blood.

GDM incidence increased from 5% (SCOPE) to 15.2% (STOP), in line with the national GDM rise, and was associated with increased maternal folate status and altered levels of placental hormones that regulate maternal glucose homeostasis. Compared to women pre-FA food fortification, women post-FA food fortification had higher serum folate (↑18%, p<0.0001), RCF (↑259%, p<0.0001), hPL (↑29%, p<0.0001) and GH2 (↑12%, p=0.01), but lower insulin resistance (↓24%, p=0.003). Although women post-FA food fortification had overall lower insulin resistance, those with maternal folate excess (concentrations exceeding the RCPA reference range) were 23% more likely to be insulin resistant (p=0.002) and had 11% higher PRL (hormone that promotes insulin secretion, p=0.03). Furthermore, obese women post-FA food fortification had 20% higher GH2 (hormone that promotes maternal insulin resistance; p=0.0006) compared to obese women pre-FA food fortification.

Whilst adequate maternal folate may protect against insulin resistance, excess maternal folate may perturb secretion of placental hormones which regulate maternal insulin resistance and glucose homeostasis during pregnancy, to increase insulin resistance and risk of GDM, particularly in the setting of maternal obesity.

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Exploring the therapeutic potential of proton pump inhibitor, pantoprazole, for preterm birth prevention in preclinical models

Bridget M Arman[2, 1], Natalie K Binder[2, 1], Natasha de Alwis[2, 1], Anjali Garg[2, 1], Tu'uhevaha J Kaitu'u-Lino[3, 2], Natalie J Hannan[2, 1]

1. Department of Obstetrics and Gynaecology, University of Melbourne, Therapeutics Discovery and Vascular Function in Pregnancy Group, Heidelberg, Victoria, Australia

2. Mercy Perinatal, Heidelberg, Victoria, Australia

3. Department of Obstetrics and Gynaecology, University of Melbourne, Heidelberg, Victoria, Australia

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Prolonged seminal fluid exposure before pregnancy promotes embryo survival and placental function in mice

Laura Wilkinson[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

An increasing body of research indicates that seminal fluid serves as more than simply sperm support media. Seminal fluid exposure at the time of intercourse enhances fertility and placental health. We tested the hypothesis that extended exposure to paternal seminal fluid prior to pregnancy will provide additional fertility benefits, due to the female reproductive tract being primed to male antigens in seminal fluid for a prolonged period of time. Females were primed by housing with vasectomised males for two months prior to pregnancy. Females previously housed with vasectomised males had more embryos in late pregnancy than females that had never mated, and altered placental morphology, with a significant decrease in the size of the labyrinthine zone. We have further tested whether the benefits of earlier seminal fluid exposure requires females to become pregnant with embryos from males that match the genetic and immune profile of the earlier mate. It has been theorised that the maternal immune system can be primed to recognise a male's major histocompatibility complex (MHC) antigens via ongoing seminal fluid contact, and thereby provide a better fetal/maternal (placental) relationship when the embryo presents the same antigen. While our initial study used the same male MHC for seminal fluid priming and for pregnancy, we have new data to present on the effects of switching to a novel MHC male for pregnancy, and how this impacts subsequent litter size and offspring development.

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The effects of metformin treatment for diabetes during pregnancy on placental mitochondrial function and fetal outcomes

Dayna A Zimmerman[1], Olivia J Holland[2], James SM Cuffe[1]

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

2. School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia

Metformin use during pregnancy for treatment of diabetes mellitus (DM) remains controversial despite its popularity outside of pregnancy. DM can induce placental mitochondrial dysfunction and excessive ROS production and inflammation (1). Given that metformin reduces the placental dysfunction implicated in preeclampsia (2), it is possible that by restoring mitochondrial function, metformin can improve placental function in DM and therefore fetal outcomes. Therefore, this study aimed to investigate the impact of maternal metformin treatment on placental mitochondrial function, in mice with and without diabetes in pregnancy.

Four-week-old C57BL/6J female mice were placed on either a control diet or a high fat diet (60% calories from fat) for five weeks to induce glucose intolerance. Mice then received either a daily dose of metformin (300mg/kg/day) or sterile water via oral gavage for two weeks prior to mating and throughout pregnant. Mice were culled on embryonic day 18.5 (E18.5) and placental mitochondrial respiration was measured using an Oxygraph-2k respirometer (Oroboros Instruments, Austria).

Maternal HFD reduced fetal weight by 8.5%, though brain weight was maintained. Metformin was unable to restore these changes. Metformin did increase in placental weight, however this also occurred in the LFD fed mice. Oxidative phosphorylation capacity through complex I (CI) was significantly decreased by both diet and metformin. LEAK, OXPHOS CI + CII, CIV and ETS were not affected by either treatment.

HFD lowered placental CI, which has been previously demonstrated in skeletal muscle to be linked to ROS production (3). Metformin has been shown to directly inhibit complex I activity (4), which we found to also occur in the placenta. It is possible that with reduced mitochondrial respiration, placental glucose will accumulate. Future work will investigate if the increased placental weight is driven by glycogen deposition. This study demonstrated that HFD and metformin have independent effects on placental physiology.

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Maternal circulating antioxidant function and microRNAs may influence placental gene expression and gestational diabetes pathophysiology

Hasini Rathnayake[1], Albert Liang[1], Richard Lai[1], Kshiti Patel[1], Aaron Lorimer[1], Mariama Kamara[1], Kerry Richard[2], Fabricio Da Silva Costa[3, 4], Olivia Holland[1]

1. School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, Queensland, Australia

2. Conjoint Internal Medicine Laboratory, Pathology Queensland; Department of Endocrinology and Diabetes, Royal Brisbane and Women's Hospital; School of Biomedical Sciences, Queensland University of Technology; School of Medicine, University of Queensland., Queensland, Australia

3. Maternal Fetal Medicine Unit, Gold Coast University Hospital, Gold Coast, Queensland, Australia

4. School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland, Australia

Gestational diabetes (GDM) may be controlled with diet, but requires medication if severe; however, what leads to severe GDM in some women but not others is unclear. To investigate placental involvement in GDM, we determined if the placental stress-response is altered in GDM compared to healthy pregnancies and is distinct between mild (diet treated) and severe (medicated) GDM pregnancies. To investigate if related changes can be detected during pregnancy, we profiled maternal circulating antioxidant capacity and microRNA expression.

Placental tissue (≥37 week's gestation) and maternal plasma (26–28 week's gestation) was collected from control (uncomplicated), GDM diet treated (GDMD), and GDM medicated (GDMM) pregnancies (n≥8 per group). Groups were matched for delivery mode, maternal age, maternal BMI, and infant weight. Placental expression of 239 genes was measured by qPCR. Antioxidant capacity was measured by activity assay. Maternal circulating expression of 800 microRNAs was measured using the nCounter system.

Twenty placental genes had potentially biologically meaningful changes, eight genes down-regulated and four genes up-regulated in GDM compared to control. Eight genes were up-regulated in GDMD but down-regulated in GDMM. Placental antioxidant capacity was decreased in male GDMD and female GDMM, and increased in female GDMD, relative to controls. Circulating antioxidant capacity was reduced in GDMM compared to GDMD and controls. Circulating expression of 16 microRNAs was different between GDM and controls. Nine microRNAs were upregulated in GDMD, and five in GDMM, compared to controls.

The placenta may not be adapting successfully in severe GDM, with lower expression of genes involved in antioxidants, metabolism, and drug processing in GDMM placentae, suggesting a dampened response to stimuli. This gene level response may relate to lowered antioxidant capacity in GDM in both placental and maternal compartments. Circulating antioxidant capacity and microRNA profiles may be effective in distinguishing which women are likely to require medication.

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Maternal stress, anxiety, and depression are all associated with increased placental glucocorticoid receptor D1 isoform expression in cohorts from Queensland and South Australia.

Sreeparna SB Bhaumik[1, 2], Jack JL Lockett[1, 3], Vicki VC Clifton[1, 2]

1. University of Queensland, Woolloongabba, QLD, Australia

2. Mater Research Institute, Brisbane

3. Princess Alexandra Hospital, Metro South Health, Brisbane

Mental health illness affects 15% of pregnancies. It is associated with poor perinatal and fetal outcomes which may be mediated by heightened hypothalamic-pituitary-adrenal (HPA) axis activity and elevated circulating glucocorticoids. The placenta contains thirteen glucocorticoid receptor (GR) protein isoforms that vary with maternal stress or glucocorticoid exposure. This study investigated whether mental health disorders during pregnancy alter the placental GR isoform profile. We hypothesized that stress, anxiety, and depression (SAD) may be exacerbated due to an altered placental response to glucocorticoids mediated by differential expression of placental GR isoforms that activate inflammatory pathways.

Using validated questionnaires for assessment of SAD, pregnant women from the Queensland Family Cohort (QFC) and the South Australia Cohort (SAC) were divided into controls (n=33, QFC; n=58, SAC) and a SAD group (n=33, QFC; n=71, SAC). Placental GR isoform expression was measured using Western blot. Glucocorticoid signaling genes were assessed using qPCR. Levels were compared between SAD and control groups with statistical significance considered at $p<0.05$.

GR isoforms identified in the placenta included GRα-A, GR-β, GR-C1-3, GR-P, GR-A, and GRα-D1-3. Nuclear GRα-D1 was significantly higher in women with SAD compared to controls in both cohorts (p=0.023, QFC; p=0.022, SAC). In the QFC, this increase was exclusive to the placentae of female fetuses (p=0.002), while SAC showed no sex differences. In the QFC, pro-inflammatory markers IL-6 (p=0.0006) and TNF-α (p=0.002) were elevated in women with SAD, with IL-6 specifically higher in female placentas (p=0.01). The anti-inflammatory IL-10 gene was reduced in SAD women (p=0.005), seen only in female placentas (p=0.03). Glucocorticoid-related gene expression in the SAC remains to be investigated.

In conclusion, mental health disorders during pregnancy are associated with a pro-inflammatory environment along with an upregulation of GRaD1, suggesting a role of this isoform in exacerbating materno-placental inflammation.

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Purinergic signalling in the human placenta and the functional role of purinergic receptors P1A1, P1A2A, and P2Y6 in placental development

Rose C Freemantle[1], Natalie K Binder[1, 2], Natasha de Alwis[1, 2], Tu'uhevaha J Kaitu'u-Lino[2, 3], Stephen Tong[2, 3], Lisa Hui[3], Natalie J Hannan[1, 2]

1. Dept. Obstetrics and Gynaecology, University of Melbourne, Therapeutic Discovery and Vascular Function in Pregnancy Group , Melbourne, Victoria, Australia

2. Mercy Perinatal, Mercy Hospital for Women, Melbourne, Victoria, Australia

3. Dept. Obstetrics and Gynaecology, University of Melbourne, Translational Obstetrics Group, Melbourne, Victoria, Australia

Publish consent withheld

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New perspectives on testosterone and men's health

Bu Yeap[1]

1. School of Medicine, University of Western Australia, Perth, WA, Australia

Testosterone concentrations are lower in older compared to younger men, representing both a biomarker of and a contributor to poorer health. Sociodemographic, lifestyle, behavioural and medical factors are associated with differences in testosterone concentrations. Recent analyses from UK Biobank and the Androgens In Men Study collaboration indicate that healthy men aged 40-69 years have stable testosterone concentrations over four years of follow-up, but beyond the age of 70 years, declining testosterone concentrations are accompanied by increasing luteinising hormone, indicative of Leydig cell impairment. In UK Biobank men, lower testosterone concentrations were independently associated with mortality, and with incident dementia, but not with incident cardiovascular events. The association of lower testosterone with higher mortality risk in UK Biobank and in the Health In Men Study was non-linear, with no survival advantage for men with higher testosterone concentrations. These results suggest that an optimal testosterone concentration, or range of concentrations, exists, which predicts male health and longevity. In large randomised controlled trials (T Trials, T4DM and TRAVERSE) testosterone treatment improved sexual function, anaemia and bone density, and reduced the risk of type 2 diabetes, with no increase in cardiovascular adverse events, but an increase in fracture risk. Further research is needed to clarify the extent to which non-pharmacological interventions might help men transitioning from middle to older age attain both optimal circulating testosterone concentrations and better health.

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Cystatin 6 (CST6) expression is increased in preeclamptic pregnancies

Stefan M Botha[2, 1, 3, 4, 5], Ping Cannon[2, 1], Anna Nguyen[2, 1], Tuong-Vi Nguyen[2, 1], Natasha Pritchard[2], Catherine A Cluver[2, 6, 1], Lina Bergman[6, 7, 8], Ralf Dechend[3, 4, 5, 9, 10], Olivia Nonn[3, 4, 5, 9, 10, 11], Stephen Tong[2, 1], Tu'uhevaha J Kaitu'u-Lino[2, 1]

1. Translational Obstetrics Group, The Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne, Melbourne, Victoria 3084, Australia

2. Mercy Perinatal, Mercy Hospital for Women, Melbourne, Victoria 3084, Australia

3. Experimental and Clinical Research Center, a cooperation between the Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association and the Charité-Universitätsmedizin Berlin, Berlin, Germany

4. Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

5. Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

6. Department of Obstetrics and Gynaecology, Stellenbosch University, Cape Town 7505, South Africa

7. Department of Women's and Children's Health, Uppsala University, Uppsala 751 85, Sweden

8. Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg 405 30, Sweden

9. DZHK (German Center for Cardiovascular Research), partner site Berlin, Berlin, Germany

10. Department of Cardiology and Nephrology, HELIOS Clinic, Berlin, Germany

11. Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria

Preeclampsia results from placental insufficiency, causing maternal endothelial dysfunction and multi-organ damage. In the search for preeclampsia biomarkers, our *in silico* analysis identified Cystatin 6 (CST6), a cysteine protease inhibitor, as located on the placental surface where it might be released from into maternal circulation. Therefore, we aimed to characterise CST6 in preeclampsia and assess its biomarker potential.

CST6 mRNA expression was significantly increased (~6.07-fold) in placentas from patients with early onset preeclampsia (<34 weeks' gestation, n=78) relative to gestation matched controls (n=30, p<0.0001). Plasma CST6 significantly increased (~23.45%) preceding diagnosis of term preeclampsia (36 weeks' gestation, n=23) compared to controls delivering without preeclampsia (n=181, p=0.0084).

To determine the placental cell type producing CST6, human trophoblast stem cells (hTSCs) were differentiated into syncytiotrophoblast or extravillous trophoblast (EVT). Differentiation was confirmed by significantly reduced cytotrophoblast markers *TEAD4* (p=0.0018) and *CDH2* (p=0.0096) and significantly increased syncytiotrophoblast marker, *SDC1* (p=0.0013), and EVT marker, *HLA-G* (p=0.0005). *CST6* significantly increased across 96 hours, in syncytiotrophoblast (~2.97-fold, p=0.0066) and EVT differentiation (~1.52-fold, p=0.0618 at 96 hours), confirming highest expression in syncytiotrophoblast. As preeclampsia is associated with placental hypoxia and inflammation, we next measured *CST6* in syncytiotrophoblast exposed to these conditions. *CST6* significantly increased in hypoxia exposed syncytiotrophoblast (1% O_2 vs 8% O_2) (~2-fold, p=0.0079). No significant change was observed in syncytiotrophoblast treated with inflammatory cytokines TNF-α or IL-6.

Another potential source of circulating CST6 in preeclampsia is dysfunctional endothelium. We induced endothelial dysfunction by treating human umbilical vein endothelial cells with TNF-α. This induced markers of endothelial dysfunction, *ICAM-1* (p=0.0250), *VCAM-1* (p=0.0250) and *ET-1* (p=0.0464). Interestingly, this caused a significant reduction in *CST6* (~0.5-fold, p=0.0036).

We provide the first data characterising CST6 in preeclampsia. We show it is increased in the placenta and circulation and suggest elevated circulating levels may be induced by placental hypoxia.

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Myosin Heavy Chain 10 downregulation within endometrial epithelial cells contributes to dysregulation in adhesion

Michaela Sacco[1, 2], Wei Zhou[1, 2], Poppy Downing[1, 2], Wan Tinn Teh[2, 3, 4, 5], Evdokia Dimitriadis[1, 2]

1. Gynaecology Research Centre, Royal Women's Hospital, Melbourne, Victoria, Australia

2. Obstetrics and Gynaecology, University of Melbourne, Melbourne, Victoria, Australia

3. Royal Women's Hospital, Melbourne, Victoria, Australia

4. Melbourne IVF, Melbourne, Victoria, Australia

5. Epworth HealthCare, Melbourne, Victoria, Australia

Endometrial receptivity is defined as the functional, morphological, and molecular changes of the endometrium during the window of implantation that facilitate blastocyst implantation. Luminal epithelial cell adhesive capacity is crucial for successful blastocyst attachment and implantation. Poor endometrial receptivity contributes to weak adhesion, implantation failure, and infertility. Despite this effect upon fertility, there is a lack of knowledge required to develop diagnoses and treatments. Our recent proteomics screen using an endometrial epithelial organoid model has identified a correlation between Myosin Heavy Chain 10 (MYH10) downregulation and infertility. MYH10 is a conventional non-muscle myosin and actin-dependent motor protein. Via actin cytoskeleton, MYH10 regulates cell polarity, adhesion, and migration; factors which may contribute to proper endometrial function. This study aimed to understand the role of MYH10 in endometrial receptivity. Investigation of MYH10 via immunohistochemistry revealed expression within glandular epithelium, luminal epithelium, and stroma. Fertile endometrium exhibited significantly higher MYH10 expression within glandular and luminal epithelial cells during the mid-secretory phase compared to the proliferative phase (n=11, P<0.05). Further comparison between fertile and primary infertile endometrium during the mid-secretory phase revealed a significant decrease in MYH10 within infertile luminal epithelium (n=11, P<0.05). MYH10 knockdown in Ishikawa cells (receptive endometrial epithelial cell line) significantly reduced adhesive capacity as shown via a trophoblast spheroid adhesion assay (n=4, P<0.05). xCELLigence real-time monitoring demonstrated decreased

cellular proliferation in response to MYH10 knockdown compared to controls (n=10, P<0.0001). To elucidate the mechanistic pathways by which MYH10 regulates endometrial receptivity, qPCR assessed expression of endometrial receptivity and actin cytoskeleton associated genes. Results demonstrated significantly differential expression of *PGR* and *PDLIM2*, respectively, compared to controls (n=8, P<0.05). These results convey the potential importance of MYH10 in regulation of endometrial epithelial cell adhesion, partly via regulation of actin cytoskeleton genes, therefore implying a role in implantation.

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Assessment of the impact of acute *in vivo* PFAS exposure on male reproductive health

Leah Calvert[1, 2, 3], Jacinta H Martin[1, 2, 3], Amanda L Anderson[1, 2, 3], Ilana R Berntstein[1, 2], Simone J Stanger[1, 2, 3], Geoffry N De Iuliis[1, 2, 3], Matthew D Dun[1, 4], Shaun D Roman[5], Mark P Green[6], Brett Nixon[1, 2, 3]

1. University of Newcastle, Callaghan, NSW, Australia

2. Infertility and Reproduction Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

3. Hunter Medical Research Institute, Newcastle, NSW, Australia

4. Cancer Signalling Research Group, School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW , Australia

5. NSW Health Pathology, NSW Health, Newcastle, NSW, Australia

6. School of BioSciences, Faculty of Science, University of Melbourne, Melbourne, Victoria, Australia

Due to their widespread use in consumer products and industrial applications and their inherent chemical stability, perfluoroalkyl and polyfluoroalkyl substances (PFAS) are now recognised as persistent environmental contaminants. Recent epidemiological data indicates that PFAS pose a risk to human health, prompting us to explore their effects on male reproductive function via a chronic *in vivo* exposure model wherein adult male mice received a cocktail of nine PFAS for 12-weeks via their drinking water at doses that mimic those found in the Australian environment. Thereafter, we assessed effects on overall body and reproductive organ weights, sperm functional parameters and *in vitro* fertilization. Whilst whole body, testis and epididymal weights were not affected by PFAS-treatment, both seminal vesicle ($p<0.05$) and seminal fluid weights ($p<0.01$) were significantly reduced in PFAS-treated males. Additionally, daily sperm production was also significantly reduced among PFAS treated mice ($p<0.05$). Interrogation of the impact of PFAS on sperm function revealed a significant reduction in the ability of sperm to bind the zona pellucida surrounding ovulated oocytes ($p<0.05$). More detailed assessment of the functional competence of PFAS exposed sperm in terms of their ability to support *in vivo* fertilization and embryonic development following natural mating demonstrated a significant reduction in the size of litters sired from PFAS treated males ($p<0.01$, average 10.25 vs. 12.11 pups). Notably, the pups born from PFAS treated fathers were also significantly heavier than then untreated pups at day of birth ($p<0.01$). At 7-days of age, pups sired from PFAS treated males also displayed significantly larger anogenital distances ($p<0.01$). Collectively, these studies provide new insight into the effect of PFAS on male reproductive health and have prompted us to begin to explore the mechanistic basis of such effects as well to elucidate whether they are perpetuated across generations.

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Spatial dynamics of mRNA during spermatogenesis

Jacob K Netherton[1], Rachel Ogle[1], Benjamin R Robinson[1], Mark A Baker[1]

1. The University of Newcastle, Callaghan, NSW, Australia

Mammalian spermatogenesis is a complex and highly coordinated process that drives a stem cell through meiosis and cell differentiation to form the specialised genetic cargo and shape of the spermatozoon. One of the critical features of this process is the sperm-specific packaging of DNA, that sees the normal histone organisation replaced with protamines, leading to an almost crystalline compaction of the genome and loss of transcription. To overcome this, haploid spermatids produce the necessary mRNA and hold these transcripts for up to a week prior to protein translation. While the processing and localisation of individual/total mRNA has been characterised at some stages of development, an overall picture of mRNA distribution throughout spermatogenesis has not been described.

Using RNA-FISH we have shown the localisation of mRNA during both murine and human spermatogenesis. The abundance of mRNA transcripts begins to increase in spermatocytes which subsequently form discrete foci, known as chromatoid bodies, in the late pachytene stage. Following this, the localisation of mRNA in spermatids changes dramatically during their 16 steps of development. In earlier stages, the mRNA present within nuclear speckles and colocalises in the cytoplasm with DDX4, a highly conserved RNA helicase that is critical for spermatogenesis. As the spermatid nuclei begin to elongate (steps 8-12) the cytoplasmic staining of mRNA is significantly reduced, however nuclear mRNA staining remains within the speckles. Finally, prior to the release of sperm into the seminiferous lumen, there is very little nuclear mRNA staining present.

It is likely that the tight spatial regulation of mRNA is a critical component of spermatogenesis, given that several knockout models affecting its localisation in the testis are infertile (DDX4, MIWI, PIWI). By proving a comprehensive map of the dynamics, we hope to allow easier identification of spermatogenetic disorders that disrupt normal mRNA biology.

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Characterising sperm membrane proteins and their remodelling during capacitation

Hanah M Hart[1, 2], David A Skerrett-Byrne[1, 2], Brett Nixon[1, 2], Geoffry N De Iuliis[1, 2]

1. Infertility and Reproduction Research Program, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia

2. School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW, Australia

A critical stage of sperm maturation, termed 'capacitation', occurs while spermatozoa transit the female reproductive tract, and involves a drastic remodelling of the sperm surface architecture that ultimately affords spermatozoa the ability to recognise and fertilise an egg. Intact DNA and a lack of oxidative stress damage are hallmark characteristics of spermatozoa that successfully complete this process. These cells retain the ability to organise surface receptors that permit the specialised process of binding to the zona pellucida, just prior to fertilisation. This natural gamete selection process is bypassed in higher technology assisted reproduction and remains an untapped basis for improving sperm selection in the clinic. Despite decades of research, the entities responsible for sperm-egg binding are yet to be fully elucidated, which has greatly limited the translation of this platform. Thus, we have utilised comparative proteomic profiling, *in silico* analysis and immuno-staining to identify membrane proteins involved in mouse sperm surface remodelling. Mouse spermatozoa were isolated both immediately from the epididymis (non-capacitated) and after *in vitro* capacitation (capacitated). For proteomic analysis, membranes were isolated and comparatively characterised via high-resolution LC-MS/MS using label-free quantification and *in silico* subcellular stratification. We identified 884 and 787 membrane proteins from non-capacitated and capacitated spermatozoa, respectively. In addition to well-characterised membrane proteins such as molecular chaperones, we identified 207 proteins not previously described or annotated in the sperm membrane. Of the proteins identified, 146 and 49 were localised uniquely to the cell membrane in non-capacitated and capacitated spermatozoa, respectively. Subsequent validation of membrane proteins feature the carbohydrate binding protein, malectin, an egg recognition candidate with affinity for the sugars adorning the zona pellucida. This targeted characterisation of the sperm membrane during capacitation is providing a valuable resource for advancing our understanding of sperm maturation and the rational development of new sperm selection materials for ART.

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The role of insulin-like growth factor binding protein-3 in mouse embryo implantation

Kate B Carey[1], Monique H Rayhanna[1], Michael B Morris[1], Margot L Day[1]

1. School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, New South Wales, Australia

Preimplantation embryo development *in vitro* is poorer and results in decreased implantation rates compared to those developed *in vivo*. A contributing factor to these problems *in vitro* may be the lack of maternally derived growth factors, such as insulin-like growth factor binding protein-3 (IGFBP3) (1-2). Exogenous IGFBP3 improves mouse preimplantation embryo development by increasing cell division rate between the 2-cell and 8-cell stage, blastocyst formation and hatching (3-4). IGFBP3 can act via insulin-like growth factor-independent mechanisms, such as via the sphingosine-1-phosphate (S1P) signalling pathway (5) and this may occur in mouse embryos to promote preimplantation embryo development (3-4) and implantation. In this study, embryos were exposed to IGFBP3 during preimplantation development and to IGFBP3 and/or S1P at the embryonic-maternal interface during implantation. Two measures of implantation were assessed; attachment where blastocysts were co-cultured with Ishikawa cells, a receptive human endometrial adenocarcinoma cell line for 48 h, and invasion where blastocysts were transferred to coverslips for outgrowth for 96 h. Exposure to IGFBP3 during preimplantation embryo development improved blastocyst attachment but had no effect on outgrowth. Exposure to IGFBP3 only during the implantation process reduced blastocyst outgrowth area and had no effect on attachment. Exposure to S1P during implantation had no effect on blastocyst attachment or outgrowth and did not prevent the reduced outgrowth caused by IGFBP3. In conclusion, adding IGFBP3, only during implantation, is not beneficial but it improves attachment when added to embryo culture medium during preimplantation development, and thus should be considered as a media component to improve the outcomes of assisted reproductive technologies.

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Determining the pathogenesis of endometriosis by utilising a single cell atlas of endometrial stem/progenitor cells

Harriet C Fitzgerald[1, 2], Sally Mortlock[3], Caitlin Filby[1, 2], Fiona Cousins[1, 2], Brett McKinnon[3], Elizabeth Marquez-Garcia[1, 2], Grant W Montgomery[3], Caroline Gargett[1, 2]

1. Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia

2. The Ritchie Centre, Hudson Institute of Medical Research, Clayton, VIC, Australia

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

Adult stem cells present in human endometrium; N-cadherin$^+$ (*CDH2*) epithelial progenitors and SUSD2$^+$ mesenchymal stem cells (eMSC) are shed during menstruation and refluxed into the peritoneal cavity. SSEA1$^+$ basalis epithelial cells resurface the denuded endometrium to generate the luminal epithelium. Alterations in the gene expression of these cells may enable them to

initiate endometriotic lesions. Our aim was to determine the gene expression atlas of single endometrial stem/progenitor cells and identify new markers of endometrial stem cell populations.

Human hysterectomy endometrium (n=5) was digested to single cells for 6-way FACSorting to collect stem/progenitor cell subpopulations and their differentiated progeny using N-Cadherin$^{+/-}$, SSEA1$^{+/-}$ and SUSD2$^{+/-}$. For single cell RNA sequencing (scRNA seq), the 6 cell fractions were combined, processed using 10X Genomics, libraries generated, and analysed using Cell Ranger and Seurat pipelines. Immunofluorescence on full thickness endometrium examined the co-localisation of SSEA-1, N-Cadherin and Indian Hedgehog.

scRNA seq identified 16 cell clusters; 10 of epithelial and 6 of mesenchymal origin. *CDH2*$^{+}$*SOX9*$^{+}$ cells localised to two clusters suggesting a basalis glandular epithelial population, with high expression of *IHH*. *SUSD2* expression was localised to two main clusters, representing MSC with high *MYH11* expression, transitioning to more mature stromal fibroblast clusters. Immunofluorescence identified all 4 endometrial stem/progenitor cell subpopulations and IHH^{+} basalis epithelial cells. Ligand-receptor analysis indicated key interactions between *IHH* in the basalis progenitor epithelial cells with its co-receptors *BOC* and *CDON* in the basalis stromal and smooth muscle cells respectively, reflective of a potential new stem cell niche environment in the human endometrium.

The identification of endometrial stem/progenitor cell subgroups and investigation of their gene expression from patients with endometriosis may assist our understanding of endometriosis pathogenesis for developing new treatments targeting the disease.

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RNA-seq to identify activin and TGFβ target genes in Testicular Germ Cell Tumours

Sarah C Moody[1, 2], Benedict Nathaniel[1], Ciara Conduit[3], Nathan Lawrentschuk[4, 5, 6], Ben Tran[3, 7], Kate L Loveland[1, 2]

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

3. Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

4. Department of Urology, Royal Melbourne Hospital, Melbourne, VIC, Australia

5. EJ Whitten Centre for Prostate Cancer Research, Epworth Healthcare, Melbourne, VIC, Australia

6. Department of Urology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

7. Division of Personalised Medicine, Walter and Eliza Hall Institute, Parkville, VIC, Australia

Testicular germ cell tumours (TGCTs) are the most common solid tumour affecting young men (19-44 yo). TGCTs arise when fetal germ cells fail to differentiate, forming germ cell neoplasia *in situ* (GCNIS) and transforming into seminomas or non-seminomas around puberty. As in many cancers, altered TGFβ superfamily has been implicated. To identify activin A and TGFβ superfamily target genes, fresh TGCT and adjacent tissue was obtained from two consented patient orchidectomies (non-seminoma, HTCa8; seminoma, HTCa9). Each was divided in half (A and B), then further divided for histology (4% PFA), transcript analysis (snap-frozen) at collection (T=0) and culture (1 mm^3 fragments, 48 hours, 30 µL drops (0.1% BSA/DMEM:F12 + ITS + PS) with 50ng/mL activin A, 10µM SB431542 (TGFβ/activin/Nodal inhibitor) or vehicle controls (n=3-5). By immunohistochemistry, HTCa8 tumour appeared heterogeneous (classical non-seminoma); HTCa8 adjacent contained GCNIS (OCT4+). HTCa9 tumours displayed classic homogeneous seminoma (OCT4+), with immune infiltrates (CD68+); HTCa9 adjacent tubules were abnormal. Bulk RNA-sequencing and DAVID analysis was performed on HTCa8 adjacent and HTCa9 tumour fragment cultures. HTCa8A and HTCa8B exhibited distinct transcript profiles (A: normal germ cells, *DDX4, TNP1*, minimal GCNIS; B: GCNIS-rich, *POU5F1*), and were analysed separately. HTCa9A and B fragments consistently expressed seminoma markers (*SOX17, POU5F1, TFAP2C*) and were combined for analysis. Changes following culture in untreated samples (vs T=0) included downregulation of cholesterol and steroid biosynthesis-associated genes in HTCa8, and downregulation in chemokine-related transcripts in HTCa9 (FDR<0.05, LogFC>1). Activin A and SB431542-treated samples each displayed differentially expressed genes (DEGs; vs vehicle; p<0.05, LogFC>1), with reciprocal expression of some between these opposing treatments, and DEGs common across HTCa8A, HTCa8B and HTCa9 tissue following activin A or SB431542 treatment. Interrogation of DEGs is ongoing, informing future culture experiments in conjunction with spatial transcriptomics to delineate cell targets and outcomes associated with disrupted pathway activity.

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Assessing the impact of cryopreservation on Merino ram sperm using three DNA integrity assays

Caitlin G. Chambers[1], Theresa-Fina Barker[1], Jessica P. Rickard[1], Simon P. de Graaf[1]

1. The University of Sydney, Faculty of Science, School of Life and Environmental Sciences, 2006, NSW, Australia

Sperm DNA integrity assessment can employ various techniques, each yielding distinct outputs. The sperm chromatin dispersion test (HALO) is often used for its simplicity, cost-effectiveness, and minimal specialised equipment requirements. The sperm chromatin structure assay is a high-throughput technique utilising flow cytometric analysis of the metachromatic shift of acridine orange (AO) fluorescence from green (double-stranded DNA) to red (single-stranded DNA). Both techniques present DNA integrity as a percentage of sperm with fragmented DNA within a population. While less common due to its resource-intensive nature, the alkaline single-cell gel electrophoresis (COMET) assay examines the degree of DNA fragmentation within individual sperm, making it useful for detecting subtle changes in DNA integrity. This study employed these three assays to investigate alterations in merino ram (n=9 biological replicates) sperm DNA integrity post-cryopreservation. Cryopreservation is essential for disseminating elite genetics in the sheep production industry; however, the cryopreservation process, including cold shock or the chemical components of the media, may negatively impact the integrity of critical intracellular components, including DNA.

The alkaline COMET assay revealed a statistically significant but minor decline in sperm DNA integrity after cryopreservation. Cryopreserved sperm exhibited higher mean tail DNA percent intensity (37.9%; $p<0.01$) compared to liquid-extended sperm (35.1%). Decreased DNA integrity of cryopreserved sperm was further confirmed by an increased mean tail moment, a parameter that considers both DNA fragment number and size by formulating comet tail DNA percent intensity and length for cryopreserved sperm (18.6; $p<0.001$) compared to liquid-extended sperm (16.6).

In contrast, neither AO nor HALO could distinguish the DNA integrity of cryopreserved sperm (3.98%; 2.16%) from liquid-stored sperm (4.66%; 2.65%), likely due to their population-based analysis methods. This research highlights the disparities among DNA integrity assays and their capacity to detect subtle changes following cryopreservation.

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The *PRKACB* locus is imprinted in marsupials

Trent Newman[1], Teruhito Ishihara[1, 2], Phoebe Rizzoli[1], Lucinda Xiao[3], Quentin Gouil[3], Donna Bond[4], Tim Hore[4], Geoff Shaw[1], Marilyn B Renfree[1]

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

2. Epigenetics programme, Babraham Institute, Cambridge, United Kingdom

3. Epigenetics and Development Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia

4. Department of Anatomy, University of Otago, Dunedin, New Zealand

Genomic imprinting has largely been studied in eutherians, particularly humans and mice, but marsupials also have imprinted genes, albeit a smaller number so far. For many imprinted genes, parental identity is marked by a differentially methylated region (DMR) in which the DNA methylation status differs between the two alleles. We detected several candidate DMRs in a publicly-available koala whole genome bisulfite sequencing dataset[1], using a custom computational pipeline. One candidate DMR was associated with *PRKACB*, a gene encoding a catalytic subunit of the cAMP-dependent protein kinase. Nothing is known about the imprinting status of *PRKACB* in eutherians, even though mutations of this gene can cause multiple developmental abnormalities. Intriguingly, the G-protein Gsα functions upstream of PRKACB in the cAMP signalling pathway and is encoded by the *GNAS* gene which is imprinted in eutherians[2] but not marsupials[3]. Comparison of the *PRKACB* start site indicated a longer 4 kb CpG island (CGI) in marsupials relative to the 1 kb CGI in eutherians. In the brushtail possum, direct nanopore DNA sequencing detected allele-specific methylation over the *PRKACB* CGI. In the tammar wallaby, using bisulfite PCR, the maternal allele was 90% methylated (12 reads) and the paternal allele was 1% methylated (5 reads), across two animal replicates. Transcriptional analysis using nanopore sequencing of a collection of tammar tissues identified two antisense lncRNAs and nine *PRKACB* transcript isoforms produced from the locus. Allele-specific expression analysis identified paternal expression of an antisense *PRKACB* lncRNA in tammar pouch young liver and muscle tissue. We conclude that there is DMR-associated imprinting of the *PRKACB* locus in marsupials. Lineage-specific imprinting of *GNAS* in eutherians and *PRKACB* in marsupials could indicate a conserved selection pressure for imprinting of the cAMP signalling pathway in therian mammals.

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Identifying alternative hydroxysteroid dehydrogenases capable of testosterone biosynthesis in *Hsd17b3*-deficient mice

Ben M Lawrence[1], Liza O'Donnell[2], Anne-Louise Gannon[1], Imogen Abbott[1], Diane Rebourcet[1], Lee B Smith[1, 2]

1. The University of Newcastle, Callaghan, NSW, Australia

2. Griffith University, Southport, QLD, Australia

Testosterone regulates androgen-dependent functions including male sexual development and spermatogenesis. In the adult testis, Leydig cells synthesise testosterone via the canonical androgen biosynthesis pathway, where the HSD17B3 enzyme catalyses the conversion of the androgen precursor androstenedione into testosterone. Consequently, loss of function mutations in human *HSD17B3* results in a disorder of sexual development. 46,XY *HSD17B3*-deficient individuals retain internal Wolffian structures however genitalia is undermasculinised, appearing as female or ambiguous.

Two independent research groups have generated *Hsd17b3*-deficient mice [1, 2]. Surprisingly, in contrast to human cases of *HSD17B3*-deficiency, male *Hsd17b3* knockout mice are masculinised from birth and fertile in adulthood. Although *Hsd17b3* knockout mice exhibit high androstenedione/testosterone ratios (indicative of HSD17B3 dysfunction), intratesticular testosterone is normal. This suggests the existence of alternative testosterone biosynthetic enzymes in mice. We aimed to identify hydroxysteroid dehydrogenase (HSD) enzymes that may be responsible for continued testosterone biosynthesis in *Hsd17b3* knockout mice.

We have identified mouse HSD enzymes that can convert androstenedione into testosterone. We have demonstrated that a key amino acid in a particular HSD allows it to synthesise testosterone, in contrast to the human enzyme which has a different amino acid and is unable to produce testosterone. To model human androgen production in mice, we developed a transgenic mouse line expressing the HSD that is altered to express the human amino acid and is thus unable to produce testosterone. We are cross breeding this humanised HSD mouse line with *Hsd17b3* knockout mice to identify whether the mutated HSD is unable to compensate for the lack of *Hsd17b3*. The validity of the mouse model has been established and the phenotype is

currently being characterised. In conclusion, we are generating mouse models that can be used to better understand human disorders of androgen biosynthesis and can be exploited to identify novel therapies for androgen deficiency.

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Clinical validation of a lenvatinib assay

Monica Majumder[1], Lucy Ding[1, 2, 3], Cameron Wood[1, 3], Christopher Hodgkins[1, 3], Eleanor White[1], Bruce G Robinson[1, 2, 4], Roderick J Clifton-Bligh[1, 2, 4], Paul Bonnitcha[2, 5], Matti L Gild[1, 2]

1. Royal North Shore Hospital, St Leonards, NSW, Australia

2. University of Sydney, Sydney , NSW, Australia

3. NSW Health Pathology, Sydney, NSW, Australia

4. Kolling Institute, St Leonards, NSW, Australia

5. Royal Prince Alfred Hospital, Sydney, NSW , Australia

Background: Lenvatinib improves progression-free and overall survival for radioiodine refractory thyroid cancer. Significant treatment-related adverse effects (TRAEs) can necessitate dose interruptions and suboptimal dosing. Plasma lenvatinib levels are not routinely employed as there is no validated assay for clinical use.

Purpose: To develop a mass-spectometry assay that accurately measures lenvatinib levels and identify normal range peak and trough levels which may correlate with TRAEs.

Methods: A pilot prospective single centre study was performed at Royal North Shore Hospital Sydney. An in-house LC MS/MS assay on a reverse phase column using a 4 minute gradient run was developed to measure lenvatinib levels. Extraction by protein precipitation of plasma samples using methanol with removal of the supernatant before introduction to the column yielded a drug level (ug/L). Available patient dosage, TRAEs and disease progress were recorded.

Results: We collected trough lenvatinib levels in nine and peak (2 hours post administration) levels in eight patients with radioiodine refractory thyroid cancer. Doses ranged between 4-14mg daily. Duration on lenvatinib ranged between 7-63 months (mean 29 months). Duration of treatment on dose of medication at time of testing ranged between 1-14 months. Trough levels ranged between 4.60-30.53ug/L (mean 18.97ug/L). Peak levels for patients on 10mg (n=3) ranged between 78.50- 237.72 ug/L (mean 148.59 ug/L) and on 14mg (n=4) ranged between 65.10-263.64 ug/L (mean 174.80 ug/L). Of note, the case with the lowest trough level was the only participant without hypertension.

Discussion: Access to accurate drug levels may improve monitoring of patients on lenvatinib. Potential to adjust dosing prior to TRAEs occurring and so facilitate more sustained treatment courses still requires exploration.

Conclusion: A novel lenvatinib assay was developed to evaluate peak and trough levels. A large range within the same dose was demonstrated.

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The effect of menstrual cyclicity on brown adipose tissue activity in eumenorrheic women

Anne Nicole De Jesus[1], Belinda A. Henry[2]

1. Monash Biomedicine Discovery Institute, Department of Physiology, Monash University, Clayton, VIC, Australia

2. Monash Biomedicine Discovery Institute, Department of Physiology, Monash University, Clayton, VIC, Australia

Aims: Brown adipose tissue (BAT) expends energy via adaptive thermogenesis. Recently, BAT activation has also been shown to improve glucose clearance. However, it remains unknown as to whether BAT function changes across the menstrual cycle in women. We aimed to characterise changes in BAT activity, glucose tolerance and circulating levels of 17β-estradiol and progesterone in women during the luteal and follicular phases of the menstrual cycle.

Methods: Menstrual cyclicity was monitored in women (18-39 years) and studies were conducted in the follicular (n= 10) and luteal (n= 13) phases of the cycle. Weight, height, BMI, waist circumference and fat mass were measured. In fasted participants, resting energy expenditure (REE) and respiratory quotient (RQ) were determined. Infrared thermography was used to measure cutaneous supraclavicular temperature as an index of BAT temperature in response to either cold exposure (15^{o}C) or an oral glucose tolerance test (oGTT). Continuous glucose monitors (Freestyle Libre) were used to measure transcutaneous glucose concentrations. A single blood sample was collected to measure 17β-estradiol, progesterone and insulin concentrations.

Results: There was no effect of the menstrual phase on REE, RQ and anthropometric parameters. The plasma concentrations of progesterone (p<0.01), 17β-estradiol (p<0.05) and insulin (p<0.01) were increased during the luteal phase of the menstrual cycle. Cold-induced BAT temperature was lower during the luteal phase (p<0.0001). Furthermore, glucose levels were increased (p<0.05) during the oGTT in luteal phase women, and this coincided with an attenuated increase in BAT temperature, but this was not statistically significant.

Conclusion: Our findings suggest that thermogenesis in BAT is attenuated during the luteal phase of the menstrual cycle. Moreover, this coincided with impaired glucose tolerance and reduced insulin sensitivity. This study shows that innate variation in BAT activity in women across the menstrual cycle may not only influence energy expenditure but also glycaemic control.

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Exploring the fragility of liver glycogen alpha particles in diabetes

Ziyi Wang[1], Mitchell Sullivan[2], Liang Wang[1, 3], Robert Gilbert[4, 5]

1. Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Saint Lucia, QLD, Australia

2. Glycation and Diabetes, Mater Research Institute, The University of Queensland-Translational Research Institute, Brisbane, QLD, Australia

3. Laboratory Medicine, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, Guangdong, China

4. Key Laboratory of Plant Functional Genomics of the Ministry of Education/Jiangsu Key Laboratory of Crop Genomics and Molecular Breeding, College of Agriculture, Yangzhou University, Yangzhou, Jiangsu, China

5. Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou, Jiangsu, China

Aims:The formation mechanism of glycogen α particles, derived from smaller β particles, remains elusive. In wild-type mice during glycogen synthesis (active state), extracted liver glycogen is vulnerable to DMSO-induced degradation into β particles [1]. Conversely, glycogen particles remain stable during glycogen breakdown (resting state) [1]. However, diabetic mice exhibit fragile liver glycogen in both synthesis and breakdown phases [2]. This α particle fragility could be common among diabetic mammals, linked to features like hyperglycemia. Prior research has mainly focused on diabetic mice, limiting human relevance. Our primary goal was to probe hepatic glycogen fragility susceptibility in individuals with and without diabetes, illuminating potential universality among diabetic mammals. Additionally, we aimed to identify proteins within the livers of diabetic and non-diabetic mice and humans, which might contribute to α glycogen particle vulnerability.
Methods:We employed diabetic and non-diabetic mice, alongside human liver tissues, as experimental materials. Fluorophore-assisted carbohydrate electrophoresis, size-exclusion chromatography, and transmission electron microscopy revealed structural glycogen features. Proteomics analysis explored differential protein expression in liver tissues of diabetic and non-diabetic subjects.
Results:Diabetic mice and humans displayed significantly larger average glycogen chain lengths during glycogen breakdown, compared to non-diabetic counterparts. Both groups exhibited liver glycogen fragility after DMSO treatment [3]. Seven overlapping proteins emerged between differential expression proteins in diabetic and non-diabetic liver tissues. Notably, the glycogen-associated protein PPP1R3G was significantly downregulated in diabetics, consistent with diurnal healthy mouse protein expression patterns. Specifically, PPP1R3G upregulation during glycogen breakdown implied stable α glycogen, while its downregulation during synthesis suggested fragile α glycogen dominance.
Conclusion:Liver glycogen is more fragile in diabetic individuals, indicating widespread fragility of α glycogen among diabetic mammals. The PPP1R3G protein may contribute to α glycogen structure fragility.

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Effects of myocyte-specific deletion of Vitamin D receptors on muscle function and structure in mice

Miguel Lorenzo Sales Agoncillo[1], Jennifer Chen[1], Josephine Yu[1], Christian Girgis[1, 2, 3], Jenny Gunton[1, 2, 3]

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

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

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

Vitamin D deficiency is associated with falls, muscle weakness, and sarcopenia. Vitamin D receptor (VDR) deletion in mice reduces their grip strength and endurance.

This project investigates the effects of myocyte-specific Vitamin D receptor deletion (mVDR) on muscle regeneration and function after injury.

Floxed VDR mice and human skeletal actin Cre-recombinase mice were bred to generate mVDR mice. The controls were floxed mice without Cre. Ten-month old males (n = 11-13/group) were injected with Notexin in their right tibialis anterior (TA) to induce myocyte death. Each mouse also had a control injection of saline into the Left TA.

mVDR mice had decreased grip strength (rmANOVA, $p<0.01$), but there were no significant differences in endurance-distance or voluntary wheel-running. At 28 days, the notexin-treated TA mass in mVDR was 25% lighter than their left-TA control ($p=0.045$) and 18% heavier than notexin-treated FC mice TA.

Histological analysis of mVDR notexin-treated muscles showed a high proportion of central nuclei, indicating ongoing myocyte regeneration. Two of the mVDR mice had very pronounced fibrosis in their R TA muscle, whereas none of the controls had increased fibrosis.

In summary, mVDR mice showed decreased grip strength but increased TA weight after a notexin-model of muscle injury and regeneration. Further histological assessment will be performed to investigate increased TA mass and muscle morphology.

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Trend of IGF-1 levels over time - is it time to adjust our reference intervals?

Caroline Bachmeier[1], David Kanowski[1], Lee Price[1], Gemma Daley[1], Robert Flatman[1], Greg Ward[1]

1. Chemical Pathology, Sullivan Nicolaides Pathology, Bowen Hills, QLD, Australia

IGF-1 is a hormone that is used for diagnosing and monitoring acromegaly. There has been discussion regarding the appropriateness of current reference intervals and positive flagging rates of IGF-1 concentrations over the past few years - this included an Australia-wide IGF-1 survey. A recent paper suggested that in up to 5.2% of cases IGF-1 levels were elevated without a clinical diagnosis of acromegaly. [1] We reviewed IGF-1 concentrations and associated age and gender associated reference intervals as measured on the Diasorin Liaison at a large referral laboratory over the last five years. Over 100,000 results were available for review. Our data indicates that there has been an earlier peak of IGF-1 concentrations for both men and women, which may have led to increased flagging rates over time. Patient based quality control over the study period also suggested that these observations are based on physiological factors rather than analytical performance of the assay.

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Glycogenic hepatopathy is associated with hepatic steatosis in type 1 diabetes

Stephanie Teasdale[1]

1. Mater Hospital, South Brisbane, QLD, Australia

Metabolic associated fatty liver disease (MAFLD) is associated with multiple metabolic-vascular disorders and is now viewed as an independent risk factor for cardiovascular disease [1]. A high prevalence of hepatic steatosis has been reported with T1DM based on ultrasonographic characteristics [2], however when the technique of 1H-MRS was used, a lower rate of steatosis was seen in T1DM compared with controls [3]. Part of this inconsistency may be related to liver glycogen, which has a similar ultrasonographic appearance to fat [3].

Proton magnetic resonance spectroscopy (1H-MRS) was used to examine liver fat and glycogen content in five clinical groups defined based on metabolic and liver disease phenotypes, each containing five participants: 1. type 1 diabetes (T1DM) with glycogenic hepatopathy, 2. satisfactorily controlled T1DM with no liver disease, 3. suboptimally controlled T1DM without liver disease, 4. a control group of BMI- and age-matched individuals without diabetes or liver disease, and 5. no diabetes but hepatic steatosis (biopsy-proven), unmatched for BMI or age.

Fat content was highest in the hepatic steatosis group (median 15.4%, IQR 10.0-19.3). Compared with the control group (median 1.0%, IQR 0.7-1.1), fat content was much higher in the individuals with glycogenic hepatopathy (median 6.5%, IQR 4.5-9.1), lower in the T1DM with satisfactory control (median 0.3%, IQR 0.2-0.6), and not significantly different in the group of T1DM with suboptimal control without liver disease (median 1.1%, IQR 0.9-1.1).

1H-MRS glycogen content could not be interpreted in the majority of those with glycogenic hepatopathy because of interference from the fat signal.

The findings from this pilot study confirm and extend current evidence suggesting that the hyperechoic ultrasonographic liver changes seen in T1DM are often termed “fatty liver”, but may relate to glycogen accumulation and not fat, however in cases diagnosed with glycogenic hepatopathy there may be significant concomitant fat accumulation.

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Dysnatraemia in the era of climate change: a global systematic review of the association between serum sodium and climate

Elizabeth Wootton[1], Mathis Grossmann[1], Annabelle M Warren[1]

1. Department of Endocrinology and Diabetes, Austin Health, Melbourne, Victoria, Australia

Background: Serum sodium abnormalities are common and associated with increased morbidity and mortality(1, 2). Both hyponatraemia and hypernatraemia have been reported to occur more frequently in high ambient temperatures, although the underlying mechanisms are not well understood. Global temperatures are rising secondary to climate change, which may impact the incidence of dysnatraemia worldwide.

Aim: To identify, collate and critically appraise studies analyzing the relationship between climate measures (outdoor temperature, humidity) and serum sodium levels.

Methods: Systematic review, reported in accordance with PRISMA guidelines. MEDLINE and EMBASE were searched with relevant key terms (“hyponatr(a)emia”, “hypernatr(a)emia”, “water-electrolyte imbalance”, “dehydration”, “water intoxication”, “electrolyte abnormality” and “climate”, “temperature”, “humidity”, “seasons”, “heatwave”). Studies assessing the effect on serum sodium measurement of raised temperature or humidity versus a comparator were included.

Results: 1354 potentially relevant studies were identified, with 34 meeting the inclusion criteria. Included studies originated from 23 countries spanning all inhabited continents. The majority (30 of 34, 88%) reported a significant association between outdoor temperature and dysnatraemia, predominantly a reduction in serum sodium levels during periods of increased temperature. Humidity did not have a substantial effect on serum sodium level. The eleven largest population-based studies are characterised in Table 1. Specific populations with increased vulnerability to dysnatraemia were identified: the elderly, as well as children, individuals taking medications such as diuretics and antidepressants, with chronic renal impairment or undertaking physical exertion.

Conclusion: An increased incidence of dysnatraemia, particularly hyponatraemia, in association with higher ambient temperature is consistently reported. It can be inferred that hyponatraemia presentations are likely to rise with increasing global temperatures and frequency of extreme heat events secondary to climate change. Evidence-based public health messages, clinician education and reduction in fossil fuel consumption are necessary to reduce the expected burden on healthcare services worldwide(3).

Table 1: Largest population-based studies investigating the impact of high ambient temperature on serum sodium.

Author, year	Location (climate zone*)	Population	Comparison	Association
Bucher, 2014(4)	St Gallen, Switzerland (temperate)	13,277 patients admitted to internal medicine	Temp/humidity for the day preceding hospital admission or averaged over a period of 1 week, and 1 month before hospital admission, 2002-2004	Yes Both higher air humidity (sodium: +0.017 mmol/L per % higher humidity, p<0.0001) and higher ambient temp (sodium: +0.033 mmol/L per °C, p<0.0001) in the week preceding admission
Giordano, 2017(5)	Caserta, Italy (temperate)	15,049 patients presenting to ED	Seasons/months (warmer vs cooler), 2014-2015	Yes (in elderly only) Prevalence of hypona highest in summer for elderly (12.5% vs 9.4-10.5% other seasons, p<0.05) Severe hypona (<125mmol/L) more common in summer (4.1% vs 0.28-1.4% other seasons, p<0.05). No seasonal effect in adults overall.
Huwyler, 2016(6)	Geneva, Switzerland (temperate)	28,734 adults presenting to ED	Seasons, daily temperature and humidity, 2011-2013	Yes Incidence of profound hypona (<125mmol/L) 0.54% winter and 1.29% summer; significant association with daily temp (p<0.001).
Imai, 2018(7)	Kawasaki, Japan (temperate)	8377 adults presenting to ED	Seasons and months (mean temp range 4.6°C in Jan to 26.8°C in August), 2015-2016	Yes (in elderly only) In elderly, increased prevalence of hypona in summer (p<0.001 vs spring and winter) with linear correlation for monthly temp. No association in adults overall.
Imai, 2019(8)	Kawasaki, Japan (temperate)	12,598 patients presenting to ED	Seasons and months, 2015-2017	Yes (in elderly only) In elderly, increased prevalence of mod/severe hyperna (>150mmol/L) in winter (p=0.04 vs summer) with linear correlation for monthly temp. No association in adults overall.
Josseran, 2009(9)	Multicentre, France (temperate /continental)	415,862 visits to ED including children	Heatwave vs. Control (daily temp during 18-day heatwave period vs control period, 2006	Yes 11 visits/day for hypona during heatwave vs 3.9 in control period, p<0.001, with elderly disproportionately affected. Hyperna not reported.
Kutz, 2020(10)	Nationwide, Switzerland (temperate /continental)	84,210 medical inpatients with hypoosmolar hypona	Seasons/months, Mean daily outdoor temp, 2009-2015	Yes Highest prevalence of hypona in July (4.5%) vs December (2.7%), p=0.005. Age (elderly) and sex (F>M) differences more pronounced with increasing temp.
Mannheimer, 2022(11)	Nationwide, Sweden (temperate /continental)	11,213 adults hospitalised with hypona	Daily mean air temp and humidity, 2005-2014	Yes Small increase in incidence of hypona from 10-15°C then rapid increase to max temp 25°C. Greater risk in women and elderly. Small influence of humidity.
Pfortmueller, 2014(12)	Bern, Switzerland (temperate)	22,239 adults admitted to ED	Daily temp, humidity and sunshine, 2009-2010	Yes Weak correlation between sodium and daily maximum temp (inverse), humidity and sunshine (inverse), p<0.05. Increased prevalence of hypona if ≥30°C (11 vs 9%, p0.04).
Sailer, 2019(13)	Aarau, Basel, Switzerland (temperate)	222,217 patients with sodium ≤145mmol/L	Daily outdoor temp (3-25°C) and humidity (69-85%), 2011-2016	Yes Significant correlation with profound hypona and temp, with 1.2% increased risk per °C, p<0.001. Diuretic-induced hypona increased by 4% per °C, p= 0.02.
Yong, 2015(14)	Melbourne, Australia (temperate)	Hospital admissions with hypona (unknown number)	Monthly excess heat factor (a high mean temp over a 3-day period compared with historical reference), 2006-2014	Yes Positive association with hypona admissions and increasing excess heat factor.

ED – emergency department, hyperna – hypernatraemia, hypona – hyponatraemia

*The Köppen system divides climates into five groups based on rainfall and temperature: tropical, dry, temperate, continental or polar(15).

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Screening for primary aldosteronism underutilised in stroke and transient ischaemic attack: a multi-centre cohort study

Josephine McCarthy[1, 2, 3, 4], Jocelyn Widjaja[1], Frank M. Gao[5, 6], Ignatius J.H. Lee[7], Jordan H.N.C. Lai[8], Sanjana Gupta[9], Jason Vuong[1, 10], Philip M.C. Choi[11, 12], Thanh G. Phan[1, 10], Jun Yang[1, 2, 3]

1. Department of Medicine, School of Clinical Sciences at Monash Health, Faculty of Medicine Nursing and Health Sciences, Monash University, Clayton, Victoria, Australia

2. Department of Endocrinology, Monash Health, Clayton, Victoria, Australia

3. Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, Victoria, Australia

4. Department of Endocrinology, Eastern Health, Box Hill, Victoria, Australia

5. Department of Medicine, Austin Health, Melbourne Medical School, The University of Melbourne, Melbourne, Victoria, Australia

6. Department of Endocrinology, Austin Health, Melbourne, Victoria, Australia

7. Goulburn Valley Health, Shepparton, Victoria, Australia

8. Department of Medicine, Eastern Health, Box Hill, Victoria, Australia

9. The Royal Melbourne Hospital, Melbourne, Victoria, Australia

10. Department of Neurology, Monash Health, Clayton, Victoria, Australia

11. Department of Neuroscience, Eastern Health, Box Hill, Victoria, Australia

12. Eastern Health Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Box Hill, Victoria, Australia

Primary aldosteronism (PA) is the most common endocrine cause of hypertension. People with hypertension due to PA have a higher rate of adverse cardiovascular events including stroke compared to those with blood pressure matched essential hypertension. (1) Targeted treatment with mineralocorticoid receptor antagonists or surgical resection for an aldosterone producing adenoma can effectively reduce blood pressure and ameliorate the increased cardiovascular risk. (2)

Aim: The aim of this study was to evaluate the proportion of patients admitted for stroke or transient ischaemic attack (TIA) who had an indication for PA testing according to the Endocrine Society 2016 Guideline and the proportion who were actually tested. (3) **Method:** This retrospective cohort study was conducted at two tertiary health services in Melbourne. Patients were included in analysis if they were admitted for stroke or TIA in 2019 and had documentation of blood pressure and concurrent medications within 2 years of admission. We screened 1,110 patient records and excluded 710 due to missing data. **Results:** Of the 400 patients analysed, 298 (75%) had a history of hypertension or were hypertensive at the time of discharge and 75 (19%) had indications for PA testing. Only 8 (2%) were considered for PA testing. Seven patients had an aldosterone to renin ratio and one had an adrenal CT. Six of the seven patients with an aldosterone to renin ratio were on interfering medications which can cause false negative results. One patient was lost to follow up and no patients underwent formal testing as recommended by the Endocrine Society. **Conclusion:** In conclusion, a quarter of patients admitted with stroke or TIA and hypertension have an indication for PA testing. However, few are ever tested appropriately. Appropriate PA screening in this population may identify a potentially curable cause of hypertension, improve blood pressure control and reduce stroke recurrence.

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Establishment of normal reference values for ^{99m}Tc-pertechnetate thyroid uptake in the local population, an Australian first

Melanie Tempest[1], Bethany Wornes[1], Emily Karahalios[2], Joanna Gong[1]

1. Western Health, Melbourne, VIC, Australia

2. Centre for Epidemiology and Biostatistcs, The University of Melbourne, Melbourne, VIC, Australia

AIM: The aim of this study was to establish normal reference values for ^{99m}Tc-pertechnetate thyroid uptake in the local patient population.

To our knowledge, there have been no published reports on normal reference values for the Australian population. The current normal reference values for thyroid uptake used at our institution of 1 – 4% are based on data from a study performed in 1971. Uptake values may change with geographical location and from one decade to the next with studies from Namibia, Brazil and the United Kingdom establishing updated normal reference values for local populations of 0.15 – 1.69%, 0.4 – 1.7%, and 0.2 – 2.0% respectively.

METHODS: A prospective study was conducted alongside a retrospective audit for 271 patients undergoing parathyroid scans in our Nuclear Medicine department between January 2017 and December 2021. Anterior thyroid static images acquired following intravenous injection of ^{99m}Tc-pertechnetate were reviewed to calculate thyroid uptake. Patients with known and / or prior thyroid conditions, abnormal biochemical thyroid function tests and / or abnormal appearances on ^{99m}Tc-pertechnetate thyroid scans were excluded from the study.

RESULTS: Of the 271 patients a total of 151 studies met the eligibility criteria and were included for analysis. The median uptake value in our patient population was 0.97% with a 95% reference range of 0.4 – 2.4%.

CONCLUSION: The calculated normal reference range in this study was found to be considerably different to that of the 1971 study findings of 1 – 4 %. The results demonstrate the importance of periodic evaluation of normal uptake values for geographically varied populations. Based on this finding we recommend updating the normal range for ^{99m}Tc-pertechnetate thyroid uptake to reflect values relevant to the local population.

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Hierarchical Cluster Analysis and Principal Components Analysis confirm metabolic and reproductive subtypes in PCOS

Kharis Burns[1, 2], Alexander Stuckey[4, 3], Scott Wilson[6, 5], Gerald Watts[7, 2], Bronwyn Stuckey[6, 2, 8]

1. Department of Endocrinology and Diabetes, Royal Perth Hospital, Perth, WA, Australia

2. Medical School, University of Western Australia, Crawley, WA, Australia

3. School of Agriculture and Environment, University of Western Australia, Crawley, WA, Australia

4. Pink Lake Analytics, Nedlands, WA, Australia

5. School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia

6. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

7. Lipid Disorders Clinic, Department of Cardiology, Royal Perth Hospital, Perth, WA, Australia

8. Keogh Institute for Medical Research, Nedlands, WA, Australia

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Polycystic Ovary Syndrome (PCOS), characterised by hyperandrogenism and oligomenorrhoea, is an umbrella term encompassing notable heterogeneity.

We aimed to explore the heterogeneity of PCOS and potential subtypes by statistical analysis of biochemical and anthropometric data using two statistical methods.

We studied 1035 women with NIH-defined PCOS including data on BMI, serum LH:FSH, testosterone, DHEAS, androstenedione,17-OH-progesterone, SHBG, HOMA_IR, lipids and blood pressure. Unsupervised agglomerative hierarchical cluster analysis was used to group a) phenotypic variables and b) patients into clusters. Principal components analysis (PCA) was used to resolve correlated variables (excluding BMI) into independent factors. The relationship between resultant components and BMI was then explored.

Study subjects had a median age 29.0y (23.0, 37.0). 28% were lean, 20.5% overweight, and 51.5% obese. Analysis of phenotypic variables revealed two main clusters - one characterised by blood pressure, BMI, HOMA_IR and triglycerides, and a second by LH:FSH, androgens, SHBG, and lipids. There were 3 separate patient clusters: Cluster A (40.4% of women) demonstrated lower BP, BMI, HOMA_IR, triglycerides, testosterone, FAI, and higher LH:FSH, DHEAS, androstenedione, 17-

OH-progesterone, SHBG and HDL. In contrast, cluster C (45.4%) had higher BP, BMI, HOMA_IR and triglycerides and lower LH:FSH, androgens, SHBG and lipids. Cluster B (14.2%) was intermediate.

PCA, excluding BMI, found two components that aligned with the cluster analysis. Variables with greatest weight in principal component 1 (PC1) included HOMA_IR, triglycerides, systolic and diastolic BP, FAI, and SHBG. PC1 demonstrated positive correlation with BMI (R^2=0.26) and aligned with cluster C. Principal component 2 (PC2) was strongly influenced by LH:FSH, testosterone, FAI, DHEAS and androstenedione, with loadings in the opposite direction from LDL and cholesterol, and aligned with cluster A. There was little relationship between BMI and PC2 (R^2=0.028).

Our analysis revealed “metabolic” and “reproductive” subtypes. BMI was influential in the metabolic subtype but not the reproductive subtype.

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Short-term effects of testosterone therapy on quality of life in trans individuals seeking masculinisation: a prespecified secondary analysis of a randomised clinical trial

Brendan J Nolan[1, 2], Sav Zwickl[2], Jeffrey D Zajac[1, 2], Ada S Cheung[1, 2]

1. Endocrinology, Austin Health, Heidelberg, Victoria, Australia

2. Austin Health, University of Melbourne, Heidelberg, Victoria, Australia

Background:

Testosterone treatment is a necessary component of management for some transgender and gender diverse (trans) individuals. Observational studies have demonstrated improvements in quality of life (QoL) following commencement of testosterone but no randomised trial has been performed. We aimed to assess the impact of testosterone therapy compared to no treatment on QoL in trans people seeking masculinisation. We hypothesised that testosterone treatment would improve QoL.

Methods:

This was a prespecified secondary analysis of a 3-month open-label RCT in 64 trans individuals commencing testosterone. Participants were randomised to immediate testosterone commencement (intervention group) or no treatment (standard care waiting list of 3 months prior to commencement). This design ensured no individual would be waiting longer than standard care. QoL was assessed using EQ-visual analogue scale (EQ-VAS) and EQ-5D-5L at 0 and 3 months. EQ-VAS is a visual analogue scale with values between 100 (best imaginable health) and 0 (worst imaginable health). EQ-5D-5L is a questionnaire measuring 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression with values between 0 (a state as bad as being dead) and 1 (full health). ANCOVA was used to compare mean differences between groups.

Results:

In the overall cohort at baseline, mean (SD) EQ-VAS and EQ-5D-5L utility score were 56.8 (20.6) and 0.72 (0.19), respectively. Fifty-eight (93.5%) reported anxiety/depression. Compared to standard care, in individuals receiving testosterone, there was a clinically significant increase in EQ-VAS over 3 months follow-up (mean difference +11.6 points (4.9, 18.3), *p*=0.02). The between-group change in EQ-5D-5L utility score did not reach statistical significance (mean difference +0.07 (-0.07, 0.21), p=0.06).

Conclusion:

This RCT demonstrates impaired QoL at baseline and clinically meaningful improvement in QoL over the first 3 months of testosterone therapy for gender affirmation. This study supports the use of testosterone therapy to improve QoL in trans individuals desiring masculinisation.

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New insights into adrenal cortex development using a novel preclinical model of Adrenal Hypoplasia Congenita

Imogen Abbott[1], Anne-Louise Gannon[1], Daniel Bird[2], Andreas Schedl[3], Dagmar Wilhelm[2]

1. The University of Newcastle, Newcastle, NSW

2. The University of Melbourne, Melbourne, VIC

3. University of Nice Sophia Antipolis, Nice, France

Publish consent withheld

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What's new with LDL-cholesterol calculations?

Caroline Bachmeier[1], Timothy Foster[1], Gemma Daley[1], Robert Flatman[1], Lee Price[1], David Kanowski[1], Greg Ward[1]

1. Chemical Pathology, Sullivan Nicolaides Pathology, Bowen Hills, QLD, Australia

LDL-cholesterol is a major risk factor for cardiovascular disease and is still a major target in clinical practice guidelines. [1] While the gold standard for lipid measurement is beta-quantification this is expensive, labour-intensive and not practical for routine use. Instead most laboratories use the Friedewald formula to calculate LDL-cholesterol. [2] This comes with several limitations, including inaccuracy when triglycerides are greater than 4.5 mmol/L, the presence of chylomicrons or IDL-cholesterol or very low LDL-cholesterol values. [3] Various new calculations have been developed, with the Sampson equation being a promising alternative to the Friedwald equation, allowing calculation of LDL-cholesterol with triglycerides up to 9 mmol/L and showing a significantly better correlation to gold standard methods. [4] We applied the Sampson equation to a local dataset of 337,138

patient samples with calculated LDL-cholesterol results via the Friedewald formula. The Sampson equation showed excellent correlation with the Friedewald formula for triglycerides < 4.5 mmol/L, but additionally allowed calculation of of LDL-cholesterol in cases of triglycerides 4.5 - 9 mmol/L. In our laboratory this will result in the ability to provide calculated LDL-cholesterol results for an additional 2% of patients (or 18,826 samples per year).

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It's all in the CTX

Caroline Bachmeier[1], Ben Teis[1], Lee Price[1], David Kanowski[1], Gemma Daley[1], Greg Ward[1]

1. Chemical Pathology, Sullivan Nicolaides Pathology, Bowen Hills, QLD, Australia

Denosumab is a common and convenient treatment option for patients with osteoporosis. The cessation of denosumab can be associated with a transient but significant increase in bone turnover associated with bone loss and it is recommended that patients should be administered alternative anti-resorptive agents to mitigate this risk. [1,2] One approach suggests that CTX could be used to guide timing of administration of bisphosphonates such as zoledronic acid. [3] The cut-off for commencement of zoledronic acid ranges from 'above the mean found in age- and sex-matched cohorts', [3] to twice the upper limit of normal in premenopausal women [4] to absolute cut-offs. We measure CTX on the Roche Cobas analyzer in our laboratory. Reference intervals for CTX change depending on age and gender. We reviewed the data of over 200,000 CTX values measured from 2017 to 2023 to derive age specific percentiles in our local population.

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Adverse Cardiac Remodelling Following *Foxe1* Deletion in the Adult Mouse

Alexander Widiapradja[1], Martyn Bullock[2, 3], Grace Lim[2, 3], Roderick J Clifton-Bligh[2, 3, 4], Scott P Levick[1]

1. Department of Physiology, Pharmacology and Toxicology, West Virginia University, Morgantown, WV, USA

2. Cancer Genetic Laboratory, Kolling Institute, St Leonards, NSW, Australia

3. University of Sydney, Sydney, NSW, Australia

4. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

Cardiovascular disease, encompassing conditions such as heart failure with diastolic dysfunction, is a leading cause of mortality in adult populations of Australia and the United States. Diastolic dysfunction, characterized by the heart's failure to relax properly, is often associated with cardiac fibrosis, which is marked by excessive deposition of extracellular matrix components like collagen. Currently, there is a lack of effective targeted treatments for heart failure and the underlying fibrosis, underscoring an urgent need to explore novel therapeutic avenues for prevention and regression. In this context, our research investigates the *FOXE1* gene, which encodes a transcription factor vital for thyroid gland development and function. While its extrathyroidal function remains largely unexplored, our study of a novel tamoxifen-induced *Foxe1* knockout mouse model has revealed that the Foxe1 loss leads to adverse cardiac remodelling, including fibrosis.

We hypothesized that a loss of Foxe1 promotes a pro-inflammatory macrophage state that induces a pro-fibrotic phenotype change in cardiac fibroblasts to ultimately cause fibrosis.

Immunohistochemical (IHC) staining revealed a 10% increase (P<0.05) in collagen volume in *Foxe1*$^{flox/flox}$*/Cre* mouse hearts compared to *Cre* controls (n=10 per group), harvested at 20 weeks post-tamoxifen treatment. Additionally, we observed a 60% increase (P<0.05) in pro-inflammatory (M1) macrophages and a 90% reduction (P<0.05) in anti-inflammatory (M2) macrophages. Consequently, the overall M1/M2 macrophage ratio was markedly reduced in *Foxe1*$^{flox/flox}$*/Cre* hearts. Immunoblotting and IHC further detected Foxe1 nuclear staining in the *Cre* hearts, but not in the *Foxe1*$^{flox/flox}$*/Cre* hearts.

Collectively, these findings constitute the first evidence that Foxe1 plays a cardio-protective role, specifically opposing cardiac inflammation and fibrosis. While the loss of Foxe1 shows detrimental effects on the heart, the cellular mechanisms underlying how Foxe1 loss impacts the phenotype of cardiac fibroblasts and macrophages remain to be elucidated. These insights may pave the way for innovative interventions against cardiac fibrosis.

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Exploring FOXE1's Role in Thyroid Cancer: Insights from ENCODE Data

Martyn Bullock[1, 2], Roderick J Clifton-Bligh[1, 2, 3]

1. Cancer Genetic Laboratory, Kolling Institute, St Leonards, NSW, Australia

2. University of Sydney, Sydney, NSW, Australia

3. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

Recent studies have revealed a strong association between *FOXE1* and susceptibility to differentiated thyroid cancer. However, the mechanisms through which FOXE1 promotes thyroid tumorigenesis remain elusive. Our recent work on the mechanisms behind telomerase reverse transcriptase (*TERT*) activation demonstrated that FOXE1 activates *TERT* in thyroid cell models by

cooperating with either ETS-factor ELK1 (1) or ETV5 (2). We posited that FOXE1, in conjunction with ETS-factors, might also regulate other thyroid cancer-associated genes.

Leveraging publicly available ENCODE (https://www.encodeproject.org/) ATAC-seq data from four normal thyroid tissues (Thy), we performed a transcription factor motif enrichment analysis using MEME-ChIP. Our analysis showed that ATAC peaks (indicative of open chromatin) with predicted FOXE1 motifs were significantly enriched for both ETS and CTCF binding motifs, with 80% and 90% located within 300 bp of the FOXE1 motif, respectively. Subsequently, we employed the GREAT tool for ontological enrichment analysis. Top terms included: “increased thyroid tumour incidence” (FDR *Q*-value: 0.000439), covering genes like *BRAF* (+366 bp from transcriptional start site), *PTEN* (-665 and +508), and *TP53* (+7, +809); and "abnormal telomere morphology" (*Q*-value: 0.0406), that included *TERT* (-21,370, -20,738, +47,906) and genes associated with the telomere-binding Shelterin complex, including *POT1* (-25) and *TERF1* (+53, +336). Next, focusing in on the *TERT* locus, we further evaluated Thy HiC data. Our results suggest that FOXA2 and ETV5, in tandem with CTCF binding, delineate topological associating domain (TAD) boundaries throughout this region and promote long-range interactions with the *TERT* promoter.

Our findings offer new insights into the relationship between *FOXE1* and thyroid cancer and will necessitate experimental validation.

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Investigating the aetiology of early-onset osteoporosis in Down Syndrome using a human stem cell model

Huong Q Duong[1], Michelle Maugham-Macan[1], Georgia Kafer[1]

1. University of the Sunshine Coast, Sippy Downs, QLD, Australia

Introduction

Trisomy 21 (T21) or Down syndrome (DS) is a common genetic anomaly caused by the triplication of chromosome 21. Individuals with DS face significant bone health challenges, an area not extensively studied. As the DS population ages, the prevalence of osteoporosis rises.

Early-onset osteoporosis (EOO), characterised by reduced bone density and altered bone structure, increases fracture risk. Recognized as a "silent epidemic," osteoporosis has substantial health and economic implications. There is an urgent need to understand the factors exacerbating bone issues in DS, given their increased fracture risk and reduced healing capacity, impacting their overall quality of life.

Aims

We aim to understand how T21 affects bone health, particularly osteoblast differentiation and function. EOO in DS is believed to stem from compromised osteoblastic activities, leading to decreased bone mass. The study will explore osteoblast efficiency, maturation, and function using hiPSCs from WT and individuals with T21, seeking insights into how T21 influences osteoblasts and EOO in DS. Findings may guide future specialized treatments.

Methods

This study examines hiPSCs as a new model to explore bone health in T21. We use cell lines from WT individuals and those with T21. Through *in vitro* cell culture experiments, we investigate osteoblastic differentiation via WST-1 and qPCR analysis, maturation with Alizarin Red staining, and functionality via immunofluorescence. We also plan to assess proliferation, senescence and apoptosis using Western Blot.

Expected outcomes

We hypothesise that osteoblast differentiation will be reduced as evidenced by a decrease osteoblast markers expression at both the mRNA and protein levels. We anticipate reduced osteoblast maturation, functionality, and cellular proliferation with an increase in osteoblast senescence and apoptosis.

Conclusion

By elucidating the cellular processes altered in the bone of T21 individuals, these findings will improve bone health management for individuals with DS, thus significantly enhancing their quality of life.

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The Utility of AAVs to target the Adrenal Gland

Akhil Gajjpara[1], Annalucia Darbey[2], Pamela Brown[3], Diane Rebourcet[1], Ben Lawerence[1], Liza O'Donnell[4], Lee B Smith[4], Anne-Louise Gannon[1]

1. School of Environmental and Life Sciences, The University of Newcastle, Newcastle, New South Wales, Australia

2. UCL Queen Square Institute of Neurology, University College London, London, UK

3. College of Medicine and Veterinary Medicine, The University of Edinburgh, Edindurgh, UK

4. Office for Research, Griffith University, Gold Coast, Queensland, Australia

Background: Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disorder which results in disruption to hormone production in adrenal cortex. There are multiple hydroxylase and lyase enzymes which are responsible for hormone conversion at different stages of the steroid biosynthesis pathway. A loss of function in one of the genes that encodes for these enzymes, results in CAH. Inadequate hormone production needs hormone replacement at regular intervals. However, this replacement

can not meet the demand of periodic secretion especially in stress. Ultimately, these short-term treatments may cause metabolic disturbances and adrenal crisis. Hence, there is an extreme requisite of alternate solutions for long-time treatment of CAH. Gene based therapies attracts significant attention due to its lifelong effectiveness in which mutated gene is replaced by normal gene using vector system. While AAVrh10 has previously shown to have successful delivery to adrenal cortex, its delivery was short term. While this demonstrates proof of principle for adrenal gene delivery, enhanced targeting and improved longevity needs further investigation.

Methods: To determine if alternative AAV serotypes can target the adrenal cortex we assessed four different AAV serotypes to deliver green fluorescence protein (GFP) to determine cargo delivery efficiency to adrenal cortex. In this experiment, genes encode for GFP were encapsulated in AAV and injected into mice. After two weeks, tissues were collected, morphology and immunohistochemistry were performed to analyse GFP accumulation and biodistribution.

Results: Our preliminary data shows successful GFP delivery in adrenocortical cells in all serotypes analysed with a greater efficiency from what been previously described. Adrenal morphology is found to be normal when compared to control tissues indicating that AAVs are biocompatible for such treatments.

Conclusion: This data demonstrates novel AAV serotypes capable of delivering genetic cargo to the adrenal cortex, opening utility for improved gene therapies for the adrenal, and CAH disorders.

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Using SAFA Technology to Develop a Long-Acting FSH for the Treatment of Female Infertility

Daham Kim[1], Yoon Hee Cho[1], Min Jeong Kang[1], So Jeong Lee[1], Cheol Ryong Ku[1], Hyunjin Chi[2], Jungsuk Ahn[2], Kyungsun Lee[2], Jaekyu Han[2], Susan Chi[2], Moo Young Song[2], Sang-hoon Cha[2], Eun Jig Lee[1]

1. Department of Internal Medicine, Institute of Endocrine Research, Yonsei University College of Medicine, Seoul, Republic of Korea

2. AprilBio Co., Ltd., Rm 602, Biomedical Science Building, Kangwon National University, Chuncheon, Republic of Korea

Recombinant human follicle-stimulating hormone (rhFSH) is a commonly used treatment for female infertility, but its short half-life requires multiple doses. Even corifollitropin alfa, which has a longer half-life, still requires additional injections of rhFSH after 7 days. Our goal was to use anti-serum albumin Fab-associated (SAFA) technology to develop a long-acting FSH that eliminates the need for additional injections.

SAFA-FSH was produced using a Chinese hamster ovary expression system, and its biological activity was confirmed through tests measuring its ability to stimulate cAMP production, estradiol synthesis, and the expression of FSH receptor, hCYP19α1, and hSTAR in KGN cells. The effects of SAFA-FSH and rhTSH on ovarian weight gain and serum estradiol levels were compared in SD rats.

The results showed that SAFA-FSH stimulates cAMP synthesis in KGN cells and increases the expression of FSH receptor, hCYP19α1, and hSTAR with increasing concentrations of SAFA-FSH. Female SD rats aged 21 days, receiving daily subcutaneous hCG injections for 5 days, showed a significant increase in ovarian weight with SAFA-FSH administered on the first day or with 9 injections of rhFSH over 5 days. Notably, the group receiving SAFA-FSH on the first and third day showed an even greater increase in ovarian weight. In addition, the SAFA-FSH group showed a significant increase in estradiol levels, while the rhFSH group did not.

These findings suggest that SAFA-FSH could be a viable alternative to current rhFSH treatments for female infertility. However, further research is needed to fully evaluate its safety and effectiveness in clinical settings.

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Characterisation of inhibin-binding to its novel co-receptor, TGFBR3L

Haitong Luan[1], William A Stocker [2], Evan RS Buddle[3], Daniel J Bernard[3], Craig A Harrison[1], Kelly L Walton[4]

1. Department of Physiology, Monash Biomedicine Discovery Institute,Monash University, Clayton, Victoria, Australia

2. Monash University, Clayton, VIC, Australia

3. Department of Pharmacology and Therapeutics, McGill University, Montreal, QC , Canada

4. School of Biomedical Science, University of Queensland, Brisbane, QLD, Australia

The gonadotrope-restricted transmembrane protein, transforming growth factor-β receptor 3-like (*TGFBR3L*), has emerged as an inhibin B-specific co-receptor involved in the regulation of follicle-stimulating hormone (FSH) synthesis. This novel co-receptor shares a high degree of similarity with known inhibin co-receptor, betaglycan (*TGFBR3*). As TGFBR3L specifically binds inhibin B (α/β_B dimers) and not inhibin A (α/β_A dimers), it is hypothesised that inhibin B binding to TGFBR3L is mediated via discrete residues in the b_B-subunit. Here, we aimed to identify the key residues in the β_B-subunit that permit binding of inhibin B, and not inhibin A, to TGFBR3L. Using site-directed mutagenesis and luciferase reporter assays, we identified a cluster of residues within the inhibin β_B-subunit (Leu^{340}, Thr^{352} and Ala^{353}) critical for TGFBR3L interactions. Substitution of these β_B-subunit residues with the corresponding residues from the β_A-subunit disrupted the ability of inhibin B to dock onto TGFBR3L and suppress activin-mediated signalling in COV434 granulosa cells. Interestingly, the Leu^{340}Ile inhibin B variant retained *in vitro* bioactivity in the presence of betaglycan, despite being non-functional in the presence of TGFBR3L. Conversely, Thr^{352}Ser and Ala^{353}Thr β_B-subunit mutations disrupted inhibin B *in vitro* bioactivity in both the presence of TGFBR3L and betaglycan, likely indicating cross-over with ligand/co-receptor contact sites. Importantly, mutation of these β_B-subunit residues did not impair the ability of resultant activin B forms ($\beta_B/_B$ dimers) to signal via the activin type II receptors in these cells. Introducing the identified key residues in the β_B-subunit into the inhibin β_A-subunit permitted inhibin A activity via TGFBR3L (IC_{50}=1.9nM), albeit at lower potency than wild-type inhibin B (IC_{50}=0.15nM). Together, our results have identified key residues in the inhibin β_B-subunit that mediate the specific binding of inhibin B to itsnewly identified co-receptor, TGFBR3L. Understanding the mechanisms by which inhibin B modulates FSH will advance the development of reproductive technologies.

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Fracture risk in Breast Cancer Bone Metastases: Does Obesity have an effect?

Michelle Maugham-Macan[1], Huong Duong[1]

1. University of the Sunshine Coast, Sippy Downs, QLD, Australia

Obesity is now recognised as a key risk factor for cancer with Breast Cancer one of thirteen obesity-driven cancers identified by the World Cancer Research Fund. Breast Cancer bone metastasis is a debilitating disease and recent research has demonstrated a link between obesity and poorer outcomes from bone metastatic breast cancer. The aim of this study is to investigate the impact of obesity on breast cancer bone metastases outcomes, specifically fracture risk and overall survival. We conducted a scoping review of existing literature to investigate the effect of obesity on fracture risk in bone metastatic breast cancer. At the time of review there was no literature examining this association. We therefore are conducting a retrospective analysis of female patients diagnosed with breast cancer bone metastases reviewed by a multidisciplinary team on the Sunshine Coast between 2008 and 2021. Patient BMI, evidence of fractures during the study period and survival 5 years and 10 years after diagnosis are manually extracted from CHARM Oncology database. Anonymised data will then undergo correlation analysis. We hypothesise that there is a correlation between body mass index and fracture risk as well as overall survival. The results of this study along with results of our scoping review will support future research into strategies that may reduce the impact of breast cancer bone metastases such as exercise physiologist-prescribed, personalised exercise plans to manage obesity and reduce bone loss that occurs with osteolytic lesions of bone metastatic breast cancer and therefore decrease bone pain and fracture risk and increase quality of life.

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Factors associated with engagement with blood glucose monitoring using a cloud-based platform (NetHealth) for monitoring Gestational Diabetes and associated outcomes

Bethany Lou[1], Beth Lacy[1], Chelsea W Tan[1], Jessica M Triay[1], Amy Harding[1]

1. Bendigo Health, Bendigo, VIC, Australia

Gestational diabetes mellitus (GDM) diagnosis brings expectant mothers additional burdens regarding glucose monitoring. Remote monitoring can reduce unnecessary appointments, or treatment delays, but may not be appropriate for all users. Identifying factors associated with lower engagement with such platforms enables treating teams to direct work flows and accommodate for standard and low-engagement users.

56 women with GDM were linked to the NetHealth application for remote blood glucose monitoring enabling results to be automatically uploaded and immediately visible to the treating team. Users were stratified into categories of standard (> 75% daily readings available), or low-engagement (< 75% daily readings available). Demographics, medical history and birth outcomes were collected.

Overall, 75% of the cohort achieved standard glucose monitoring. Low-engagement users completed a mean of 51% of required tests and were less responsive to direct messaging (33% returned messages vs 57%).

Associated factors in the low-engagement group included (1) lower rates of family or friend support (53% vs. 74%), (2) lower rates of employment (53% vs. 90%), (3) higher rates of pre-existing anxiety and/or depression (47% vs. 24%), (4) previous diagnosis of postnatal depression (27% vs. 5%), and (5) current or previous concerns regarding family safety (33% vs. 0%). Babies born to low-engagement users had higher admission rates to the special care nursery (seven with respiratory distress, five with jaundice, and one with neonatal hypoglycaemia).

This cohort demonstrates that glucose remote monitoring remains a challenge for some expectant mothers. Awareness of these factors associated with low-engagement with remote monitoring platforms should encourage clinicians to have open and informed discussions with women about their preferred method of team interactions. Low-engagement with remote monitoring platforms can also be considered a signal for increased complications after delivery.

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Method validation and trueness estimation of thyroglobulin by LC-MS/MS.

Chris Hodgkins[1], Maria Charan[1], Peter Ward[1], Lucy Ding[1], Douglas Chesher[1], Cameron Wood[1]

1. NSW Health Pathology, St Leonards, NSW, Australia

Introduction

Accurate quantification of serum thyroglobulin (TG) is crucial for monitoring patients after treatment for Differentiated Thyroid Carcinoma (DTC). However, challenges arise from potential interference by Thyroglobulin Abs (TGAB) with immunoassays (IA), leading to potentially falsely low or negative results in TG measurements. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) has emerged as a reliable analytical technique offering high sensitivity and specificity for TG measurement, addressing limitations associated with traditional IA methods.

Methods

An LC-MS/MS method was developed based on a published, validated clinical TG mass spectrometry (TG-MS) assay[1], with minor adjustments to sample preparation and instrument settings made to suit local laboratory infrastructure.

Sufficient analyses were conducted to meet the criteria for "pre-validation"[2], demonstrating the local TG-MS assay's suitability in terms of sensitivity, linearity, and precision for full analytical and clinical validation.

In this study, we present the early stages of method validation and trueness estimation for TG quantification using LC-MS/MS, with thyroglobulin standardised to the Roche Cobas E601 assay using TGAB-negative human serum pools.

Results

A cohort of TGAB negative patient samples analysed by both IA and LC-MS/MS showed good concordance in both Passing-Bablok and Bland-Altman comparisons, with bias <5%.

In a cohort of TGAB positive samples, LC-MS/MS consistently measured higher Tg values than IA and correlation was overall poor.

When TGAB positive and TGAB negative samples were mixed before analysis, TG-MS demonstrated closer alignment to the theoretical concentration than IA.

Conclusion

Quantification of TG by LC-MS/MS addresses the challenges posed by positive TGAB, providing accurate and precise TG measurement in the context of DTC. The demonstrated robustness and reliability of this LC-MS/MS-based assay, establish it as a suitable tool for routine clinical use, supporting informed clinical decision-making in the management of DTC patients.

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Diagnostic accuracy of the water deprivation test combined with fasting copeptin in the polyuria-polydipsia syndrome

Claudia Ashkar[1], Maresa Derbyshire[1], Balasubramanian Krishnamurthy[1, 2], Richard MacIsaac[1, 3], Nirupa Sachithanandan[1]

1. Endocrinology and Diabetes, St Vincent's Hospital, Melbourne, VIC, Australia

2. St Vincent's Institute of Medical Research, Melbourne, VIC, Australia

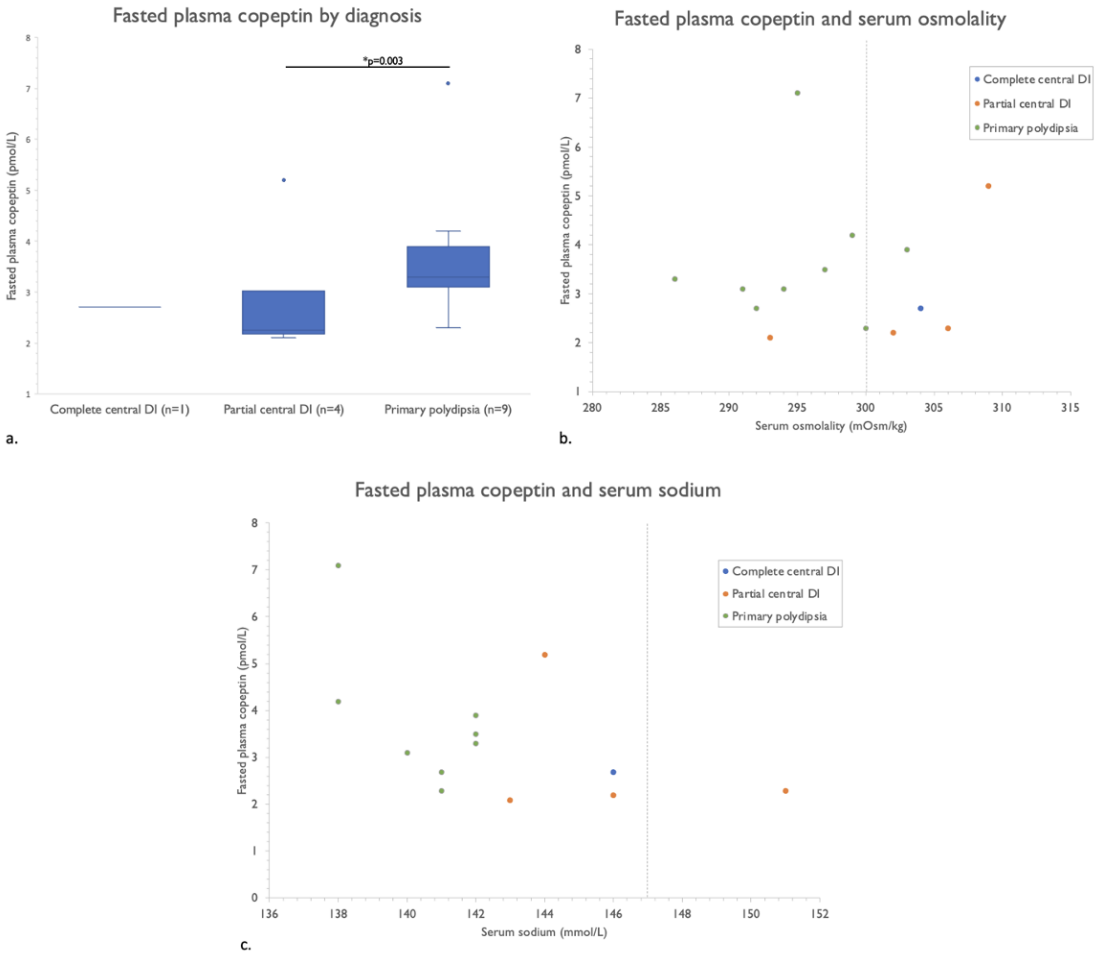
3. University of Melbourne, Melbourne, VIC, Australia

Aims: The water deprivation test (WDT) is the gold standard investigation to distinguish the diagnosis for the polyuria-polydipsia syndrome. Fasted plasma copeptin has emerged as a useful adjunct to distinguish the diagnosis. The aim of this study was to assess the diagnostic accuracy of the WDT and the utility of 8hr fasted copeptin.

Methods: This was a retrospective cohort study of 63 outpatient WDTs completed between 2012-2022 in a tertiary referral centre. A standardized WDT was undertaken. In 12 cases, plasma copeptin was measured at 8 a.m. following 8hr overnight fast. Diagnosis was based on standard biochemical diagnostic criteria and, where results were equivocal, independent review of clinical features and response to treatment was undertaken by two Endocrinologists.

Results: Sixty-one patients underwent 63 WDT's and 37 (60.7%) were diagnosed with primary polydipsia, 17 (27.9%) were diagnosed with partial central diabetes insipidus (DI), 6 (9.8%) with complete central DI and 1 (1.6%) was inconclusive. The diagnosis from WDT alone was concordant with predefined biochemical diagnostic criteria in 53 (84.1%) tests. Where a fasted copeptin was measured, the corresponding serum sodium was >147 mmol/L in one patient (1/12, 8.3%) and serum osmolality was >300 mOsm/kg in 6 patients (6/12, 50%). In patients with primary polydipsia the median 8hr fasted copeptin (3.3 pmol/L, IQR 3.1-3.9) was significantly higher compared to patients with partial central DI (2.25 pmol/L, IQR 2.1-4.5, p=0.003). A fasted copeptin (pmol/L) to serum sodium (mmol/L) ratio >0.02 demonstrated a sensitivity of 77.8%, specificity of 75% and positive predictive value of 87.5% in diagnosing primary polydipsia.

Conclusion: WDT identifies a clear diagnosis in the majority of presentations with polyuria-polydipsia syndrome. The plasma copeptin following 8hr overnight fast did not improve the diagnostic accuracy of WDT, as most patients had not yet reached the osmotic threshold to stimulate copeptin release.



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A retrospective study on clinical characteristics treatment approaches and outcome data of patients with prolactinoma followed up at a tertiary care center in Sri Lanka

Tharaka Gayaththri Athukorala[1], Nethrani Sameera W Pathirana[1], Pavani G Punchihewa[2], Sivatharshya Pathmanathan[1], Uditha Bulugahapitiya[1], Manilka Roshan Sumanatilleke[1]

1. Diabetes and Endocrine Unit, National Hospital of Sri Lanka, Colombo 10, Colombo, Sri Lanka

2. Diabetes and Endocrine Unit, Sri Jayawardenapura General Hospital, Sri Jayawardenapura, Sri Lanka

Prolactinoma accounts for the majority of pituitary adenomas and can be successfully treated with dopamine agonists. However, data looking at treatment modalities and outcomes are scarce in the South Asian region. This Knowledge can bring new insights into managing prolactinomas in resource-poor settings.

A descriptive cross-sectional study was conducted at the Diabetes and Endocrine Unit at the National Hospital of Sri Lanka to assess clinical characteristics, therapeutic approaches and their outcomes in patients with prolactinoma followed up at a tertiary care center in Sri Lanka. Socio-demographic and clinical data were gathered through an interviewer-administered questionnaire and medical records.

Data was collected from 54 patients and 51.9 % (n=28) were female. The mean age of the population was 43.3 ± 12.98 years. Females had a mean age at presentation of 35.95 ± 13.53 years whereas among males it was 33.32 ± 9.31 years. In females 75%(n=21) presented with headache, 67.8% (n =19) with galactorrhea and 64.2% n=18) with menstrual irregularities. Most males, 88.4% (n=23), presented with visual impairment due to the mass effect, whereas 73% (n=19) presented with headache. Majority of the population had macroprolactinomas (53.7% n=29) with a mean prolactin level of 36015.7 mIU/L. Microprolactinomas accounted for only 29.6% (mean prolactin-5952.87mIU/L) while 16.8% (n =9) had giant prolactinomas (mean prolactin-78588.62 miu/L) 2 patients who had surgery as the first-line treatment (3.7%) failed to achieve remission. 37 patients received cabergoline as first-line treatment and 19 are tumour free. 12 patients received bromocriptine and 8 are tumour-free. 5 received radiotherapy without achieving remission.

Among Sri Lankan patients with prolactinoma ,dopamine agonists as first-line treatment have a good response. However, further studies are recommended to decide on the best treatment strategy in a resource-poor setting like Sri Lanka when current management is shifting towards surgery.

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Vitamin D levels and outcomes of diabetes related foot ulceration

Nada Bechara[1, 2, 3], Jenny Gunton[2, 3, 4], Peta Tehan[5]

1. Department of Diabetes and Endocrinology, Blacktown-Mt Druitt Hospital, Blacktown, NSW, Australia

2. Westmead Hospital, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia

3. Centre for Diabetes, Obesity and Endocrinology Research (CDOER), The Westmead Institute for Medical Research, The University of Sydney, Westmead, NSW, Australia

4. Faculty of Medicine and Health, Sydney School of Health Sciences, The University of Sydney, Camperdown, NSW, Australia

5. Monash University, Melbourne

Diabetes-related foot ulcers are a common complication of diabetes, with 19-34% of people with diabetes developing a foot ulcer over their lifetime. Vitamin D is known to be important for many bodily processes, however, impacts on healing, specifically for foot ulcers are unknown.

This study aimed to assess serum vitamin D with healing trajectory, amputation, and ulcer size in those with DFU. Participants were sent for routine bloods at baseline encompassing usual blood metrics as well as vitamin D levels. Vitamin D deficiency was classified as less than 50 mmol/L.

One hundred adults (72 males and 28 females) were recruited from Blacktown High Risk Foot Service (HRFS) from August 2021 to October 2022. Average age was 62.47 years and duration of diabetes was 20.6 years. The DFU was present for a median of 60 days (IQR 30-120) prior to initial presentation at the HRFS. There was a 50% deficiency rate of vitamin D, with a median vitamin D of 50 mmol/L. Median HbA1c was 8% and CRP 7 mg/L. CRP was significantly correlated with size at baseline ($p=0.022$), and amputation or death at end of follow up period ($p=0.007$). Participants who were vitamin D deficient were more likely to have an increased weight ($p=0.033$). Serum vitamin D was also correlated with age ($p<0.001$), HbA1c ($p=0.009$), and sodium ($p=0.025$). WIfI score was correlated with amputation or death at end of follow up ($p<0.001$). There were no correlations between vitamin D and ulcer healing trajectory.

In conclusion, those who are vitamin D deficient are more likely to be heavier, older, and have an increased HbA1c. WIfI scores were indicative of amputation at the end of the study period, confirming the use of the WIfI scoring system for DFU. Larger sample sizes are required to assess any correlations between vitamin D and DFU.

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Less than subtotal parathyroidectomy is an effective surgical approach for multiple endocrine neoplasia type 1–primary hyperparathyroidism

Jessica Bindra[3, 1, 2], Lyndal J Tacon[3, 1], Roderick Clifton-Bligh[3, 1, 2]

1. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

2. The Kolling Institute, St Leonards, NSW, Australia

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

Background: Primary hyperparathyroidism (pHPT) is the most common and usually first endocrinopathy diagnosed in patients with multiple endocrine neoplasia type 1 (MEN1). Less than subtotal parathyroidectomy (LSTP) with selective gland removal can mitigate the risk of developing permanent hypoparathyroidism, which is often seen following total and subtotal parathyroidectomy (STP), while effectively correcting hypercalcaemia.[1,2] This retrospective cohort study aims to compare the outcomes of LSTP, STP and total parathyroidectomy in MEN1-associated pHPT.

Methods: Patients who underwent parathyroidectomy for MEN1-related pHPT were included and informed consent was obtained. Data was extracted from electronic medical records including demographics, type of surgery, length of follow-up, and rates of disease persistence (hyperparathyroidism <6 months), recurrence (hyperparathyroidism >6 months), and hypoparathyroidism.

Results: Ten patients were included with a median age of 39 years (range 16-61), 3 males and 7 females. One patient underwent total parathyroidectomy upfront and developed permanent hypoparathyroidism despite autotransplantation. An additional patient required completion parathyroidectomy and autotransplantation following LSTP due to persistent hyperparathyroidism, and also developed permanent hypoparathyroidism. STP with autotransplantation was the initial surgical choice in one patient and was complicated by permanent hypoparathyroidism. LSTP was the initial surgery in eight (80%) patients, including 2-gland-removal in three patients (37.5%) and 3-gland-removal in five patients (62.5%). LSTP was complicated by persistent disease in two patients and transient hypoparathyroidism in a further two patients, with both cases resolving within 6 months. All cases of disease recurrence occurred in the LSTP group with a rate of 30% and a median time to recurrence of 52 months (range 15-56).

Conclusion: This single-institution retrospective study describes LSTP as an effective method for managing MEN1-related pHPT, with no reported cases of permanent hypoparathyroidism. Though disease recurrence occurred in 30%, the median time to recurrence was close to 4.5 years, making it an appealing option for younger patients.

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The Genetic Landscape of Familial Non-Medullary Thyroid Cancer (FANTOM): Protocol for a multi-centre cohort study

Jessica Bindra[3, 1, 2], Roderick Clifton-Bligh[3, 1, 2], Matti Gild[3, 1, 2]

1. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

2. The Kolling Institute, St Leonards, NSW, Australia

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

Background: Familial non-medullary thyroid cancer (FNMTC) can be either syndromic or, more commonly, non-syndromic. In syndromic disease a driver germline mutation is known, such as Cowden syndrome, familial adenomatous polyposis and DICER1 syndrome, while in non-syndromic disease there is limited understanding of its genetic basis. There is an unmet need to investigate the genetic landscape of non-syndromic FNMTC as it carries poorer prognosis when compared to its syndromic and sporadic counterparts.[1-5] Identifying contributing genetic factors may pave the way to create appropriate screening guidelines and eventually identify targeted therapies to improve outcomes.

Objectives: (1) To describe the multi-centre experience of patients with syndromic FNMTC, in turn alerting clinicians to facilitate timely recognition, surveillance, management and genetic counselling and testing of family members where required; and (2) To identify novel genetic contributors in patients with non-syndromic FNMTC through whole genome sequencing (WGS).

Design and Methods: Royal North Shore Hospital (RNSH) will coordinate a two-part multi-centre study, involving retrospective collection of clinicopathologic data and prospective collection of blood samples for WGS. Recruitment will commence at RNSH. We will include adults > 18 years with FNMTC (i.e. differentiated thyroid cancer, DTC) based on the presence of at least TWO first-degree relatives with DTC. The control group will include unaffected family members > 40 years with benign-appearing thyroid ultrasound and/or fine needle aspirate biopsy. Outcome measures will include pathogenic and likely pathogenic mutations in FNMTC identified by WGS and the association between genetic mutation(s) identified and clinical phenotype of non-syndromic FNMTC.

Analysis Plan: We will perform WGS on FNMTC cases and familial unaffected controls. If syndromic loci negative, we will examine for shared variants within each nuclear family case. Analysis will include shared variants across all FNMTC cases and exclude these identified shared variants in controls (via GnomAD, 1000g, sporadic DTC cases from TCGA).

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Peptide receptor radionuclide therapy (PRRT) for management of ectopic Cushing's syndrome due to metastatic gastroenteropancreatic neuroendocrine neoplasia (GEPNEN): A single centre experience

Emma Boehm[1], Terry Hung[1], Tim Akhurst[1], Anthony Cardin[1], Ramin Alipour[1], Cherie Chiang[1], Rod Hicks[2], Michael Hofman[1], Michael Michael[1], Nirupa Sachithanandan[1], Javad Saghebi[1], Grace Kong[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

2. St Vincent's Hospital, Melbourne, Vic, Aus

AIM: Metastatic GEPNEN can cause ectopic Cushing's syndrome (ECS) due to secretion of ACTH or CRH. ECS is highly morbid and challenging to control with medical therapy(1). Bilateral adrenalectomy carries perioperative risk and long-term morbidity. Patients who are unsuitable for surgery have dismal outcomes(2). We describe the biochemical and clinical outcomes of patients with metastatic GEPNEN and ECS treated with PRRT in our centre.

METHODS: Single-centre, retrospective analysis of the molecular imaging, biochemistry, and clinical outcomes of 7 consecutive patients with ECS caused by metastatic GEPNEN treated with radionuclide therapy (PRRT) from 2006-2023.

RESULTS: See table 1 for cohort demographic and clinical details. The primary site of disease was pancreas (5/7) and rectum (2/7). Prior to PRRT all patients had high somatostatin receptor (SSTR)-expressing and FDG-avid imaging phenotype of positron emission tomography (PET) scans. All patients (except one who had bilateral adrenalectomy performed prior to PRRT) were on medical therapy for ECS prior to PRRT. Five patients had clinical and biochemical flare of ECS within one week of the first cycle of PRRT. Following PRRT, five of seven patients had complete biochemical resolution of ECS (four ongoing at last follow-up; one ECS recurrence after 14 months); bilateral adrenalectomy was avoided in three and delayed in two patients (17 and 9months respectively). Following PRRT, three patients had a complete metabolic response (CMR) on FDG-PET imaging, two had a partial metabolic response, two progressed. Biochemical and imaging responses were durable, the longest ECS regression and CMR is ongoing at 14 years.

CONCLUSION: PRRT is effective in controlling ECS caused by metastatic SSTR-positive GEPNEN. PRRT may avoid or delay adrenalectomy and lead to durable biochemical responses. Careful multidisciplinary management to consider treatment sequencing and management of post treatment Cushing's flare is required.

Age/ Sex	Primary site	Grade (Ki-67 %)	PRRT regimen	Treatment of ECS prior to PRRT (CLT)	Pre-treatment ACTH (range)	Post-treatment ACTH (range)	FDG response post initial PRRT	Time to normal ACTH (months)	Time to cessation of CLT	Survival (months)
17F	Pancreas	G1/2 (ND)	^{111}InTate +5FU ^{177}LuTate	Bilateral adrenalectomy	Unknown	1.5pmol/l (1.6-13.9)	CMR	Unknown	NA	Alive (169)
25F	Pancreas	G3 (50*)	^{177}LuTate +cap	Metyrapone	40.0 pmol/L (1.6-13.9)	30.5ng/L (7.2-63.3)	CMR	1.25	8.25	Alive (38)
54F	Pancreas	G3 (50*)	^{177}LuTate +cap	Metyrapone	269ng/L (7.2-63.3)	17.4ng/L (7.2-63.3)	PMR	3.25	3.75	Alive (26)
75M	Rectum	NEC (>95)	^{177}LuTate	Ketoconazole	22 ng/L (9-51)	317ng/L (9-51)	Progression	NA	NA	Deceased (23)
53F	Pancreas	G2 (15*)	Y-Tate/ ^{177}LuTate+5 FU	Metyrapone	440ng/L (9-51)	4.6pmol/l (1.6-13.9)	CMR	7	7	Deceased (117)
60F	Rectum	G1 (2)	^{177}LuTate +cap	Metyrapone	158ng/L (9-51)	18.4ng/L (9-51)	PMR	2.5	continuing	Alive (22)
58F	Pancreas	G2 (15)	^{177}LuTate	Ketoconazole / metyrapone	563ng/L (7.2-63.3)	179pmol/ L (1.6-13.9)	Progression	NA	continuing	Alive (9)

Table 1: Summary of selected clinical characteristics of ECS caused by GEPNEN patient cohort. *liver biopsy; ^{111}InTate ^{111}Indium octreotate, ^{177}LuTate ^{177}Lutetium DOTA-octreotate, cap capecitabine, CLT cortisol lowering therapy; CMR complete metabolic response, G1/2/3 Grade1/2/3, NA not applicable, ND not done, PMR partial metabolic response, PRRT peptide receptor radionuclide therapy.

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Improving adrenal vein sampling with point-of-care cortisol assays

Christopher Chan[1, 2], Brian Tran[1, 2], Yu-Fang Wu[2], Cankayan Suthaharen[3], Luke Baker[4], Joshua Ryan[3], Christian Girgis[1, 2]

1. Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

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

3. Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Sydney, NSW, Australia

4. Department of Radiology, Westmead Hospital, Sydney, NSW, Australia

Background: There is an increasing prevalence of primary aldosteronism in recent decades[1]. Adrenal vein sampling (AVS) remains the gold standard in identifying unilateral surgically curable causes of primary aldosteronism[2,3]. AVS is a time-consuming, expensive, invasive and technically challenging procedure[1,4,5] with success rates historically dependent on centre-specific expertise and ranging between 30% and 96%[6]. Rapid point-of-care cortisol assays (POCCA) have been shown to improve success rates even in less experienced hands[2,7-9].

Aims: Review effectiveness of POCCA (Trust Medical, Japan) for successful bilateral cannulation

Methods: AVS was performed using ACTH stimulation protocol (50 micrograms/hr continuous cosyntropin). In procedures undertaken between January 2019 and March 2022 (n=33), successful intraprocedural cannulation was determined using standard radiological criteria and POCCA were not utilised (pre-POCCA group). From April 2022 to August 2023, the use of POCCA was implemented to confirm successful intraprocedural cannulation for all patients (n=25) (POCCA group).

Successful cannulation was confirmed post-procedurally using standard biochemical criteria with adrenal/paired peripheral cortisol levels (Selectivity index= SI) ≥ 3.0

Assessment of cannulation success using POCCA was evaluated on-site by 3 independent reviewers blinded to the lab results.

Result: The pre-POCCA group had 16 successful bilateral cannulations (48.5%), 12 unsuccessful on the right vein only, and 5 unsuccessful bilaterally on post-procedure biochemistry. Two patients accounted for 4/5 bilaterally unsuccessful cases.

For the POCCA group, 22/25 cases (88%) had intraprocedural and post-procedure biochemical confirmation of successful bilateral cannulation. The 3 unsuccessful cases were related to difficulties in aspirating the right adrenal vein only.

POCCA imparted a 2 fold improvement in successful bilateral cannulation between the groups (p = 0.002).

Conclusion: This demonstrates an improvement in success rates in AVS over a 3-year period with the use of POCCA. The use of POCCA allowed for earlier treatment decisions when considering sample collection and repeat attempts.

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An audit on clinical characteristics and hospital course of patients admitted with hyponatremia.

Divij Sharma[1], Sukesh Chandran[1]

1. St John of God Hospital Perth, Midland, WESTERN AUSTRALIA, Australia

No. of patients
Age
Sex

Aims

1. To investigate the frequency, clinical, biochemical characteristics, underlying diagnosis and clinical outcomes, and patient profiles of 30 hyponatremic patients.
2. To classify which treatment regime and correction rate was followed as per European Society of Endocrinology Guidelines.

Methods

We classified patients according to the clinical severity of Na as mild, moderate and severe. They were also classified into acute vs. chronic as per the time of development (48 hours is cut off), symptomatic vs. asymptomatic, and hypovolemic vs. euvolemic vs. hypervolemic. This classification was done to evaluate directions for diagnosis and treatment. In addition, the records of these patients were reviewed for relevant demographic, clinical, and laboratory data. The underlying diagnoses and complications after treatment were also sought.

Result

The Image represents data as per clinical, demographic characteristics and treatment administered in 30 hyponatremic patients.

Interestingly, rare conditions like autoimmune encephalitis and glioblastoma were the underlying cause of 4 euvolemic hyponatremic patients.

On the treatment front, 12 patients had more than one cause behind the hyponatremia; hence more than one treatment modality was administered in these patients. European guidelines recommend a rate of around 10 mmol/L per day. However, we kept the range of 6-8mmol/L as our reference due to the risk of Osmotic Demyelination Syndrome in high-risk patients. This table illustrates that the correction rate did not exceed recommended limits.

Conclusion

Following European guidelines, no treatment modality stood as the absolute gold standard or benefit in our audit; hence we saw that more than one regime was meticulously used in most patients to reach the Na target. More than 50% of patients had hyponatremia detected incidentally on routine biochemical tests, consistent with the literature. This audit is directed for doctors across all sub-specialties to know recent treatment guidelines for diagnosing and managing hyponatremia patients.

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The comparison of pregnancy outcomes between diet and insulin treatment in women with GDM: a case-control study

George Q Chen[1], Lin Wang[2], Yue Wang[2], Minghui Qin[2], Dengxin Zhang[2], Yanfang Gu[2], Qi Chen[3], Min Zhao[2]

1. Royal Liverpool University Hospital, , Liverpool, the United Kingdom

2. Wuxi Maternity and Children Health Hospital, Wuxi, China

3. The University of Auckland, Grafton, Auckland, New Zealand

Gestational diabetes mellitus (GDM) affects 15% of all pregnancies, with serious consequences for mothers and babies. GDM women normally receive lifestyle intervention or insulin treatment. However, there were no criteria for the option of treatment. We undertook this study to compare pregnancy outcomes between GDM women managed by lifestyle intervention and insulin treatment after diagnosis. Additionally, we investigated the impact of normal and abnormal glucose levels of oral glucose tolerance tests (OGTT) on pregnancy outcomes in GDM women with lifestyle intervention.

Data on maternal age at diagnosis, gestational age at diagnosis and delivery, birthweight, Apgar score, pregnancy complications, and weekly weight gain were collected. Pregnancy outcomes were compared between the lifestyle intervention (n=309) and insulin treatment (n=45) groups. We also analysed pregnancy outcomes in GDM women with lifestyle intervention based on normal or abnormal glucose levels of an OGTT.

The mean gestational age at delivery was no difference between the two groups. There was no difference in glucose level in 0h-OGTT or 2h-OGTT between the two groups. No statistical difference in the incidence of LGA, should dystocia, preeclampsia, and planned or emergency caesarean sections between the two groups. Additionally, there were no differences in pregnancy outcomes in GDM with lifestyle intervention, whether they had a normal or abnormal glucose level of an OGTT. Furthermore, there was no difference in the number of GDM women with abnormal fasting glucose levels at delivery between lifestyle intervention and insulin treatment, and there was no difference in adverse pregnancy outcomes between these two groups.

Although there is no international guideline for insulin treatment in GDM, our data demonstrate no difference in adverse pregnancy outcomes between lifestyle intervention and insulin treatment. Additionally, there were no differences in adverse pregnancy outcomes in GDM women with abnormal glucose levels at delivery between lifestyle intervention and insulin treatment.

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Incidence and risk factors of transient and permanent post-thyroidectomy hypocalcaemia at a rural referral hospital in Australia.

Aiden Man Hei Cheung[1], Aye Thin[2]

1. South Western Sydney Local Health District, Campbelltown, NSW, Australia

2. Endocrinology, Tamworth Rural Referral Hospital, Tamworth, NSW, Australia

Aims

Transient (<12 months) and permanent (>12 months) post-thyroidectomy hypocalcaemia (PTHC) are known complications following thyroidectomies. Their reported incidences vary widely at 19-38% and 0-3% respectively [1]. Different preoperative and postoperative factors have been reported to be associated with PTHC. This study aims to evaluate the incidence and risk factors of PTHC at an Australian centre.

Methods

This was a single-centre retrospective cohort study of a rural referral hospital in New South Wales, Australia. 399 cases of total or completion thyroidectomies between 01/06/2012 and 31/05/2022 were identified using electronic medical records. Hypocalcaemia was defined as a corrected serum calcium of <2.10 mmol/L.

Results

The incidences of transient and permanent PTHC were 15.3% and 3.3% respectively. PTHC was first detected beyond 24 and 48 hours postoperatively in 8.5% and 2.5% of patients. No patients required readmission for symptomatic hypocalcaemia.

Transient PTHC was associated with preoperative and 24-hour postoperative hypoparathyroidism ($p = 0.008$ and $p < 0.001$ respectively), and malignant histology (14.3% in benign and 21.3% in malignant, $p<0.001$). Amongst surgical indications, the incidence of transient PTHC was higher in suspicious thyroid nodules (22.2%), Grave's disease (20%), and thyroid cancer (18.9%) than multinodular goitre (13.4%) and obstructive goitre (13.2%) ($p<0.001$).

Hypoparathyroidism 24-hour postoperatively was the only factor associated with permanent PTHC, with the incidences being 8.9%, 0.45% and 0% in hypoparathyroid, euparathyroid and hyperparathyroid patients ($p<0.001$).

Patients' sex, age, preoperative thyroid status and immediate postoperative parathyroid status were not significantly associated with PTHC.

Conclusion

PTHC remains potentially life-threatening complications despite advancement in surgical techniques. No perioperative factors can accurately predict PTHC. Therefore, all post-thyroidectomy patients should undergo routine monitoring for hypocalcaemia for at least 24-48 hours postoperatively.

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Non-Alcoholic Steatohepatitis Is Associated With Diabetic Cardiomyopathy in Type 2 Diabetes

Dong-Hyeok Cho[1], Jin-Ook Chung[1], Dong-Jin Chung[1]

1. Endocrinology and Metabolism, Chonnam National University Hospital, Dong-ku, Gwangju, South Korea

Background: Abnormalities in cardiac structure and function in type 2 diabetic patients may develop in the absence of ischemic heart disease. These abnormalities are attributed to diabetic cardiomyopathy. Impaired diastolic heart function has been observed in persons with non-alcoholic fatty liver disease (NAFLD) and/or with type 2 diabetes. We investigated the association between liver fibrosis and left ventricular (LV) diastolic dysfunction in type 2 diabetes.

Methods: We studied 92 patients with type 2 diabetes (51 men; mean age 62 ± 6 years) who had undergone liver ultrasonography and conventional Doppler echocardiography. Presence of NAFLD and/or advanced liver fibrosis was determined by abdominal ultrasonography and NAFLD fibrosis score (NFS). LV diastolic dysfunction was defined according to transmitral peak early to late ventricular filling (E/A) ratio and deceleration time (DT), using echocardiography.

Results: Fifty-four patients (58.7%) had NAFLD. On echocardiography, LVEF was within normal range in both groups, whereas LV mass index (P=0.035) and LA diameter (P=0.021) were significantly greater in the NAFLD patients. The systemic vascular resistance and arterial elasticity were not different. NAFLD patients had lower E/A ratio (P=0.014) and longer DT (P=0.042) than those without steatosis. When NAFLD was stratified by NFS, subjects with advanced liver fibrosis exhibited a higher prevalence of diastolic dysfunction (46.2%, 55.6%, 60.0%; none, simple steatosis, advanced fibrosis, respectively; P for trend = 0.025). In multivariate logistic regression, liver fibrosis was independently associated with diastolic dysfunction (OR=1.46, 95% CI=1.02-2.46, P=0.041) after adjusting for cardiometabolic risk factors.

Conclusions: Our data show that in patients with type 2 diabetes and NAFLD, liver fibrosis was associated with LV diastolic dysfunction and may be an independent risk factor for diastolic dysfunction.

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The role of adipsin for pancreatic beta-cell function in type 2 diabetes

Hochan CHC Cho[1], Jae-Hyung PJH Park[2], Ji Hong YJH You[1]

1. Keimyung University Dongsan Hospital, Daegu, DAEGU, South Korea

2. Department of Physiology, Keimyung University School of Medicine, Daegu, South Korea

Adipsin, as an adipokine, has an important role in maintaining beta cell function. However, the association between adipsin and clinical parameters in type 2 diabetic(T2DM) patients remains unclear. We investigated plasma adipsin concentrations in three distinct patient groups: normoglycemia, T2DM, and T2DM with possible beta cell failure treated with insulin. Plasma adipsin concentrations were significantly decreased in T2DM treated with insulin, compared to T2DM without insulin treatment.

Furthermore, adipsin had significantly positive correlations with fasting serum C-peptide concentration, 2h C-peptide, and 2h C-peptidogenic index. Interestingly, the normoglycemic and T2DM groups did not exhibit a significant correlation between adipsin and other clinical parameters. Also, we found significant T2DM-associated variants within adipsin, Using the TIGER portal. Our findings suggest that adipsin may be a potential biomarker of pancreatic beta-cell function in T2DM with possible beta cell failure.

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Radiation-induced bone loss in women treated for gynaecological malignancies

Terry Diamond[1], Charles Godber[1], Catherine Clark[1], Reza Rahbari[1], Carl Bryant[2]

1. St George Hospital, Sydney, NSW, Australia

2. Interventional Radiology, St George Private Hospital, Sydney, NSW, Australia

Pelvic insufficiency fractures commonly occur in women receiving external beam radiotherapy (EBRT) for gynaecological malignancies (GM). Our study was to determine the effects of EBRT on the skeleton.

24/127 women treated from 2020-23 and who met the entry criteria were evaluated. Their mean age was 63.2 (45-85) years, 21/24 were post-menopausal and 19/24 were non-smoker. Endometrial cancer was the most common primary site (18/24). 9/24 received EBRT to the pelvis and para-aortic nodes and 15/24 only to the pelvis. The total radiation dose and the mean radiation exposure to the third lumbar vertebrae (L3) and left femoral neck (FN) were calculated. BMD of lumbar spine (LS) and FN was performed prior to and within 18 months of EBRT by quantitative computed tomography (QCT). Precision error was 1% for LS and 1.5% for FN.

The mean time from EBRT to follow up QCT was 6.2 (range 1.0-15.1) months. BMD decreased significantly in 20/24 (83.3%) patients at the LS and 13/24 (54.2%) at the FN. The mean change in BMD was -29.1% (95% CI; -17.7% to -40.6%) at LS and -4.1% (95% CI; -0.5% to -8.7%) at FN. The mean change in LS BMD was significantly more pronounced ($t(13) = 3.354$, $p = 0.05$) when the treatment field was extended above the L4 (-50.2%, 95% CI; -29.8% to -70.5%) compared to treatment at L4 or below (-16.5%, 95% CI; -6.1% to -16.5%). There was a significant correlation between radiation dose and %change in BMD at L3 ($r=-0.739$, $p>0.001$), but not at the FN ($r =-0.2$, $p=0.30$). The change in the LS BMD correlated weakly with time since radiotherapy ($r=-0.36$, $p=0.08$).

These data demonstrate that women treated with EBRT for GM develop severe radiation-induced osteoporosis. The radiation dose, field size and time since radiotherapy are important variables contributing to skeletal bone loss.

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Impact of the current lack of standardisation on the validity of pathological assessment of pituitary tumours: What clinicians need to know.

Prishila Fookeerah[1, 2], Winny Varikatt[3, 4], Mark Mclean[1, 2]

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

2. School of Medicine, Western Sydney University, Sydney, NSW, Australia

3. Department of Tissue Pathology and Diagnostic Oncology, Westmead Hospital, Sydney, NSW, Australia

4. Westmead Clinical School, University of Sydney, Sydney, NSW, Australia

Background

The application of transcription factor(TF) analysis has transformed understanding of the biology of pituitary tumours. However, there remains a high degree of heterogeneity in epidemiological and clinical patterns seen in different studies, precluding the development of clinical guidelines for histological subtypes. We conducted a systematic review and meta-analysis of methodological and diagnostic criteria in recent studies, exploring inconsistencies in the literature.

Methods

A PubMed search was conducted with keywords “pituitary tumour”, “classification”, “transcription factor” and “clinicopathological”. Abstracts of studies involving pituitary tumours, published in English between 2015 to February 2023 were screened for relevance. Studies including all 3 TF were evaluated for methodology, antibody type and scoring strategy. Proportion of tumour subtypes was assessed.

Results

Nine of thirteen identified studies were retrospective and used stored tissue blocks. Mean storage time of oldest specimens used in retrospective studies was 17.1 +/- 6.8 years at time of publication. There was a positive linear correlation between percentage of null cell (NC) tumours and the age of tissue analysed ($R^2= 0.71$, $p=0.03$). Threshold definitions for positive identification of tumour types varied from 5% to 80% positive tumour cells. Higher cut-off did not allow detection of plurihormonal tumours. Five studies reporting clinical data indicated that 33-100% of NC tumours showed radiological invasion. In the non-functioning tumour category, estimated proportion of lineage-based subtypes was: 5.6% (95% CI 4.6-6.7%) silent PIT1, 18% (16.7-20.1%) silent corticotroph tumours, 68% (66-70%) gonadotroph tumours. Significant heterogeneity was observed, with I^2 of 81.7%, 71.7% and 75.7% in each group ($p<0.0001$).

Conclusion

There is marked heterogeneity in proportion of tumour subtypes in studies. Higher incidence of NC tumours is seen with older tumour specimens, possibly representing false negative staining. Plurihormonal tumours can be missed if higher positivity cut-off is applied. Non-standardization of methods poses problems for clinical interpretation of results.

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Clinical behaviour of multilineage tumours expressing PIT1 and SF1 in a cohort of tumours at Westmead Hospital

Prishila Fookeerah[1, 2], Winny Varikatt[3, 4], Meena Shingde[3, 4], Mark AJ Dexter[4, 5], Mark Mclean[1, 2]

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

2. School of Medicine, Western Sydney University, Sydney, NSW, Australia

3. Department of Tissue Pathology and Diagnostic Oncology, Westmead Hospital, Sydney, NSW, Australia

4. Westmead Clinical School, University of Sydney, Sydney, NSW, Australia

5. Department of Neurosurgery, Westmead Hospital, Sydney, New South Wales, Australia

Background

Multilineage PIT1 and SF1 pituitary neuroendocrine tumours (PitNETs) are a rare morphological subtype consisting of a single tumour population co-expressing transcription factors PIT1 and SF1. These tumours may also demonstrate variable combinations of hormonal immunostaining corresponding to both lineages. Clinical manifestations can vary depending on the dominant hormone secreted by the tumour. The overall clinical behaviour and prognosis of these PitNETs is not known.

Methods

We performed a retrospective assessment of the histological characteristics and clinical outcomes of a series of nine multilineage PIT1 and SF1 PitNETs identified from a cohort of 246 pituitary tumours at Westmead Hospital. Tumours were assessed for the presence of pituitary transcription factors, hormonal expression and somatostatin receptor (SSTR) status using immunohistochemistry. Clinical data for each tumour was reviewed.

Results

These tumours represented 3.7% of our PitNET cohort. Eight tumours (88.9%) expressed growth hormone and caused acromegaly at presentation. One tumour did not express any PIT1 lineage hormones. Of the 7 macrotumours that caused acromegaly, only one had radiological cavernous sinus invasion. Ki 67 labelling index was low, ranged from 0.6 to 3.6%. 88% of tumours secreting excess growth hormone exhibited strong immunostaining for SSTR2 and all tumours displayed weak immunoreactivity for SSTR5. In 62.5% of patients with acromegaly, cure was achieved after surgical resection. Somatostatin receptor ligands resulted in clinical remission in all cases where medical treatment was initiated. There was no new tumour recurrence or regrowth over an overall mean follow-up period of 62.5 months (12-132 months).

Conclusion

The majority of multilineage PIT1 and SF1 tumours were macrotumours expressing growth hormone and causing acromegaly. Surgical cure was achieved in over 60% of tumours causing acromegaly. Overall prognosis appears favourable over a mean follow up of approximately 5 years.

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New histological variants of pituitary tumours vary in their patterns of expression of somatostatin receptors.

Prishila Fookeerah[1, 2], Winny Varikatt[3, 4], Mark AJ Dexter[4, 5], Mark Mclean[6, 2]

1. Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

2. School of Medicine, Western Sydney University, Sydney, NSW, Australia

3. Department of Tissue Pathology and Diagnostic Oncology, Westmead Hospital, Sydney, NSW, Australia

4. Westmead Clinical School, University of Sydney, Sydney, NSW, Australia

5. Department of Neurosurgery, Westmead Hospital, Sydney, New South Wales, Australia

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

Introduction

The 2022 WHO classification of pituitary neuroendocrine tumours(PitNETs) recognises 17 histological variants of pituitary tumours. These PitNETs differ in their clinical manifestations and response to medical therapy. Tumour subtypes may express somatostatin receptors (SSTR), a potential drug target to achieve tumour reduction and control of hormone secretion. There is a well-established role for somatostatin receptor ligands(SRL) in the management of acromegaly and Cushing's disease. Previous studies have not assessed SSTR expression in the new histological variants. Our aim is to determine patterns of SSTR expression to understand differences in clinical behaviour and response.

Methodology

We conducted a retrospective study of PitNETs at Westmead Hospital resected from 2011 to 2018. Immunohistochemistry was performed on all tumours to evaluate expression of pituitary hormones, transcription factors(PIT1, SF1 and TPIT), co-factors (GATA3, oestrogen receptor) and somatostatin receptors (SSTR2, SSTR5). Patterns of expression of SSTR2 and 5 were assessed.

Results

246 pituitary tumours were included in this study. Positive SSTR2 expression was seen in 69.2% PIT1, 45.3% gonadotroph and 3.9% corticotroph tumours. The corresponding expression of SSTR5 was 67.3% of PIT1, 0.9% gonadotroph tumours and 23.5% corticotroph tumours. Among lactotroph tumours, 7.7% showed positive immunostaining for SSTR2; 38.5% expressed SSTR5. Among the histological variants that can cause acromegaly(GH-PitNETs), SSTR2 expression was seen in 94.7% and SSTR5 in 92.1%. When grade(intensity and extent) of immunoreactivity was assessed, 100% densely granulated somatotroph and mature plurihormonal PIT1 PitNETs showed strong SSTR2 expression compared to 11.1% sparsely granulated

somatotroph, 50% immature PIT1 and 45.4% mammosomatotroph tumours. 95% GH PitNETs showed only weak or moderate staining for SSTR5.

Conclusion

Not all GH-PitNETs strongly expressed SSTR2 or SSTR5, possibly accounting for differences in clinical response. A small proportion of corticotroph and lactotroph tumours expressed SSTR5. Our findings may be useful in predicting therapeutic response to SRL in the future.

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Real World Teriparatide Experience in a Tertiary Centre

Courtney Streeter[1], James Hill[1], Mitchell Pederson[2], Christian Girgis[2, 3], Roderick Clifton-Bligh[4, 2, 1], Matti Gild[4, 2, 1]

1. Northern Clinical School, Faculty of Health and Medicine, University of Sydney, Sydney, NSW

2. Endocrinology Department, Royal North Shore Hospital, St Leonards, NSW, Australia

3. University of Sydney, Sydney

4. Kolling Institute of Medical Research, St Leonards, NSW, Australia

Background: Osteoporosis has a significant morbidity and mortality burden. In Australia, antiresorptive medication is first line and PBS criteria limit accessibility to the anabolic agent Teriparatide.

Aims: To quantify the real-world effectiveness of 18 month teriparatide treatment in a clinical cohort. Secondary aims include (1) analysis of clinical characteristics that predict BMD improvement; (2) comparison of teriparatide cohort (TP) to a matched teriparatide-naïve cohort (control cohort CT) and assessment of follow up fracture rates.

Methods: Analysis of a retrospective cohort was performed utilising a database of DEXA scans performed on Hologic/Lunar scanners at RNSH in Sydney. EMR data was collected and analysed for 115 patients. 54 patients met the inclusion criteria for the TP cohort; receiving 18-24 months of teriparatide at RNSH from 2010-2022. A BMD-matched cohort of 61 patients was conceived as the CT, which had similar baseline T-scores but were teriparatide-naïve and primarily treated with bisphosphonates or denosumab between 2017-2022.

Results: Mean BMD increase in the TP cohort was associated with a statistically significant improvement of 5.99% at the lumbar spine and 2.04% at the total hip ($p<0.05$). The CT cohort similarly showed BMD improvements at only the lumbar spine and total hip of 6.47% and 4.39%, respectively ($p<0.001$). There were no statistically significant differences between the TP and CT cohorts ($p>0.05$). We were unable to predict BMD changes based on demographic and clinical characteristics. At one-year follow up, 11.11% of the TP cohort and 8.20% of the CT cohort had refractured, with no difference between groups ($p>0.05$).

Conclusions: This study showed that teriparatide has no benefit over a matched cohort treated with primarily bisphosphonates or denosumab. This may reflect that the current osteoporosis treatment sequence in Australia may require refinement.

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Heterogeneity of clinical behaviour of phaeochromocytomas in an MEN 5 kindred

Gregory I Hockings[1, 2], Amanda J Love[3], Sofia B Velosa[4], Roderick J Clifton-Bligh[5, 6], Emma L Duncan[7, 8]

1. Endocrinology Unit, Greenslopes Private Hospital, Brisbane, Qld , Australia

2. School of Medicine, University of Queensland, Brisbane, Qld , Australia

3. Department of Endocrinology, Royal Brisbane & Women's Hospital, Brisbane, Qld , Australia

4. Department of Endocrinology, Sunshine Coast University Hospital, Birtinya, Qld , Australia

5. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW , Australia

6. Faculty of Medicine and Health, The University of Sydney, Sydney, NSW , Australia

7. Department of Endocrinology, Guy's and St Thomas' NHS Foundation Trust, London SE1 9RT, UK

8. Faculty of Life Sciences and Medicine , King's College , London SE1 7EH, UK

MEN5[1], caused by germline mutations in the *MAX* gene, has been added to the 2022 revision of the WHO international diseases classification[2]. We describe the clinical, biochemical and radiological features of pheochromocytomas (PCCs) in nine affected family members from a single large MEN5 kindred, and the clinical course of these tumours.

Although all these patients have the same *MAX* mutation, they show considerable heterogeneity in their catecholamine and hormonal secretory profiles, nuclear medicine characteristics and the clinical behaviour of their tumours over an extended period of follow-up.

Eight of the affected family members had predominantly noradrenergic catecholamine secretion and presented with hypertension, while the ninth showed a predominantly adrenergic catecholamine profile and presented with paroxysmal symptoms and myocardial ischaemia. These secretory patterns did not change with recurrences in individual patients.

One patient has chronic acromegaly; their GH excess occurred synchronously with their catecholamine excess, and persisted after pituitary surgery, suggesting ectopic GHRH secretion. Another does not have phenotypic features of acromegaly, but has an intermittently elevated IGF-1 and a markedly asymmetric pituitary gland; her PCC tissue stained positive for GHRH.

The radionuclide scanning agents used varied because of the long time span involved in the diagnosis and follow-up of the various family members. I-MIBG uptake was variable among these patients. Ga-DOTATATE and/or F-DOPA were always taken up by the PCCs, but with some difficulties in interpretation regarding possible lymph node metastases.

Three of the nine patients have developed recurrences in an adrenal bed, plus/minus lymph node metastases; one of these has died from progressive metastatic disease. Two more have possibly abnormal isotope uptake in lymph nodes, of uncertain significance. The clinical variability, difficulties in interpretation of nuclear medicine imaging and relatively high malignancy risk should be carefully considered in the diagnosis and management of PCCs in patients with MEN5.

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The utility of a protocol for prophylactic Zoledronic Acid in patients undergoing bone marrow transplantation

Annabel Jones[1], Leon A Bach[1, 2], Sushrut Patil[3, 4], Mitsi Blazos[1], Krisha Solanki[1], Emily Robertson[1], Shoshana Sztal-Mazer[1, 5]

1. Department of Endocrinology and Diabetes, Alfred, Melbourne, Victoria, Australia

2. Department of Medicine (Alfred), Monash University, Melbourne, Victoria , Australia

3. Department of Haematology, Alfred Health, Melbourne, VIC, Australia

4. Department of Haematology, Monash University, Melbourne, VIctoria, Australia

5. School of Public Health and Preventative Medicine, Monash University, Melbourne, VIC, Australia

Osteoporosis post allogenic stem cell transplantation (alloSCT) is common (1-2), with an estimated prevalence of 50% (3), and is accelerated by GVHD prophylaxis and premature hypogonadism (4). The majority of bone loss occurs in the year following alloSCT (5-6), most commonly in the femur but also at the lumbar spine (7). Despite evidence for bone protection with Zoledronic Acid (ZA) in alloSCT (8-11), use is not universal. In 2015, a protocol for prophylactic zoledronic acid was commenced at our institution in all patients prior to alloSCT, regardless of bone density. We aimed to investigate protocol uptake and the utility of prophylactic ZA to prevent bone loss and fracture by comparing bone mineral density (BMD) and atraumatic fracture incidence in patients who received ZA compared to those who did not.

We conducted a retrospective cohort study of all patients who underwent an alloSCT at the Alfred Hospital between 2008 and 2021. Exclusion criteria comprised multiple myeloma, age <18, pre-existing anti-resorptive therapy and <1 year survival post-transplant. Patients who received prophylactic ZA (2015-2021) were compared to historical controls who did not (2008-2014). Demographics, biochemistry, ZA dosage and timing, DEXA results and fracture incidence were extracted from medical records with follow up until 31/10/22.

Of the 271 patients who underwent alloSCT, 249 patients were included (129 in pre protocol group and 120 patients post protocol). Implementation of the protocol significantly increased ZA administration (53 vs 3.9%, $p<0.0001$). We are currently analysing changes in BMD at the lumbar spine and femoral neck over the follow up period as well as fracture outcomes.

This is the largest study presented in the literature to date evaluating the role of prophylactic ZA post alloSCT on bone density and fracture outcomes. A protocol for bone health in alloSCT patients effectively increases use of prophylactic anti-resorptive therapy.

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Ultrasonographic features predicting lateral cervical lymph node metastases in patients with papillary thyroid microcarcinoma

Ho-Cheol Kang[1, 2], Jee Hee Yoon[2], Ji Yong Park[1], Hee Kyung Kim[2]

1. Internal Medicine/Endocrinology and metabolism, Chonnam National University Bitgoeul Hospital, Gwangju, South Korea

2. Chonnam National University Medical School, Gwangju, South Korea

Background: Papillary thyroid microcarcinoma (PTMC) is characterized by its favorable prognosis and potential for active surveillance (AS) as a management option. However, the presence of cervical lymph node (LN) metastasis, especially lateral LN metastasis, significantly impacts management and prognosis. This study aims to identify predictors of lateral LN metastasis by analyzing pre-operative ultrasonographic findings alongside clinicopathological factors.

Methods: A retrospective review of medical records was conducted for PTMC patients who underwent surgery at Chonnam National University Hwasun Hospital between 2004 and 2013. This is a case-control study that compares patients with lateral LN metastasis (N1b) to age and sex-matched patients without LN metastasis (N0).

Results: The study included 90 PTMC patients with N1b and 268 age and sex-matched patients with N0. The mean age was 49.3 years, and females were dominant in both groups. Structural recurrences of 4.4% (4/90) were observed only in the N1b group. The N1b group exhibited a higher frequency of upper lobe tumor location compared to the N0 group (38.9% vs. 16.0%, p<0.001). There was no significant difference in the locations with the presence of invasion to adjacent organs. A higher proportion of non-parallel shape was observed in the N1b group than the N0 group (80.0% vs. 66.0%, p=0.013). There were no differences in echogenicity, sonographic feature, margin, and AP diameter of the thyroid gland between the two groups. In multivariate analysis, independent risk factors for lateral LN metastasis included extra-thyroidal extension, multiplicity, upper lobe tumor location, and non-parallel shape.

Conclusion: Lateral cervical LN metastasis is a significant risk factor for structural recurrence in PTMC patients. Detailed ultrasound examinations, evaluating tumor location, number, orientation, and the presence of ETE, are crucial in accurately predicting lateral LN metastasis. These evaluations can help guide the decision between active surveillance and immediate surgery in PTMC patients.

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Establishment of a new adrenal vein sampling service in a tertiary referral centre: experience over a 21-month period with a prospective quality improvement audit

Tavleen Kaur[1], Tsui Yue Ong[1], Michelle Kerr[1], Hong Lin Evelyn Tan[2], Shamasunder Acharya[1, 3], Richard Ruddell[4], Christian Abel[5], Michael Carey[5], Christine O'Neill[3, 6, 7], Emma E Croker[1, 3]

1. Department of Endocrinology, John Hunter Hospital, New Lambton, NSW, Australia

2. Department of Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

3. School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

4. Department of Anatomical Pathology, John Hunter Hospital, Newcastle, NSW, Australia

5. Department of Medical Imaging, John Hunter Hospital, Newcastle, NSW, Australia

6. Department of Surgery, John Hunter Hospital, Newcastle, NSW, Australia

7. Hunter Medical Research Institute, Newcastle, NSW, Australia

Primary aldosteronism (PA) is a common cause of secondary hypertension in Australia(1). Adrenal vein sampling (AVS) is considered gold standard to lateralise surgically curable disease (2). Our large tertiary referral hospital covers a local health district population of nearly one million people. We identified a need for a local AVS service to meet referral demand and reduce travel costs for patients.

Aim: A quality improvement audit of a new multi-disciplinary supported protocolised AVS service, John Hunter Hospital (Newcastle, NSW).

Method: Prospective data was collected from November 2021 to August 2023. Diagnosis of PA was defined as per Australian and International guidelines(2, 3). AVS was performed after overnight recumbency via sequential cannulation with continuous IV ACTH-infusion (50mcg/hr). Point-of-care rapid cortisol assay was used to predict adrenal vein cannulation success. Successful AVS defined as selectivity index (SI)≥5.0 (adrenal vein cortisol:peripheral vein cortisol). Unilateral aldosterone secretion was defined as lateralisation index ≥4 (dominant aldosterone:cortisol (A:C)/non-dominant A:C) with contralateral suppression <1.0 (non-dominant A:C/peripheral A:C). Patients were treated as per routine standards of care and followed up 3-12 months post-operatively.

Results: Baseline characteristics and results shown **Table. 1**. Of thirty-two AVS procedures performed to date, overall success rate was 78% (n=25) with no complications. 60% (n=15) of successful procedures demonstrated lateralisation. Those who lateralised were more likely to have hypokalaemia (60% versus 0%,p<0.01,Fisher's Exact Test) and adrenal nodule on imaging (60% versus 33%,p=0.04,Fisher's Exact Test) compared to bilateral. AVS and imaging were discordant in 24% (n=6). Following

MDT confirmation of AVS results, 16 have been referred for adrenalectomy. Ten have undergone adrenalectomy. Six are ≥ 3 months post-operation. All have biochemical cure and 3 have clinical cure.

Conclusion: We have demonstrated successful establishment of an AVS service with procedural success rates comparable to centres worldwide and good preliminary surgical cure data(4, 5).

TABLE 1 BASELINE CHARACTERISTICS AND RESULTS

Baseline Characteristics	
Participants - no.	29
Sex female - % (no.)	48 (14)
Age years - median (IQR)	59.5 (IQR 46.8-67.3)
Spontaneous/diuretic induced hypokalaemia - % (no.)	62 (18)
Adrenal Adenoma on CT - % (no.) - Left - (no.) - Right - (no.)	69 (20) (11) (9)
Mean no. of anti-hypertensive agents	2 (SD±1.2)
Mineralocorticoid receptor antagonist use during AVS % (no.)	17 (5)
Baseline aldosterone* - mean (SD)	1000 pmol/L (±592.6)
Baseline direct renin concentration** - mean (SD) (reference interval 4.0 – 40 mU/L)	2.7 mU/L (±1.9)
Baseline ARR (reference range <70)	481 pmol/mU (±593)
Results	
Number of AVS	32
Successful – % (no.)	78% (25)
Results - % (no.) - Left Unilateral - Right Unilateral - Bilateral - Bilateral suppression - Unsuccessful	 28% (9) 19% (6) 28% (9) 0.03% (1) 22% (7)
Relationship between AVS and imaging - % (no.) - Concordant imaging and AVS result - Adrenal nodule on imaging with bilateral result AVS - No adrenal nodule on imaging with unilateral result AVS	 76% (19) 16% (4) 8% (2)

*Aldosterone and Cortisol- Abbot Architect Immunoassay **Direct Renin Concentration - Diasorin Immunoassay

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Fetal Abdominal Obesity in Women with One Value Abnormality on Diagnostic Test for Gestational Diabetes Mellitus

Wonjin Kim[1]

1. CHA University, CHA Gangnam Medical Center, Gangnam-gu, SEOUL, South Korea

Background: Fetal abdominal obesity (FAO) was already observed at the time of diagnosis of GDM in our previous study. We investigated whether fetuses of women with hyperglycemia milder than GDM showed accelerated abdominal growth, leading to adverse pregnancy outcomes.

Methods: 7,569 singleton pregnant women were universally screened using a 50-g glucose challenge test (GCT) and diagnosed by a 3-h 100-g oral glucose tolerance test (OGTT) with GDM, one value abnormality (OVA), and normal glucose tolerance (NGT, NGT1: GCT negative, NGT2: GCT positive & OGTT negative). Fetal abdominal growth at 24-28 gestational weeks was assessed by fetal abdominal overgrowth ratios (FAORs) of the ultrasonographically estimated gestational age (GA) of abdominal circumference per biparietal diameter, femur length, and actual GA by the last menstruation period, respectively. FAO was defined as FAOR ≥90th percentile.

Results: FAORs were significantly higher in OVA subjects compared to NGT subjects, but not in NGT2 subjects. The prevalence of LGA at birth and primary cesarean delivery rates were significantly higher in OVA (9.8 and 29.7%) than in NGT (5.1% and 21.5%, $p<0.05$), especially in OVA subjects with FAO (33.3% and 66.7%).

Conclusion: Fetal abdominal growth was accelerated in OVA subjects and OVA subjects with FAO were strongly associated with adverse pregnancy outcomes.

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An Internal Audit of the Sutherland Hospital Gestational Thyroid Clinic: Changes in referral process and clinical outcomes before and after implementation of a referral management algorithm.

Jeremy A Knott[1], Michael J Bennett[2], Malgorzata M Brzozowska[2]

1. Endocrinology, St George Hospital, Sydney, NSW, Australia

2. Endocrinology, Sutherland Hospital, Sydney, NSW, Australia

Background:

Hypothyroidism is the most common pregnancy-related thyroid condition, associated with increased risks including: miscarriage, preterm delivery, reduced birthweight, and offspring intellectual impairment. Such risks may be reduced through timely diagnosis and thyroxine initiation to achieve maternal euthyroid state. To streamline access to Endocrinology services for women with thyroid dysfunction in pregnancy, Sutherland Hospital implemented a simplified referral management algorithm in February 2021.

Aims:

The primary outcome was to compare the proportion of referred patients who achieved target TSH during pregnancy, before and after algorithm implementation. Secondary outcomes included change in (a)referral numbers, (b)rate of guideline-appropriate investigations, and (c)mean time to Endocrinology consultation.

Methods:

A retrospective database search was performed for the first one-hundred consecutive patients referred to the Sutherland Hospital Gestational Thyroid Clinic with hypothyroidism (thyroid stimulating hormone (TSH)>2.5mIU/L) between April-September 2020 (pre-intervention) and April-September 2021 (post-intervention) for the two sample populations. Detailed clinical data were compared.

Results:

All women pre- and post-intervention achieved target TSH during pregnancy (median final TSH 1.6mIU/L, IQR:1.2-2.3mIU/L). The Endocrinology Clinic received 94 referrals pre-intervention and 109 post-intervention (total 203). While there was no significant difference in guideline-discordant referrals (ie referred with normal TSH) (28% 'before' vs 18% 'after', p=0.24), significantly more women with an elevated TSH had undergone thyroid autoantibody testing post-intervention (55.5% vs 78%, p=0.035). Women post-intervention attended Endocrinology consultation earlier in pregnancy (median 19 vs 22-weeks, p=0.032) and had their TSH measured earlier in pregnancy (median 5.5 vs 6.5-weeks, p=0.011). The number of women with a raised TSH who were prescribed thyroxine prior to first appointment was unchanged (68% 'before' vs 70% 'after', p=0.83).

Conclusions:

Although service effectiveness in achieving target TSH remained unchanged post-algorithm implementation, the use of a dedicated referral algorithm can improve referral efficiency measures including earlier initial TSH measurement, increased autoantibody testing, and reduced consultation waiting time.

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Risk of endometrial cancer and change in obesity status among young women: a nationwide population-based cohort study

Kyungdo Han[1], Jae-Hyuk Lee[2], Minkyung Lee[2]

1. Soongsil University, Seoul, South Korea

2. Myongji hospital, Hanyang University College of Medicine, Goyang-si, Gyeonggi-do, South Korea

OBJECTIVE: Established risk factors for endometrial cancer include age, hormone replacement therapy, and reproductive history. However, emerging evidence suggests that obesity may play a pivotal role. Most research has primarily focused on BMI, overlooking the impact of changes in obesity status over time. Therefore, our aim was to investigate whether change in obesity status is associated with increased endometrial cancer risk in young women.

METHODS: In this large-scale prospective cohort study, we used the South Korean National Health Insurance Service between 2009 and 2012. A total of 461,639 participants aged 20–39 years who completed two different health check-ups with three-year intervals and had no history of any cancer were included. Changes in obesity (BMI >25 kg/m²) were monitored over a three-year period, and participants were classified into 4 groups according to the change in obesity status (normal/normal, obese/normal, normal/obese, and persistent obese). The primary outcome was newly diagnosed endometrial cancer, and the cohort was followed up until December 2020. The Cox proportional hazards regression model was used to estimate the hazard ratios (HRs) and 95% CIs for incident endometrial cancer.

RESULTS: During 6.57 years of follow-up, 309 patients were diagnosed with endometrial cancer. The prevalence of endometrial cancer was increased with higher BMI at baseline (*P* for trend <0.0001). The cumulative incidence of endometrial cancer was significantly different based on the four group (log-rank test, *P* <0.001). Multivariable-adjusted HRs for incident endometrial cancer were 2.067 in the obese/normal group, 2.537 in the normal/obese group, and 4.123 in the persistent obese group compared with the normal/normal group.

CONCLUSIONS: We found that change in obesity status is significantly related to the risk of endometrial cancer among young women. Persistent obesity was the highest risk of endometrial cancer, and improving obesity (obesity/normal) was associated with a lower risk than becoming obesity (normal/obesity).

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Differential effects of aerobic and muscle strengthening physical activities on non-alcoholic fatty liver disease, and cardiovascular disease

Yong-ho Lee[1], Eugene Han[2]

1. Yonsei University College of Medicine, Seoul, SEOUL, South Korea

2. Keimyung University School of Medicine, Daegu, Korea

Objective: The differential effects of physical activity (PA) types on nonalcoholic fatty liver disease (NAFLD) and their associations with NAFLD-related comorbidities, including atherosclerotic cardiovascular disease (ASCVD) has not been fully investigated.

Methods: This was a cross-sectional study using nationally representative samples of 66,021 participants from the Korean National Health and Nutrition Examination Surveys 2007–2020. Aerobic PA (A-PA) was defined as ≥ moderate-intensity 150 min/week or high-intensity 75 min/week of PA; Muscle strengthening PA (MS-PA) was defined as ≥ 2 days/week of muscle strength training. Individuals who conduct both A-PA and MS-PA were classified as multicomponent PA. The risk of ASCVD was estimated with using pooled equation score. A sensitivity analysis consisted of data from individuals who had leisure-time PA data.

Results: The individuals with NAFLD tended to less exercise than individuals without NAFLD. Multicomponent PA was associated with a lower NAFLD risk, compared with other groups (OR=0.82 for A-PA; OR=0.82 for MS-PA; OR=0.75 for multicomponent PA, all P<0.001). Multiple logistic models found that the risk of ASCVD was decreased in all PA groups in a dose-dependent manner, via the combination of A-PA and MS-PA in individuals both with and without NAFLD (OR=0.55 for multicomponent PA, OR=0.59 for MS-PA; OR=0.72 for A-PA in without NAFLD, OR=0.62 for multicomponent PA, OR=0.70 for MS-PA; OR=0.74 for A-PA in without NAFLD, all P<0.001). The multivariable models consistently found significant associations between leisure-time PA and ASCVD risk.

Conclusions: PA decreases the risks of NAFLD, and ASCVD regardless of its types. The risk reduction was greater in individuals who engaged in multicomponent PA.

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Lenvatinib for advanced thyroid cancer: Real world experience with dosing and outcomes

Tony Lian[1, 2], Monica Majumder[1], Shejil Kumar[1, 2], Venessa Tsang[1, 2], Bruce G Robinson[1, 2, 3], Anthony Glover[1, 2, 4], Roderick J Clifton-Bligh[1, 2, 3], Matti L Gild[1, 2]

1. Royal North Shore Hospital, St Leonards, NSW, Australia

2. University of Sydney, Sydney , NSW, Australia

3. Kolling Institute, St Leonards, NSW, Australia

4. The Kinghorn Cancer Centre, Garvan Institute of Medical Research, St. Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia

Aims

Lenvatinib is a multi-targeted tyrosine kinase inhibitor approved for treatment of metastatic radioiodine-refractory thyroid cancer. Phase III clinical trials demonstrated improved progression-free survival. However, real-world data has raised concerns regarding tolerability of lenvatinib's 24mg starting dose and efficacy. We aimed to assess outcomes of lenvatinib in an Australian practice setting.

Methods

We performed retrospective analysis of thyroid cancer patients on lenvatinib at a quaternary referral centre from 2010-2023. Medical records were searched for demographic data, tumour details, treatment-related adverse effects (TRAEs), biochemical and radiological response.

Results

64 patients were included (33% papillary, 22% insular, 16% medullary, 9% Hurthle, 8% follicular, 6% anaplastic, 6% mixed pathology). 20% of cases were *BRAF* mutated. Median age at diagnosis was 59 years old (range 28-90). 53% were female. Most common sites of metastases included lung (76%), skeletal (37%) and liver (12%).

Lenvatinib starting doses were 24mg (n=48, 75%), 20mg (n=4), 10mg (n=1) and unknown (n=11). 21 out of 48 patients (44%) remained on 24mg at 8 weeks. In the entire cohort, common TRAEs included hypertension (n=36), proteinuria (n=10), fatigue (n=33), nausea (n=18) and palmar-plantar erythrodysesthesia (n=9). Three patients discontinued lenvatinib due to significant TRAEs.

In a subset of 35 patients with follicular cell-derived thyroid cancer, 15 patients (43%) had analysable baseline and follow-up thyroglobulin levels. Median baseline thyroglobulin was 320 ug/L(range 10.8-13500) and nadir was 14.9 ug/L(range 1.2-1910). Median reduction in thyroglobulin was 91.3%(range 62.2%-99%). Best disease response as per RECIST criteria was available

for 17 out of 35 patients (49%). 6 (35%) achieved partial response, 10 (59%) sustained stable disease and one (6%) had progressive disease.

Conclusion

Our study demonstrates lenvatinib is effective for radioiodine-refractory thyroid cancer but fewer than 50% reach 8 weeks at full dose. Our cohort's reported TRAEs corresponds to prior studies, requiring supportive care to maximise efficacy.

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Clinical, biochemical, and hormonal effects of gender affirming hormone treatment in transgender individuals – a retrospective cohort study.

Christopher A Muir[1], Mihal Guttman-Jones[1], Elspeth J Man[1]

1. School of Clinical Medicine, University of New South Wales, Sydney, NSW, Australia

Background: Gender affirming hormone treatment (GAHT) results in measurable changes to anthropomorphic, biochemical and hormonal variables that are important to patients and their health care professionals to guide treatment. This study sought to quantify changes which occur in response to initiation of GAHT.

Methods: We performed a retrospective cohort study of outcomes in transgender and gender diverse patients starting GAHT across three practices in Sydney, Australia. The primary study outcome was time required to achieve optimal hormone levels after commencement of GAHT. Additional analyses were performed to assess whether specific clinical and biochemical factors were associated with an improved likelihood of achieving target hormone levels.

Results: A total of 346 patients were included with a median follow-up of 11 months. Among 154 transmasculine individuals, 116 (75%) achieved a target testosterone level of >10 nmol/L during follow-up. No clinical or biochemical factors were significantly associated with likelihood of reaching therapeutic testosterone concentrations. Among 192 transfeminine individuals, 131 (71%) achieved a target testosterone level of <2.0 nmol/L during follow-up. Factors associated with an increased likelihood of adequate testosterone suppression were use of subdermal estradiol implants as well as cyproterone acetate as an androgen antagonist. Changes in differing directions were observed during repeated measures of lipids, liver function, and blood count between transmasculine and transfeminine individuals, reflecting the important effects of testosterone and estradiol on biochemical tests ordered as part of routine clinical care.

Conclusions: Most (>70%) TGD patients will achieve target testosterone levels within 9 months of GAHT initiation. Adverse effects of GAHT such as polycythemia, hyperkalemia and hyperprolactinemia are exceedingly rare, and when they occur are usually mild.

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Socioeconomic correlations of diabetes in mentally ill inpatients

Chandini Murali[1], Vijaya Murali[2]

1. School of Public Health, University of Sydney, Sydney, NSW, Australia

2. Western Sydney LHD, Parramatta, NSW, Australia

Aim

This study is to look at the socioeconomic correlations of diabetes in mentally ill population.

Background

The social determinants of health are the conditions in which people are born, grow, live, work, and age. These circumstances are shaped by the distribution of money, power, and resources at global, national, and local levels. The social determinants of health are mostly responsible for health inequities—the unfair and avoidable differences in health status seen within and between countries.

Method

Literature search using Medline, Pubmed from 2010 -2022, to look at the social determinants of health and diabetes in mentally ill population.

Results

The results show that there are several contributors for increased risk of diabetes in mentally ill population including biological, clinical and non-clinical factors. Socioeconomic status is a multidimensional construct, which encompasses education, occupation and economic status. It is also known to be a strong predictor of disease onset and progression for several diseases especially diabetes. Inequities in living and working conditions and the environments in which people reside have a direct impact on biological and behavioral outcomes associated with diabetes prevention and control

Conclusion

Mentally ill patients are at risk of metabolic syndrome due to various factors. The incidence of diabetes is higher in lower socioeconomic groups, low educational status, low occupational status, poor housing stability, poorer neighborhood, high food insecurity, and ways of tackling these socioeconomic determinants is the way forward to address and combat the incidence of diabetes in mentally ill population. Hence, various primary, secondary and tertiary prevention is bound to address and improve the incidence of diabetes in mentally ill population.

The impact of metabolic control and caregiver education level on the oral health of paediatric diabetic patients

Chandini Murali[1, 2], Gnanagurudasan Prakasam[3, 1], Carly Haaland[1]

1. Pediatric Endocrinology, Center of Excellence In Diabetes and Endocrinology, Sacramento, CA, USA

2. School of Public Health, University of Sydney, Sydney, NSW, Australia

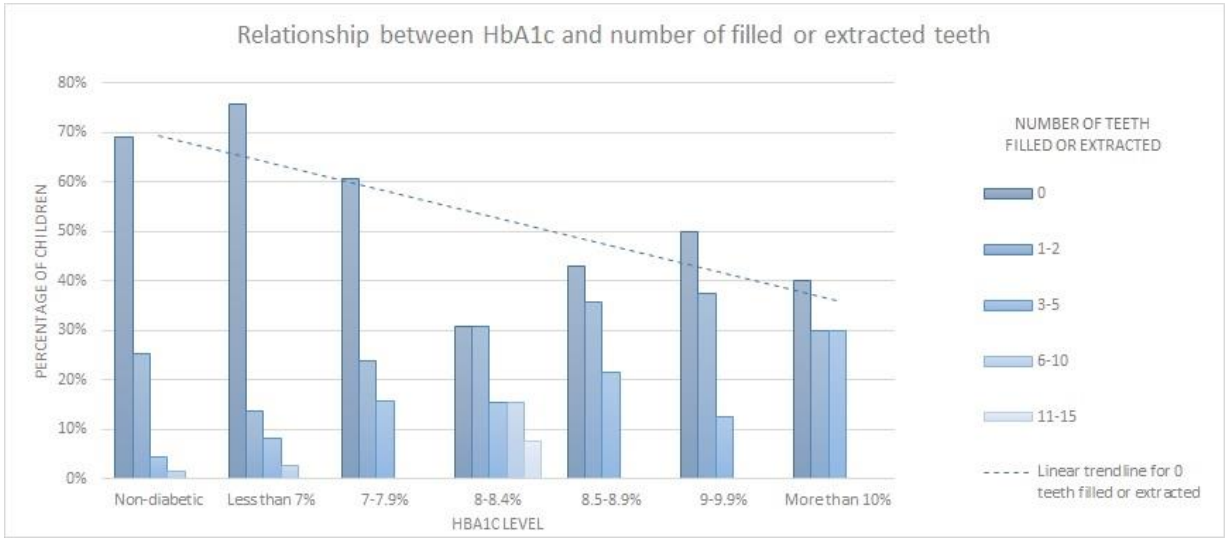
3. SUTTER MEDICAL CENTER, SACRAMENTO, SACRAMENTO, CA, United States

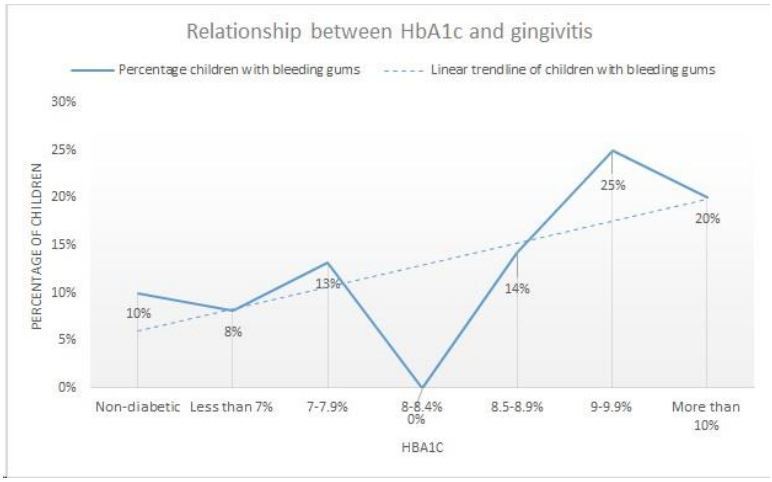
Diabetes can worsen periodontal condition[1], is a caries risk factor[2,3], and may have a bidirectional relationship with periodontitis[4]. Educating diabetic patients about these risks, promoting good oral health, and facilitating dental care access is important[5,6]. This study assessed how metabolic control affects dental health.

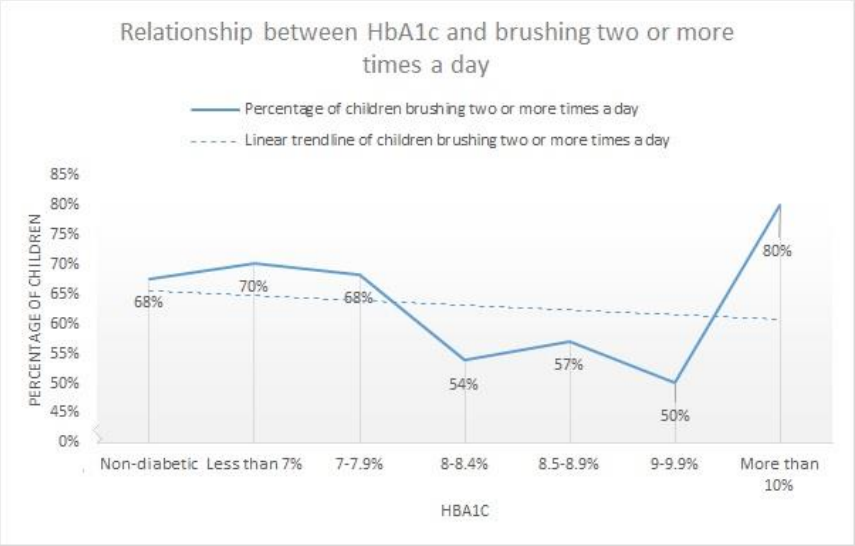
A 12 question online survey was conducted in a California paediatric endocrine clinic. Caregivers of 203 children participated. Data included 105 children with type 1 diabetes and 13 children with type 2 diabetes. Questions included insurance type, caregiver education level, and the child's current HbA1c and oral health.

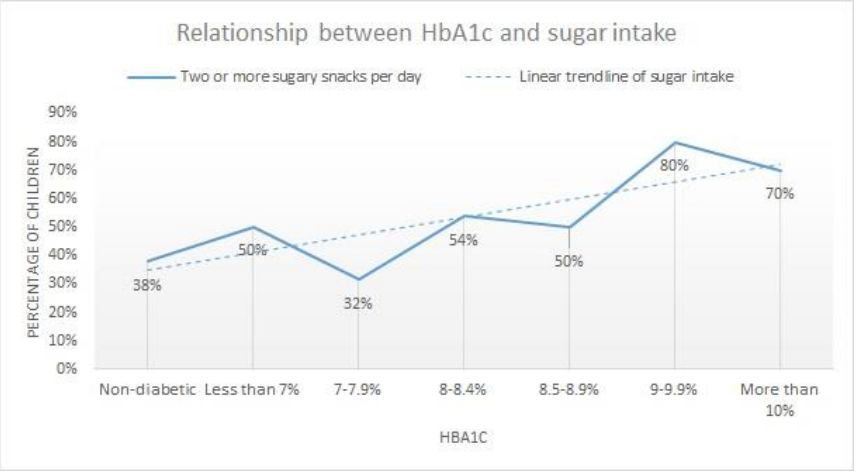
More children with higher HbA1c had at least one tooth filled or extracted, had bleeding gums, brushed less regularly, and ate more sugary snacks than children with lower HbA1c or without diabetes. At HbA1c of above 10%, oral health practices improve, perhaps due to urgent health education. Children with type 2 diabetes had a lower sugar intake, possibly due to health education linking type 2 diabetes and diet, but the sample was small. Caregiver's educational level did not affect sugar intake. More children of caregivers with higher educational levels attended a dentist at least twice a year in the past year and had at least one tooth filled or extracted, possibly due to being seen more regularly.

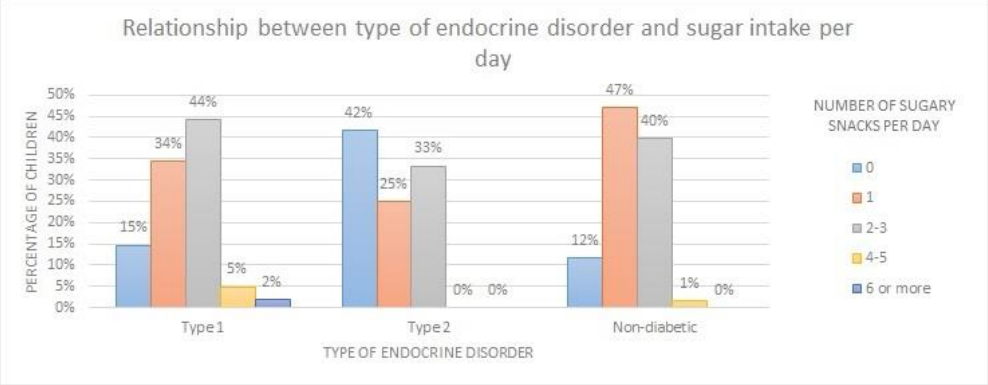
The study limitations include small sample size, lack of dental records, inaccurate recall, and false reporting. The recruitment mode excluded people without social media or internet access. Nevertheless, it underscores the need for dental health education, regardless of type or severity of disease and caregiver education level. Reducing sugar intake in type 1 diabetes is vital to combat obesity and improve oral health. Children from lower socio-economic backgrounds need better dental coverage. Social media can be an effective tool for educational intervention to improve oral health knowledge and behaviour of diabetic adults[6,7].

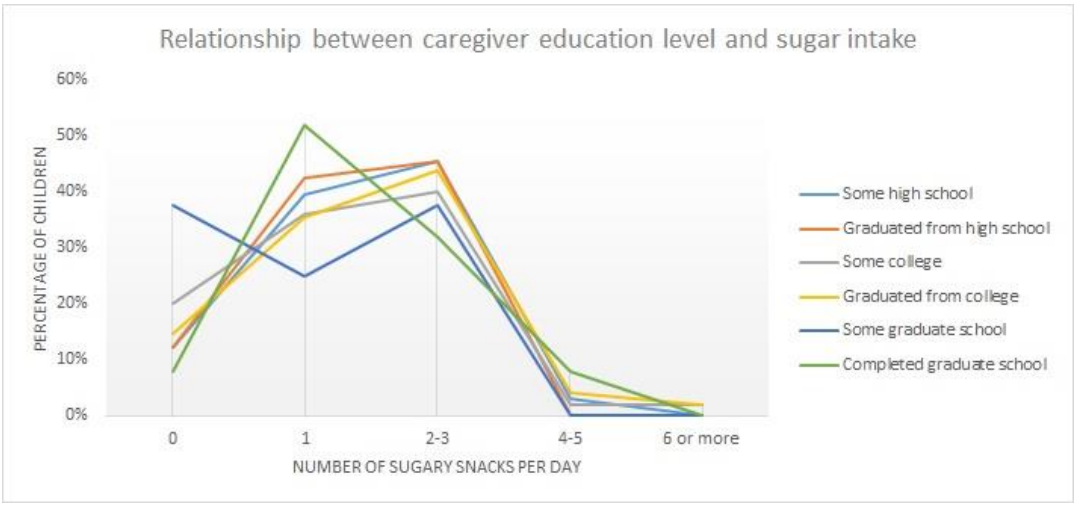


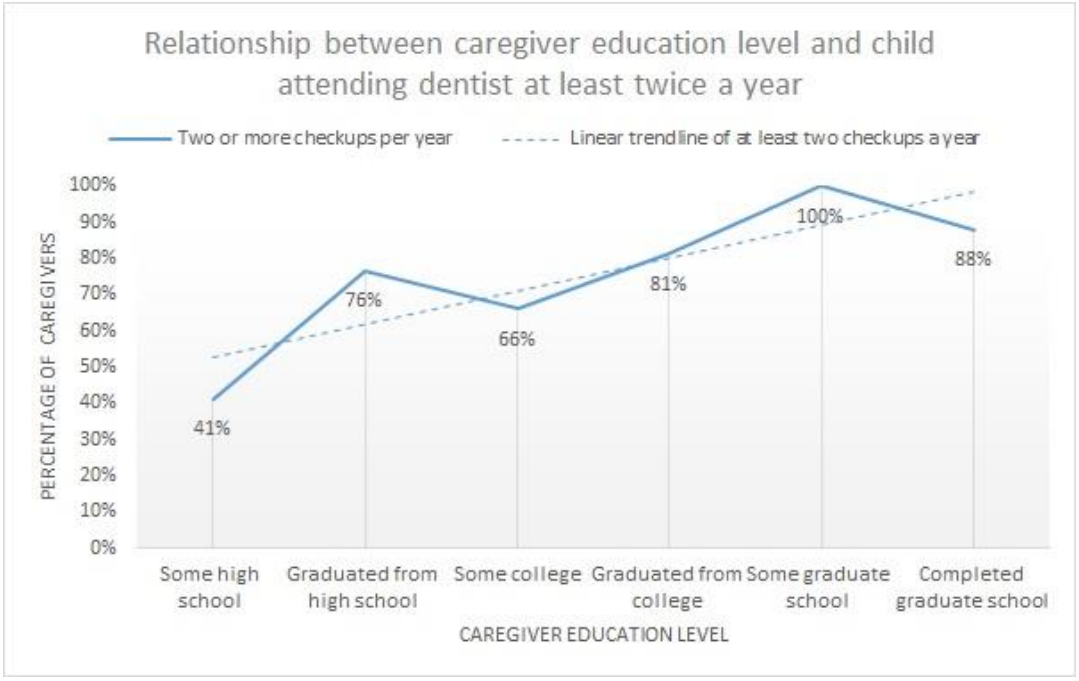


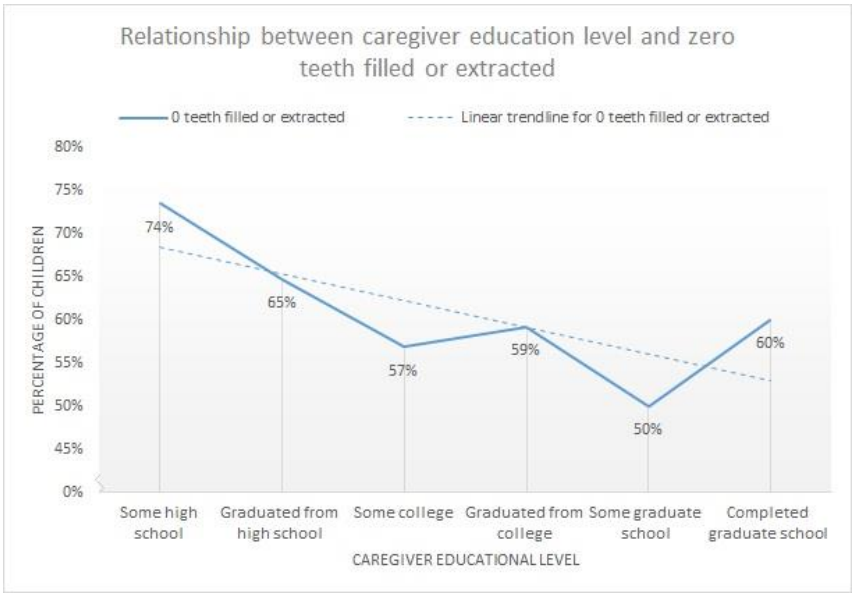

Relationship between HbA1c and brushing two or more times a day
Percentage of children brushing two or more times a day
Linear trendline of children brushing two or more times a day
PERCENTAGE OF CHILDREN
85%
80%
75%
70%
65%
60%
55%
50%
45%
0%
68%
70%
68%
54%
57%
50%
80%
Non-diabetic
Less than 7%
7-7.9%
8-8.4%
8.5-8.9%
9-9.9%
More than 10%
HBA1C


Relationship between HbA1c and sugar intake
Two or more sugary snacks per day
Linear trendline of sugar intake
PERCENTAGE OF CHILDREN
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
38%
50%
32%
54%
50%
80%
70%
Non-diabetic
Less than 7%
7-7.9%
8-8.4%
8.5-8.9%
9-9.9%
More than 10%
HBA1C


Relationship between type of endocrine disorder and sugar intake per day
PERCENTAGE OF CHILDREN
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
15%
34%
44%
5%
2%
42%
25%
33%
0%
0%
12%
47%
40%
1%
0%
Type 1
Type 2
Non-diabetic
TYPE OF ENDOCRINE DISORDER
NUMBER OF SUGARY SNACKS PER DAY
0
1
2-3
4-5
6 or more


Relationship between caregiver education level and sugar intake
60%
50%
40%
30%
20%
10%
0%
PERCENTAGE OF CHILDREN
0
1
2-3
4-5
6 or more
NUMBER OF SUGARY SNACKS PER DAY
Some high school
Graduated from high school
Some college
Graduated from college
Some graduate school
Completed graduate school


Relationship between caregiver education level and child attending dentist at least twice a year
Two or more checkups per year
Linear trendline of at least two checkups a year
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
PERCENTAGE OF CAREGIVERS
41%
76%
66%
81%
100%
88%
Some high school
Graduated from high school
Some college
Graduated from college
Some graduate school
Completed graduate school
CAREGIVER EDUCATION LEVEL


Relationship between caregiver education level and zero teeth filled or extracted
0 teeth filled or extracted
Linear trendline for 0 teeth filled or extracted
80%
75%
70%
65%
60%
55%
50%
45%
0%
PERCENTAGE OF CHILDREN
74%
65%
57%
59%
50%
60%
Some high school
Graduated from high school
Some college
Graduated from college
Some graduate school
Completed graduate school
CAREGIVER EDUCATIONAL LEVEL

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7. Improvement of oral health knowledge and behavior of diabetic patients: an interventional study using the social media BMC Oral Health. 2023; 23: 359. Published online 2023 Jun 3. doi: 10.1186/s12903-023-03007-w

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Low-dose testosterone in non-binary individuals: a retrospective cohort study

Brendan J Nolan[1, 2], Ada S Cheung[1, 2], Sav Zwickl[2]

1. Endocrinology, Austin Health, Heidelberg, Victoria, Australia

2. Austin Health, University of Melbourne, Heidelberg, Victoria, Australia

Background:

Current guidelines for initiation of testosterone for gender affirmation are based on the assumption that trans individuals desire both rapid and complete masculinisation. However, an increasing number of trans individuals, particularly those with a non-binary gender identity, desire lower testosterone doses than recommended in existing guidelines. We aimed to assess the initiation and maintenance of low-dose testosterone in non-binary individuals treated with transdermal testosterone for ≥6 months.

Methods:

We performed a retrospective cohort study of non-binary individuals initiating low-dose transdermal testosterone. We included non-binary individuals initiating low-dose transdermal testosterone with ≥6 months follow-up. Primary outcomes were transdermal testosterone dose and serum total testosterone concentration.

Results:

Forty-six non-binary individuals treated with testosterone for at least 6 months were included. Median age was 27 years (24-30) and median duration of testosterone was 14 months (9-24). Median testosterone dose at initiation was 25 mg (12.5-50) and 37.5 mg (25-50) at last follow-up ($p<0.01$). Median serum total testosterone concentration was 11 nmol/L (5.2-15.7). By last follow-up, 40 (87%) remained on low-dose testosterone and 6 (13%) had increased to full-dose testosterone. In a subgroup of 30 individuals with ≥12 months testosterone treatment, 26 (87%) remained on low-dose testosterone at last follow-up. Three (7%) individuals had polycythaemia (haematocrit >0.5).

Conclusion:

Most non-binary individuals initiating low-dose transdermal testosterone continue testosterone doses lower than those recommended in current consensus guidelines. Future research should evaluate the influence of low-dose testosterone on clinical outcomes in non-binary people.

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Predictors of metastasis in patients with pheochromocytoma or paraganglioma : a single center retrospective cohort study

ROSA OH[1], So Hyun Cho[1], You-Bin Lee[1], Gyuri Kim[1], Sang-Man Jin [1], Kyu Yeon Hur [1], Jae Hyeon Kim[1]

1. Samsung Medical center, Seoul, South Korea

Aims

The purpose of this study is to investigate predictors of metastasis in patients with pheochromocytoma or paraganglioma (PPGLs). Predictors consist of clinical, biochemical, genetic, and pathoanatomical characteristics.

Methods

This is single center retrospective cohort study with PPGLs patients in Samsung medical center from January 2013 to January 2023. Patients who have synchronous metastasis at initial diagnosis were excluded. Also head and neck PPGLs patients were excluded too. Total 232 patients were included.
All patients received genetic screening with SDHB mutation. And ages at initial diagnosis, sex, body mass index at initial diagnosis, secretory type, tumor location, largest tumor sizes, PASS score (pheochromocytoma of the adrenal gland scaled score), ASES score (age, size, extra-adrenal, secretory type), AJCC staging were extracted. Cox regression hazard model was used to analyze associations between metastasis and genetic or epigenetic factors.

Results

A total of 13 patients metastasized during the follow-up period. They showed larger tumor size (>6cm), higher PASS score (≥4), higher ASES score (≥2), Advanced stage in AJCC staging, more SDHB mutation at baseline compared to non-metastatic

patients. Especially none of the patients who were in the first stage of AJCC staging had metastasis. And none of the patients who had lower PASS score (<4) had metastasis.
In cox regression hazard model, larger tumor size (≥6cm) was significantly associated with metastasis (HR : 13.7, P=0.017). On ROC analysis, the AUC of the tumor size was 0.7829 for metastasis. Furthermore SDHB mutation was significantly associated with metastasis (HR:16.3, P=0.023). On ROC analysis, the AUC of SDHB mutation was 0.6040 But ASES score (≥2) was not associated metastasis. (HR:1.91, P=0.475).

Conclusion
Larger tumor size at initial diagnosis is independent predictors of metastatic behavior of patients with PPGLs.

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Accuracy of point of care rapid cortisol assay to predict success of adrenal vein sampling: a real-world prospective audit of the use of a rapid cortisol assay with 50mcg/hr ACTH-infusion in a tertiary referral hospital.

Tsui Yue Ong[1], Tavleen Kaur[1], Hong Lin Evelyn Tan[1, 2], Michelle Kerr[1], Shamasunder Acharya[1, 2], Richard Ruddell[3], Christian Abel[4], Michael Carey[4], Christine O'Neill[2, 5, 6], Emma E Croker[1, 2]

1. Department of Endocrinology, John Hunter Hospital, New Lambton, NSW, Australia

2. School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

3. Department of Anatomical Pathology, John Hunter Hospital, Newcastle, NSW, Australia

4. Department of Medical Imaging, John Hunter Hospital, Newcastle, NSW, Australia

5. Department of Surgery, John Hunter Hospital, Newcastle, NSW, Australia

6. Hunter Medical Research Institute, Newcastle, NSW, Australia

Adrenal vein sampling(AVS) is the gold standard to lateralise surgically-curable primary aldosteronism(1). Point-of-care semi-quantitative rapid cortisol assay(RCA) has been shown to increase rate of successful adrenal vein cannulation in this technically challenging procedure(2-4). There is limited published prospective data on accuracy of RCA with continuous intravenous(IV) cosyntropin(ACTH)-infusion.

Aim: Assessment of accuracy of RCA (AVS Accuracy Kit) in predicting successful cannulation during AVS with ACTH-infusion.

Methods: Prospective data was collected November 2021 to August 2023 from AVS procedures performed via sequential cannulation with continuous IV ACTH-infusion (50mcg/hr). RCA was used to predict cannulation success. RCA was inspected visually at 2 and 5 minutes. Appearance of test line indicated likely unsuccessful cannulation and procedure adjusted accordingly. Successful AVS was defined as selectivity index (SI)≥5.0 (adrenal vein cortisol:peripheral vein cortisol).

Results: Thirty-two consecutive AVS were performed with overall success rate 78% (n=25). 108 RCA were used (average 3.4/procedure). 59% (n=19) of procedures were adjusted based on RCA predicting failed cannulation. Of these, 63% (n=12) were subsequently successful. Selectivity index, cortisol levels and 2X2 contingency table are shown in **table 1**. Absence of RCA test line had 100% positive predictive value for successful cannulation. Presence of RCA test line had 95% negative predictive value, indicating cannulation was highly unlikely. In the 2 cases of inaccurate RCA result (2/108), a very faint line appeared at 5 minutes (adrenal venous cortisol 5004/5352/nmol/L with peripheral cortisol 901/714nmol/L) with final SI ≥5. Slight catheter adjustment was made, with subsequent samples successful with no adverse outcome.

Conclusion: The semi-quantitative RCA can confidently be used to predict successful cannulation in AVS with ACTH-infusion. The procedure should be adjusted in the presence of a definite test line. Radiologist discretion is advised when a very faint test line on RCA is detected.

TABLE 1.			
SELECTIVITY INDEX AND CORTISOL LEVELS FROM AVS SAMPLES			
Mean Selectivity Index for all successful cannulation – mean (SD) (no.)			30.5 (±18.3) (n = 174)
Mean Selectivity Index for all unsuccessful cannulation – mean (SD) (no.)			1.7 (±1.1) (n = 55)
Mean adrenal vein cortisol successful cannulation – mean (SD) (no.)			22231 nmol/L (±11516) (n = 175)
Mean peripheral vein cortisol – all peripheral venous samples			827 nmol/L ((±524) (n = 19)
No test line visible on RCA* – mean cortisol (SD); minimum; (no.) **Indicates likely successful cannulation*			22984 nmol/L (SD±11619) minimum 5715 nmol/L (n= 69)
Test line visible on RCA** – mean cortisol (SD), maximum; (no.) ***Indicates likely unsuccessful cannulation*			1512 nmol/L (SD±1265) maximum 5352 nmol/L (n=39)
2 x 2 Contingency Table – RAPID CORTISOL ASSAY versus SELECTIVITY INDEX			
	Selectivity Index ≥ 5	Selectivity Index ≤ 5	Result
RCA predicted cannulation success (No line)	69	0	Sensitivity: 97% Specificity: 100% PPV: 100% NPV: 95% Fisher's Exact Test for 2x2 table p <0.001
RCA predicted cannulation failure (Line)	2	37	

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Systematic review and meta-analysis of the association between autoimmune thyroiditis and papillary thyroid cancer with *BRAF* mutation

Sumathy Perampalam[1, 2, 3], Katherine Wu[4], Matti Gild[3, 4], Lyndal Tacon[3, 4], Martyn Bullock[3, 4], Roderick Clifton-Bligh[3, 4]

1. The Canberra Hospital, Garran, ACT, Australia

2. School of Medicine and Psychology, Australian National University, Garran, ACT, 2065

3. Faculty of Medicine and Health, University of Sydney, Camperdown, NSW, Australia

4. Department of Endocrinology, Diabetes and Metabolism, Royal North Shore Hospital, St Leonards, NSW, 2605

Autoimmune thyroid disease (AITD) co-exists in 15-25% of papillary thyroid carcinoma (PTC), with likely favourable outcome (1,2). However, the association between PTC with AITD and PTC with *BRAF* mutation is unclear. We conducted a systematic review with meta-analysis to evaluate:

- the association between PTC with *BRAF* mutation and PTC with AITD and
- the risk of:
    1. central lymph node disease (CLND) in *BRAF* mutant PTC
    2. CLND in PTC with AITD.

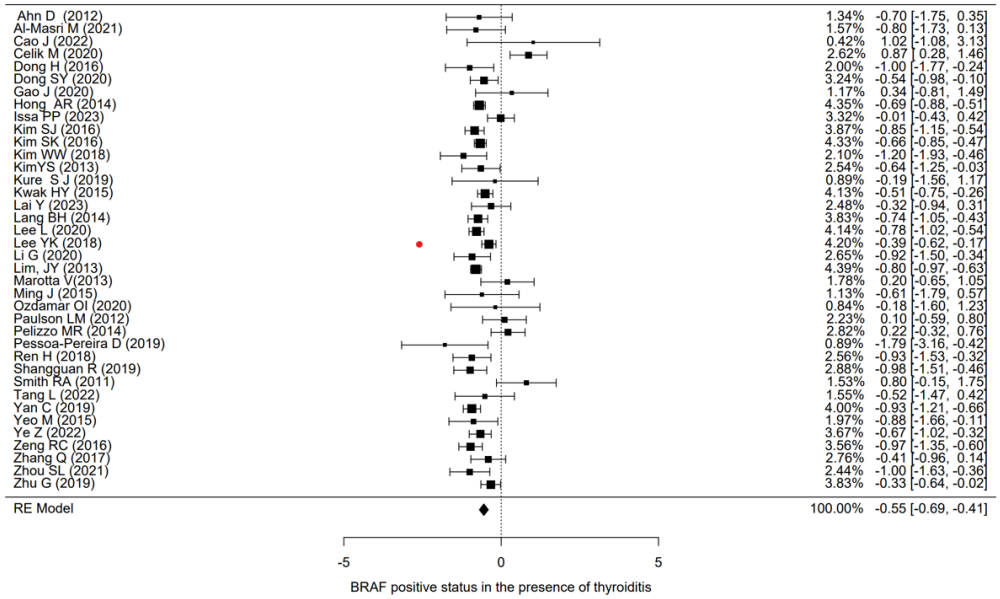
We searched PubMed, Embase and Web of Science Core Collection for observational studies published from 2010 to June 2023 on adult PTC patients. Studies with data on PTC subsets for presence and absence of *BRAF* mutation, AITD and CLND were included. For analysis, groups were categorised as "exposed":(i) *BRAF* PTC with AITD; (ii a) CLND with *BRAF* PTC; (ii b) CLND with AITD; and as "control":(i) *BRAF* PTC without AITD; (ii a) CLND without *BRAF* PTC; (ii b) CLND without AITD.

Out of 699 studies initially identified, screening resulted in 44 studies with total of 30 141 PTC patients, mean age of 44 years, 68.3% with *BRAF* mutant PTC and 24.7% with AITD. Summary statistics of 38 studies showed PTC with AITD had a lower odds of PTC with *BRAF* mutation with pooled log odds ratio (LOR) of - 0.55 (95% CI-0.69 to -0.41, p <0.001). However, there was significant heterogeneity with I^2 of 74.1 % (df 37.0;p<0.001). 10 studies showed *BRAF* PTC was associated with increased risk of CLND with pooled LOR of 0.49 (95% CI 0.18 to 0.81;p=0.002). In the same studies, PTC with AITD resulted in lower risk of CLND with LOR of -0.45 (-0.69 to -0.22;p=0.002).

Thus, concurrent AITD in PTC has a negative association with *BRAF* mutation. Risk of CLND is lower in PTC with AITD compared with PTC with BRAF mutation.

Forest plot

a) Association between PTC with BRAF mutation and autoimmune thyroiditis (AIT)

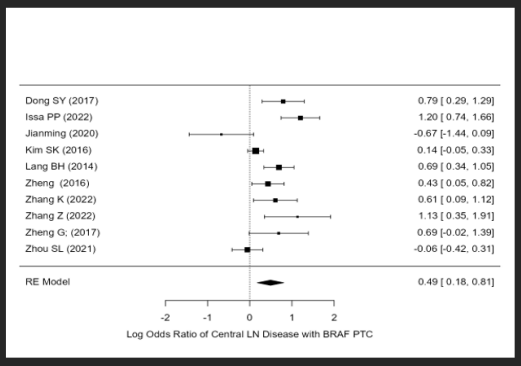


Log odds ratio = -0.551, 95% CI -0.69 to-0.41, SE 0.0732, p<0.001, using random effect model

Odds ratio =0.576, 95%CI 0.50-0.67

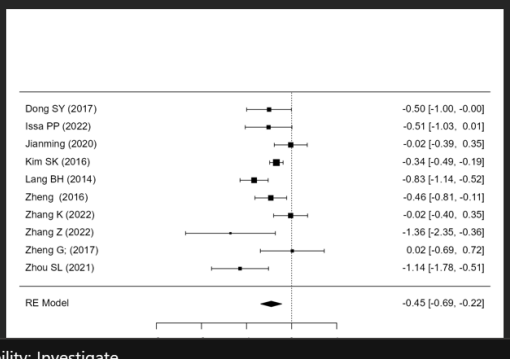
Heterogeneity statistics: Tau^2 0.11, SE 0.04; I^2 74.07%, df=37, p<0.01

b) Forest Plot: Log odds ratio of Central Lymph Node Disease in PTC with BRAF mutation



Pooled log odds ratio of 0.49 (95% CI 0.18 to 0.81, p=0.002);
Odds Ratio = 1.64 (95%CI 1.19-2.24)
I^2 =81.4%, df=9, p<0.001; random effect model was used.

Forest plot: Logs odds ratio of Central Lymph Node Disease in PTC with Autoimmune Thyroiditis



Pooled log odds ratio of -0.45 (95%CI-0.69 to -0.22, p<0.001);
Odds Ratio= 0.64(95%CI 0.50-0.80);
I^2 71.0%, df=9. P=0.002);
Random effect model was used was used

ccessibility: Investigate

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The presence of coronary artery calcification is associated with smaller increases in femoral neck bone mineral density in patients on anti-resorptive therapy

Alexander J. Rodriguez[1], Rachael Hii[2], Nitesh Nerlekar[2], Peter R. Ebeling[1]

1. Bone & Muscle Research Group, School of Clinical Sciences at Monash Health, Monash University, Melbourne, VIC, Australia

2. Victorian Heart Institute, Melbourne, VIC, Australia

Introduction

Anti-resorptive medications are first-line treatments for osteoporosis. Additionally, patients with osteoporosis are at high cardiovascular risk, partly due to vascular calcification, such as in the coronary vessels. It is uncertain if the presence of coronary artery calcification (CAC) effects bone mineral density (BMD) response to anti-resorptive treatment. We therefore assessed changes in BMD following initiation of anti-resorptive treatment for osteoporosis in patients with and without evidence of CAC.

Methods

Individuals dispensed at least one prescription for an anti-resorptive medication (bisphosphonates or denosumab) at Monash Health between 2009-2022 were identified. Unique record numbers for these individuals were then cross-matched against the cardiac CT imaging service at Monash Heart (HREC#73603). CAC was detected by CT coronary angiogram (CTCA). We

included only those patients having a baseline BMD measurement within two years of CTCA. The annualised percentage change in femoral neck BMD was calculated and adjusted for age, sex, height, weight, and number of years on anti-resorptive treatment.

Results

106 individuals were identified of which 85 (women=70 [85%], median age=73 years [interquartile range 64-79 years]) had a follow-up BMD measurement including 19 with, and 66 without, evidence of CAC. Those with CAC were older (76 years versus 64 years, p<0.001). There were 70 bisphosphonate users and 15 denosumab users. Individuals with evidence of CAC experienced, on average, a 1.2% lower increase [(0.345% (0.343 to 0.348) versus -0.881% (-0.883 to -0.879), mean difference -1.226% (-1.493 to -0.959; p<0.05)] in annualised femoral neck BMD with anti-resorptive therapy after adjusting for important clinical risk factors.

Interpretation

These preliminary data suggest that CTCA-determined CAC may negatively impact femoral neck BMD increases with anti-resorptive therapy. Analysis of the full cohort is presently underway.

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Management impact of molecular imaging in the management of phaeochromocytoma & paraganglioma: a pseudoprospective cohort study.

Simon Ryder[1, 2], Cherie Chiang[3], Roderick Clifton-Bligh[4], Christopher Yates[5], Emma L Duncan[6], David Pattison[1]

1. Royal Brisbane & Womens Hospital, Queensland Health, Brisbane, QLD, Australia

2. Logan Hospital, Meadowbrook, QLD, Australia

3. Peter MacCallum Cancer Centre, Parkville, VIC, Australia

4. Royal North Shore Hospital, St Leonards, NSW , Australia

5. Royal Melbourne Hospital, Parkville, VIC, Australia

6. King's College, London, United Kingdom

2014 international guidelines favoured anatomical imaging (AI) for phaeochromocytomas and paragangliomas (PPGL) and suggested low incremental value of [^{123}I]-MIBG in most presentations. Contemporary molecular imaging (MI) has potential diagnostic advantages and data suggest MI can have a positive management impact. We assessed the management impact of MI for individuals with possible or suspected PPGL.

We performed retrospective cohort analysis at a site with continuous access to all relevant PPGL imaging modalities including [18F]-FDOPA, [65Ga]-DOTATATE and [123I]-MIBG. We then completed a pseudoprospective study of individuals undergoing MI for possible or suspected PPGL between 2011-19. Three external reviewers with expertise in PPGL independently appraised de-identified datasets and adjudicated the management impact of scans, graded as high, medium or low impact. Analyses examined factors influencing management impact.

Of all initial MI scans, PPGL was excluded in 48% scans; PPGL was confirmed in 47% scans; while 5% scans were inconclusive. Between 2011-15 and 2016-19, there was a four-fold increased use of [68Ga]-DOTATATE, simultaneous with decreased [123I]-MIBG use. Of 128 scans, 44 (34%) were deemed high impact, 31 (24%) were moderate impact and 53 (41%) were low impact. MI had a high or moderate impact when performed for PPGL confirmation (46/51, 90%), restaging (7/8, 88%), and theranostics (6/6, 100%). A high or moderate impact was infrequently reported across other indications: PPGL staging (4/23, 17%), asymptomatic screening (5/23, 22%), and follow-up of previously treated PPGLs (7/17, 41%).

Management impact of MI performed for PPGL is dependent upon scan indication and modality. In most cases where a solitary PPGL is unambiguous on AI, MI provides low incremental value. In contrast, where there is inconclusive evidence for PPGL on the composite of clinical information, MI has a high or moderate management impact in most cases. This study supports the use of targeted MI in PPGL management.

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A Prospective Study on Hyperthyroidism Induced Dilated Cardiomyopathy and its Recovery Following Treatment

muthukumar sankaran[1], mohamed salmon[2], arunmozhi vijay[3], janakiraman ramachandran[3], syed ibrahim[1]

1. endocrine surgery, madurai medical college, madurai, Tamil nadu, India

2. general surgery, rasipuram govt hospital, rasipuram, Tamil nadu, India

3. Madurai medical College, Madurai, TAMIL NADU, India

INTRODUCTION

Dilated cardiomyopathy(DCM) is one of the deadliest complication in patients with hyperthyroidism and is the major cause of mortality.We intended to study the incidence of DCM and its recovery pattern in hyperthyroid patients.

MATERIALS AND METHODS

Three ninety one patients (age< 60 years)with newly diagnosed hyperthyroidism were evaluated with 2D Echocardiography, at the time of diagnosis(Point A),after achieving euthyroidism(Point B) with anti-thyroid drugs and 6 months after achieving euthyroidism (Point C).60 patients (age< 60 years) with nontoxic benign thyroid nodules served as controls.

RESULTS

At point A, DCM was evident in 50/391(12.7%).cardiac failure was observed in 18/391(4.6%).28/50 patients improved completely at Point B with all other cohorts showing a significant improvement in the cardiac indices. cardiac failure improved all patients at point B.At Point C dramatic improvement in DCM occurred in 41/50(82%) with remaining 9 patients also showing a significant improvement .

CONCLUSION

DCM is one of the dreaded complication of hyperthyroid cardiac dysfunction and if diagnosed early and treated properly mortality and morbidity can be prevented .

TABLE 1

IVS-Interventricular septum

Variables	**Group 1**	**Group 2**	**p value**
LV End diastolic dimension(mm)	40.72±4.07	41.95±5.21	**.002**
LV End systolic dimension (mm)	26.11±3.06	28.18±4.1	**.01**
LV End diastolic volume (ml)	68.86±12.07	75.09±9.41	**.03**
LV End systolic volume (ml)	31.09±7.34	33.12±6.73	**.04**
LV ejection fraction	64.08±4.11	61.54±3.83	**.01**
IVS diastolic thickness	9.09±1.06	9.9±1.51	.14
Posterior wall thickness	9.14±1.7	9.38±1.4	.57
pulmonary hypertension	23.05±2.3	24.08±2.35	**.07**

LV-left ventricle

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The Impact of Arterial Stiffness on Late Loss After Percutaneous Coronary Intervention According to Diabetes

Jae Bin Seo[1]

1. Boramae Medical Center, Dongjakgu, Seoul, South Korea

Background and OBJECTIVES: Increased arterial stiffness is an accepted cardiovascular risk factor. However, the effect of arterial stiffness on the performance of percutaneous coronary intervention (PCI) is not well known. The aim of this study was to evaluate the impact of arterial stiffness measured by pulse wave velocity (PWV) on late loss after percutaneous coronary intervention according to diabetes.
METHODS: Data from 242 consecutive patients who underwent PCI using drug eluting stents and pulse wave velocity study were analyzed.
RESULTS: Mean PWV and late loss were 1613 ± 371 cm s(-1), 0.10 ± 0.51 mm in the patients without diabetes and 1758 ± 404 cm s(-1), 0.16 ± 0.52 mm in the patients with diabetes, respectively. There was no significant relation between PWV and late loss in the patients with diabetes (correlation coefficient = 0.168; p=0.083). However, there was negative relation between PWV and late loss in the patients without diabetes (correlation coefficient = -0.185; p=0.032). CONCLUSION: Increased arterial stiffness is favorable for late loss of the patients without diabetes undergoing PCI. However, this is not prognostic factor for the patients with diabetes.

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Mineralocorticoid receptor antagonist treatment for low-renin hypertension: a systematic review and meta-analysis

Sonali S Shah[1, 2], **Jinghong Zhang**[2], **Stella May Gwini**[2], **Morag J Young**[2], **Peter J Fuller**[1, 2], **Jun Yang**[1, 2]

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

2. Centre of Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, VIC , Australia

Publish consent withheld

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Surgical Outcomes of Marsupialisation in Rathke's Cleft Cyst Patients

Darby J Thompson[1], Julia Low[2, 3], Nick Shoung[2], Richard J Harvey[2, 4, 5], Benjamin P Jonker[6, 8, 7], Mark Winder[2, 8], Ann I McCormack[2, 9, 10]

1. University Of New South Wales, Sydney, NSW, Australia

2. St. Vincent's Clinical School, University Of New South Wales, Sydney, NSW, Australia

3. Department of Anatomical Pathology and Cytopathology, St Vincent's Pathology, Sydney, NSW, Australia

4. Department of Ear, Nose and Throat, St Vincent's Hospital, Sydney, NSW, Australia

5. Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, NSW, Australia

6. Department of Neurosurgery, Institute of Academic Surgery, Royal Prince Alfred Hospital, Sydney, NSW, Australia

7. Brain and Mind Centre, University of Sydney, Sydney, NSW, Australia

8. Department of Neurosurgery, St Vincent's Hospital, Sydney, NSW, Australia

9. Department of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia

10. Garvin Institute of Medical Research, Sydney, NSW, Australia

Objectives: To determine the outcomes of 2 different techniques for surgical management of Rathke's cleft cysts: marsupialisation compared to fenestration.

Methods: A retrospective evaluation of all surgically resected Rathke's Cleft Cysts from St Vincent's Hospital, Sydney between the periods of 2015 – 2023. Clinical data including pre-operative symptoms, pituitary dysfunction and post-operative complications was collected from patient files. Radiology reports were reviewed for size, location, septation, fluid-fluid levels and intracystic nodules. Histopathological reports were reviewed to confirm diagnosis and determine any associated hypophysitis and cyst consistency.

Results: Forty-two patients were evaluated (37 marsupialisation, 5 fenestration) with 28.5% presenting with visual deficit, 59.5% with headaches and 16.7% with pituitary dysfunction. Marsupialisation led to an improvement in 75% of patients for visual symptoms and 73.9% of headaches which compares similarly to fenestration in the literature. Histologically, Rathke's Cleft Cysts showed a higher occurrence of hypophysitis than previously reported in the literature (38.1%). Surgical complications were similar between fenestration and marsupialisation. Cyst recurrence following marsupialisation was just 2.7% compared with fenestration (10%) and notably lower than reported in the literature.

Conclusion: Marsupialisation of Rathke's Cleft Cysts lead to a significant improvement in symptom resolution with a lower cyst recurrence rate than the traditional fenestration technique. Hypophysitis appears to be a more frequently associated with Rathke's cleft cysts that come to surgical management.

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Testosterone and the risk of incident atrial fibrillation in older men: the ASPREE study

Cammie Tran[1], Bu B Yeap[2, 3], Jocasta Ball[1, 4], Daniel Clayton-Chubb[1, 5], S Monira Hussain[1, 6], Amy Brodtmann[1, 7], John J McNeil[1]

1. School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia

2. Medical School, University of Western Australia, Perth, Western Australia, Australia

3. Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, Western Australia, Australia

4. Baker Heart and Diabetes Institute, Melbourne, VIC, Australia

5. Department of Gastroenterology, Alfred Hospital, Melbourne, VIC, Australia

6. Department of Medical Education, The University of Melbourne, Melbourne, VIC, Australia

7. Central Clinical School, Monash University, Melbourne, VIC, Australia

Background: Whether testosterone influences cardiovascular risk in older men remains uncertain. A cardiovascular safety trial of testosterone in men with cardiovascular risk factors or disease found no difference in rates of major adverse cardiovascular events (MACE) or deaths, but noted more atrial fibrillation (AF) events in testosterone-treated men.

Aim: To investigate whether endogenous testosterone concentrations are associated with risk of developing AF in healthy older men.

Methods: Post-hoc analysis of 4,570 male participants in the Aspirin in Reducing Events in the Elderly (ASPREE) study. Men had no history of cardiovascular disease (including AF), thyroid disease, prostate cancer, dementia, or life-limiting illnesses. Total testosterone was measured at baseline using chemiluminescence immunoassay. Incident AF during follow-up was ascertained using self-reported diagnosis, prescription medication and/or medical records. Risk of AF was modelled using restricted cubic splines and Cox proportional hazards regression.

Results: Mean age±SD was 75.0±4.2 years and median (IQR) of follow-up 4.4 (3.3-5.5) years, during which 286 men developed AF (15.3 per 1000 participant-years). Baseline testosterone was higher in men who developed incident AF

compared men who did not (17.3±6.7 vs 16.5±6.3 nmol/L). There was a non-linear association of baseline testosterone with incident AF. Higher baseline testosterone was associated with an increased risk of AF (per 1SD increase: fully-adjusted hazard ratio [HR]=1.17; 95% Confidence Interval [CI]=1.05-1.32). Risk of AF was similar across the lowest three quintiles of testosterone values, but higher in men with testosterone in quintiles (Q) 4&5 (Q4:Q3, HR=1.91; CI=1.29-2.83 and Q5:Q3HR=1.98; CI=1.33-2.94). Results were similar after excluding men who had MACE or heart failure during follow-up.

Conclusion: Serum total testosterone is independently associated with higher risk of incident AF in relatively healthy community-dwelling older men. Screening for AF should be considered when assessing testosterone results or testosterone treatment in older men.

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Management of patients with Graves' disease at a regional health service

Wendy Lin[1], Elizabeth Wootton[2], Amy Harding[2], Mark W Savage[2], Jessica Triay[2]

1. Faculty of Medicine, Monash University, Melbourne, VIC, Australia

2. Endocrinology and Diabetes, Bendigo Health, Bendigo, VIC, Australia

Graves' disease (GD) is typically diagnosed in primary care, with referral to Endocrinology for further management(1). During the active phase of GD, patients are often reviewed 6-8 weekly to titrate antithyroid drugs (ATDs), placing a substantial burden on specialist health services. Evidence-based recommendations could allow for development of shared care pathways between primary and secondary care(2). The interaction of GD patients with Australian health services, particularly in regional areas, is unknown.

To review the clinical course of GD patients referred to Bendigo Health (BH) and their interaction with the outpatient service

New GD patients referred to BH between 1st January 2020 and 31st December 2021 were identified via clinical coding. Data werecollected from the electronic patient record system.

22 patients were included: 17 (77%) female with a mean age of 44 years, located 10km (median, range 3-218km) from BH. 6 patients (28%) lived in the lowest socio-economic decile. For the majority (18 patients, 82%), this was their first episode of GD. Patients were reviewed in clinic for a mean 8 appointments 13.5 weeks apart. 87 (49%) appointments were telehealth, 59 (34%) in person and 30 (17%) mode not documented. Doses of ATDs were adjusted at median 3 appointments. 2 patients (9%) experienced liver derangement and 3 (14%) described an intolerance to ATDs. 3 patients (14%) became hyperthyroid following ATD cessation. 12 patients (55%) selected definitive therapy, mostly due to persistent GD; 7 (58%) underwent thyroidectomy and 5 (42%) radioactive iodine. 4 patients (18%) failed to attend appointments.

The sample size was limited by accuracy issues with clinical coding. Appointments to titrate ATDs were less frequent than expected with a high proportion opting for definitive therapy. Further work is needed to define GD-associated healthcare costs and conduct stakeholder analyses to explore the potential for shared management between Endocrinology and primary care.

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Severe and prolonged thyrotoxicosis following iodinated contrast media. A shared lesson

Vinh Hong[1], Chelsea W Tan[1], Jessica M Triay[1]

1. Bendigo Health, Bendigo, VIC, Australia

This case of iodinated contrast media (ICM)-associated hyperthyroidism with the predominant clinical feature of atrial fibrillation (AF) with rapid ventricular rate (RVR) highlights that: (1) not all episodes of ICM-associated hyperthyroidism are mild or self-limiting, (2) whilst clinical features of hyperthyroidism may be limited, decompensation may occur, and (3) intervention should be considered and may be life-saving when features are not resolving.

An 82 year old lady presented with abdominal pain on a background including chronic myeloid leukaemia (CML), treated breast and colon adenocarcinoma, and atrial fibrillation. Contrast CT of the abdomen and pelvis, and subsequently neck and chest, confirmed lymphadenopathy and splenomegaly from CML progression. On day 14 she developed AF with RVR and diarrhoea. TSH was 0.1 mIU/L, T4 28.8 pmol/L, and T3 4.4 pmol/L. Carbimazole 10 mg daily, prednisolone, and metoprolol were commenced with good effect prior to discharge. Anti-thyroid antibodies were negative. Tc99m thyroid uptake was 0%. Ultrasound revealed no abnormalities. Urine iodine was significantly elevated (2216.4 mcg/24 hours).

Five days later she represented with tachycardia (150 bpm), abdominal pain and diarrhoea. Clinical examination demonstrated AF and mild peripheral tremor. Investigations confirmed T4 > 75 pmol/L, T3 7.9 pmol/L and mild pulmonary oedema. Burch-Wartofsky score 55. Treatment converted to propylthiouracil 200 mg QID, dexamethasone 4 mg BD, propranolol 40mg TDS and digoxin 125 mcg. After 4 days of stabilisation, treatment converted to carbimazole 20 mg BD and prednisolone 37.5 mg daily.

She remained biochemically hyperthyroid and had relapses of AF with RVR and decompensation on admission days 53 and 81. She then developed a hospital acquired pneumonia and subsequently transitioned to end-of-life-care.

ICM-associated hyperthyroidism is usually mild and self-limiting. [1] In retrospect, intervention with plasma exchange to reduce thyroid hormone levels prior to total thyroidectomy should have been pursued. [2, 3]

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Systematic Review: Impact of Increasing PreOperative HbA1C Levels on Postoperative Outcomes in Adults Undergoing Major Non-Cardiac Surgery

ABBY YU[1], QUYNH TRUONG[2]

1. Department of Pharmacy , Royal Brisbane Women's Hospital, Brisbane, Queensland, Australia

2. Internal Medicine Services, Royal Brisbane & Women's Hospital, Brisbane, Queensland, Australia

People with diabetes are more likely to require surgery due to the complications that can result from the condition.1-3 Whilst there is substantial evidence that diabetes increases the risk of morbidity and mortality after major surgery,4-10 literature is conflicting on whether elevated preoperative haemoglobin A1c (HbA1c) levels, a measure of glycaemic control, is associated poorer postoperative outcomes.11-16

The aim of this systematic review is to investigate the effect of incremental increases in preoperative HbA1c levels and their impact on incidence of postoperative complications in adult patients undergoing major non-cardiac surgery.

We systematically searched EMBASE, MEDLINE and the Cochrane Library databases to identify eligible studies published between January 2012 and July 2023. Studies which measured HbA1c within 6 months before surgery and analysed it as a continuous variable or compared outcomes between at least three incremental subgroups were included.

Twenty observational studies (108,005 patients) from various surgical specialties were included in the review. Patients with higher preoperative HbA1c levels had higher odds of overall postoperative complications, postoperative acute kidney injury, anastomotic leak, surgical site infections and increased length of stay. There were no significant differences in the incidence of reoperations and mortality within 30 days of surgery between HbA1c subgroups. The literature was highly variable with respect to composite major complications, perioperative cardiovascular events, hospital readmissions, postoperative infections, pneumonia and systemic thromboembolism.

Conclusion: There appears to be a trend between higher preoperative HbA1c levels and increased incidence of postoperative complications in patients undergoing major non-cardiac surgery. High quality evidence from prospective studies is lacking, with many of the included studies lacking adequate statistical power to detect differences at an individual outcome level. Larger prospective studies are needed to confirm if the risks posed by high preoperative HbA1c levels warrant significant perioperative intervention.

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Clinical audit of Diabetic Ketoacidosis presentations to a major tertiary centre

Julia JT Tsolakis[1, 3, 2], Serena SC Chong[1, 4, 2], Mike ML Lin[1, 5, 2], Albert AH Hsieh[1, 5, 2]

1. Royal Prince Alfred Hospital, Annandale, NSW, Australia

2. Endocrinology , Royal Prince Alfred Hospital, Camperdown, NSW, Australia

3. Faculty of Health and Medicine, University of Newcastle , Callaghan , NSW , Australia

4. Faculty of Health and Medicine, University of New South Wales, Kensington , NSW, Australia

5. Central Clinical School, Faculty of Medicine and Health, The University of Sydney, Camperdown , NSW , Australia

Diabetic Ketoacidosis (DKA) is an acute hyperglycaemic emergency with life-threatening metabolic derangement. The 2022 Australian National Diabetes Audit found 7.8% of survey participants with Type 1 Diabetes (T1DM) and 1.1% with Type 2 diabetes (T2DM) experienced DKA within the preceding 24 months[1]. This highlights the issue that DKA remains an important diabetic complication, even affecting individuals under dedicated specialist care. As such, we conducted an audit to describe the clinical characteristics, treatment approaches, and outcomes for patients requiring inpatient DKA management at the Royal Prince Alfred Hospital. Retrospective analysis of all DKA admissions from July 2021 to June 2023 were identified and data relating to patient demographics, DKA triggers and severity, length of stay (LOS), and time to resolution were collected.

DKA admissions (n=99) accounted for 24% of all Endocrinology admissions in this period and there were 8 euglycaemic ketoacidosis and 25 mixed DKA/hyperosmolar hyperglycaemic state. Sixty-five percents had T1DM, 33% T2DM and 2% Type 3cDM. Most patients were female (59%) and had pre-existing diabetes (84%). The majority of DKA admissions were moderately severe (pH 7.00-7.24) for patients with both T1DM (52%) and T2DM (61%). Patients with T2DM required longer time to DKA resolution (=14.3±8.2 hours) and ICU admission (=4.5±2.8 days) compared with T1DM (=9.8±6.6 hours) and (=2.1±1.5 days) respectively. Mean overall hospital LOS for patients with T2DM was also prolonged (9.7±8.9 days) compared with T1DM (3.7±4.2 days). In 2023, insulin pump failure and concurrent SGLT2-inhibitor use both accounted for 19% of DKA admissions respectively.

In conclusion, DKA remains a significant diabetic complication and presents more severely in patients with T2DM. Insulin pump therapy failure and SGLT2-inhibitors are increasingly featured as a trigger for DKA admissions. We recommend dedicated service-linkage and sick-day management education to reduce the severity and frequency of DKA presentations.

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Physician directed low cost self help program to help patients and families manage obesity and related health programs and creating a Cardio-Metabolic Institute

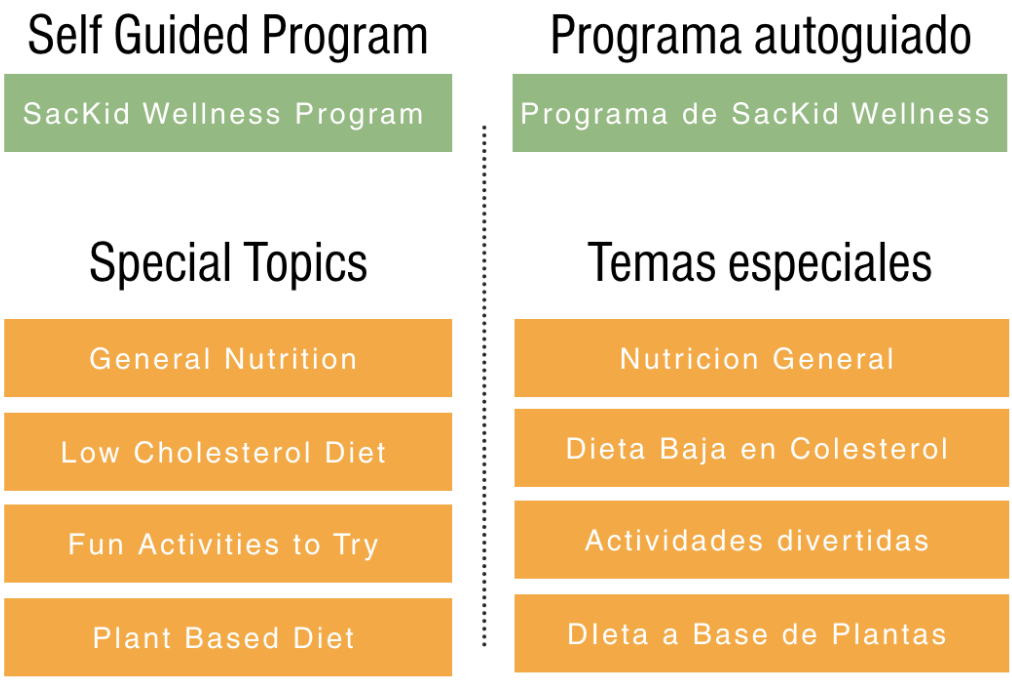
Aashi Venkat[1], GNANAGURUDASAN PRAKASAM[2, 1], Whitney Clark[2]

1. Pediatric Endocrinology, Center of Excellence In Diabetes and Endocrinology, Sacramento, CA, USA

2. SUTTER MEDICAL CENTER, SACRAMENTO, SACRAMENTO, CA, United States

There is an exponential increase in obesity, cardiometabolic morbidity and mortality globally[1].

Children and those living in lower-income regions are more likely to develop obesity, often due to their lack of exposure to obesity-related education and resources[2,3]. Despite both preventive care and secondary care effectively decreasing obesity rates, research favors accessible preventive care because it can stop obesity from occurring, decreasing the demand for secondary care[4,5]. Sackidwellness is our approach to create a multilingual, preventive resource for a motivated family to take care of their children and themselves.



To combat the paucity of resources on obesity, our group has created a self-guided, modular approach for children and their families. Our goal is to limit the patient's number of visits to a specialist and coordinate their care with primary care providers. The program consists of multilingual activities, videos, logs and booklets, and is tuned down to a weekly plan over a five month period. At the end of each week, family members meet to review their progress and plan the following weeks.

Week 1	**Material:** Introduction & schedule Healthy eating for families Booklet Activity & Eating for Adults Booklet Introduction to a Plant Based Diet (PBD): Pg 1 **Weekly Task:** ☐ Review booklets- a quick overview of each ☐ SacKid: Wellness Prescription:Complete 25% of prescription	Family meeting- ☐ have your first family meeting!

This design is helping us implement a uniform approach in educating our families with obesity, prediabetes, PCOS, metabolic syndrome, and hyperlipidemia. With a methodical regional/systemwide implementation.

This can be a partnership between primary and specialty care practices, with in-person and remote follow-up plans.

Further authentication is warranted by way of a prospective study comparing traditional diabetes prevention programs with low-cost programs like sackidwellness. We exclusively used publicly available resources, such as Wix. The capital expenditures were for the personnel time and domain registration only. Our future goal is to partner with community parks, schools and fitness centers to make the program ubiquitous.

Population Health Management is the future of Cardio-Metabolic Care. We are envisioning a Cardio-Metabolic Institute and the first step is creating this multilingual, self-directed Healthy Lifestyle program.

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Hyponatraemia is associated with deteriorated bone microarchitecture in older women

Annabelle M Warren[1, 2], Ali Ghasem-Zadeh[1, 2], Rudolf Hoermann[2], Alex Midgley[3], Ego Seeman[1, 2], Mathis Grossmann[1, 2]

1. Department of Endocrinology, The Austin Hospital, Heidelberg, VIC, Australia

2. Department of Medicine, University of Melbourne, Melbourne, VIC, Australia

3. Unaffiliated, Melbourne, VIC, Australia

Aims:

Hyponatraemia is a common electrolyte disorder that is associated with increased falls and fractures(1,2). Hyponatraemia has been associated with reduced bone mineral density (BMD) assessed using DXA(3), and reduced trabecular and cortical bone volume in an animal model(4). We aimed to quantify the morphological basis of any reduction in volumetric BMD (vBMD) in humans using High-Resolution peripheral Quantitative Computed Tomography (HR-pQCT), a non-invasive imaging technique assessing bone microarchitecture(5). We hypothesised that hyponatraemia would be associated with reduced vBMD.

Methods:

A database of 2557 adults scanned using HR-pQCT between 2007-2021 was matched to Austin Health pathology records to identify serum sodium measurements within 6 months of HR-pQCT date (closest, and nadir if multiple results). HR-pQCT outcomes across six pre-specified clinical strata by sex and age (18-50/50-75/>75 years) were assessed using repeated-measures mixed-effects models. Ethics approval was granted by Austin Health Office for Research.

Results:

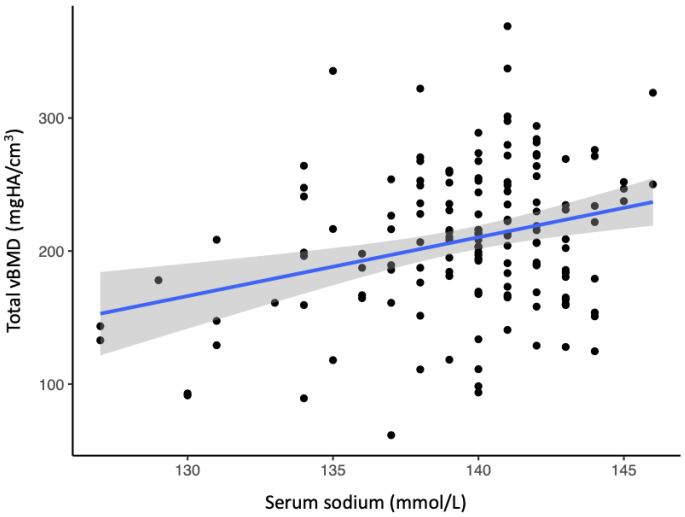
Serum sodium measurement within 6 months of HR-pQCT was available in 1462 individuals. Mean age was 59.5 years, 69% were female. The mean serum sodium closest to HR-pQCT date was 141mmol/L (range 127-148mmol/L). Forty-six individuals (3.1%) had serum sodium <135mmol/L, of which 5 (0.3%) had values <130mmol/L. Across the whole cohort, total vBMD at the radius and tibia was predicted by both the closest ($p<0.001$) and the nadir serum sodium ($p<0.001$), dependent on age and sex. The effect was most pronounced in women over 75 years, where correlation was then found between lower closest sodium and

deteriorated bone microstructure (r=0.29, p<0.001, **Fig. 1**). Limitations include the observational design, reliance on single sodium measurements, and lack of information regarding potential confounders.

Conclusion:

Lower serum sodium was associated with deteriorated bone microarchitecture in older women. Whether hyponatremia is the mechanism for impaired bone microarchitecture, and whether this correlates with fracture risk, requires confirmation in prospective studies.

Fig 1: Association between total vBMD at the tibia and serum sodium level in women over 75 years



vBMD = volumetric bone mineral density

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Application of a clinical support tool in active surveillance for low risk thyroid cancer – A Prospective Study

Eleanor White[1, 2], Anthony Glover[1, 2], Roderick Clifton-Bligh[1, 2], Matti Gild[1, 2]

1. Royal North Shore Hospital, St Leonards, NSW, Australia

2. University of Sydney, Sydney, NSW, Australia

Background: Most cases of thyroid cancer will have an excellent prognosis. There is emerging data to show we are over-treating many patients with low-risk thyroid cancer. The role of active surveillance (AS) as an alternative to surgery in the management of low-risk PTC is well established. Despite the growing body of evidence to support the option of AS, many clinicians do not feel comfortable in selecting appropriate patients.

Aims:

1) To determine if the application of a clinical support tool (CST) can identify patients who are appropriate for active surveillance

2) To assess whether the CST increases the number of patients with thyroid nodules <2cm that are managed with active surveillance?

Method: Our team will lead a randomised non-blinded cross-sectional multicentre study to evaluate the role of a clinical support tool in helping clinicians decide whether their patient is suitable for active surveillance.

Patients aged 18+ with a thyroid nodule <2cm with Bethesda III, V or VI biopsy result are eligible for inclusion. Clinicians will be block randomised in 1:1 fashion to receive either a modified clinical support tool (mCST) – control group or the clinical support tool (CST) – intervention group. The mCST will provide information about outcomes for active surveillance (including risk of nodule growth, metastases and death) and the CST will provide this information as well as whether the patient will be suitable for active surveillance based on data entered into the tool. Patients will decide their treatment in consultation with their clinician and their outcomes will be followed over time.

Outcomes: The following metrics will be assessed; rates of disease progression, rates of disease recurrence, patient quality of life outcomes (assessed through validated questionnaires EORTC QLQ C30 and THY34), and clinician acceptability of the tool. We anticipate the study duration of 36 months.

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Reversal of central obesity during the first year after bariatric surgery in Sri Lankan adults

Udai Wijetunga[1, 2], Uditha Bulugahapitiya[1], Thejana Wijeratne[3]

1. Endocrinology and Diabetes Unit, Colombo South Teaching Hospital, Colombo, Sri Lanka

2. Goulburn Valley Health, Shepparton, VICTORIA, Australia

3. Department of Surgery, Colombo South Teaching Hospital, Colombo, Sri Lanka

Introduction

Bariatric surgery is a very effective intervention to treat central obesity. Central obesity is strongly associated with increased cardiovascular risk, especially in patients of South Asian heritage. This is the first prospective study assessing efficacy of bariatric surgery, in reversal of central obesity in Sri Lankans to the best of our knowledge.

Objective

We aimed to assess the efficacy of bariatric surgery in reversing central obesity in obese Sri Lankan patients.

Methods

We followed up 50 obese patients who underwent bariatric surgery at Colombo South Teaching Hospital, Sri Lanka for 1 year. Central obesity was assessed by waist circumference (WC) measurement before and after bariatric surgery.

Results

Overall 90.0% were females. Mean age was 38.7 (±9.9) years. Mean pre-operative body weight, body mass index were 109.7 (±19.0) kg and 45.5 (±7.0) kg/m2 respectively. Body weight loss (±SD) at 1 month, 3 months, 6 months, 9 months and 12 months were 8.7 (±3.9) kg, 16.5 (±5.0) kg, 22.9 (±5.4) kg, 27.4 (±7.3) kg and 29.6 (±8.9) kg respectively. Mean WC reduction at the above follow up periods were 5.9 (± 8.1) cm, 13.3 (±5.8) cm, 18.9 (±7.1) cm, 21.6 (±5.9) cm and 23.5 (±10.2) cm respectively.

Conclusions

Bariatric surgery achieved impressive results for reversal of central obesity in Sri Lankan adults. The results were evident as early as 1 month after bariatric surgery. This is expected to translate into significant cardiovascular risk reduction in these patients, starting as early as 1 month after surgery.

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SGLT2 inhibitors and the incidence of euglycemic diabetic ketoacidosis in a specialist cancer centre

George Yang[1], Cheryl Jackson[1], Senthil Lingaratnam[1], Nirupa Sachithanandan[1], Cherie Chiang[1]

1. Peter MacCallum Cancer Centre, Parkville, VIC, Australia

Sodium-glucose co-transporter 2 inhibitors (SGLT2i) were first approved in Australia in 2014. Its indications have expanded beyond diabetes treatment to include treatment of heart failure and kidney disease. However, euglycaemic diabetic ketoacidosis (eDKA) can be precipitated by reduced oral intake, systemic illness, and infection, all of which are common in the oncological population. The incidence of eDKA in elective and emergency procedures in surgical inpatients were reported at 0.17% and 1.1% respectively. However, the rate remains unknown in the oncological setting.

This retrospective study examines the prevalence of eDKA in cancer patients who received SGLT2i at Peter MacCallum Cancer Centre, a specialist cancer centre. Pharmacy records of inpatient SGLT2i prescriptions were extracted between September 2016 and February 2023. Variables documented included SGLT2i cessation pre-elective procedure, admission category (emergency or elective), and risk factors for eDKA. eDKA was defined as glucose < 16 mmol/L, ketone > 0.6, bicarbonate ≤ 18 mmol/L or pH ≤ 7.3.

274 admissions from 180 patients (age 67 ± 9 years, 71% male, 37% on Dapagliflozin, 63% on Empaglifozin) were recorded. 58% were elective admissions; 67% had documented reduced oral intake. There were 15/274 (5.5%) episodes of eDKA. Of the 15 unique patients with eDKA (ketone = 4 ± 3.5), mortality rate at follow-up was 47% versus 34% (p = 0.048) in those without eDKA. The risk factor for eDKA was reduced oral intake (7.7% vs 1.1%, p = 0.025). Admission category, withholding SGLT2i, chemotherapy, sex, glucocorticoid use or type of SGLT2i did not alter the incidence of eDKA.

The prevalence of eDKA in oncology patients on SGLT2i was higher than reported in other populations and was associated with higher mortality, reflecting the catabolic state of this cohort. The overall mortality (26% at study cessation) might diminish the long-term cardiovascular and metabolic benefits of this drug class.

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A scoping review of adrenal vein sampling in practice: lessons for implementation

Heather Morris[1], Angela Melder[1], Elisabeth Ng[2], Helen Skouteris[1], Winston Chong[3], Jun Yang[2]

1. Health and Social Care Unit, Monash University, Melbourne, Victoria, Australia

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

3. Radiology, Monash Health, Clayton, Victoria, Australia

Adrenal Vein Sampling (AVS) is a complicated procedure requiring clinical expertise, collaboration, and patient involvement to ensure it occurs successfully. Implementation science offers unique insights into the barriers and enablers of service delivery — which are critical to the provision of quality healthcare. The primary aim of this scoping review was to identify implementation components as described within clinical studies, that contribute to a successful AVS procedure. The secondary aim was to provide informed considerations for practice to support the scale-up of AVS. A scoping review was conducted for clinical papers that also discussed factors contributing to effective AVS implementation. Implementation strategies were named and defined, allowing for implementation learnings to be synthesised in the absence of dedicated research examining implementation processes. Ten implementation components reported as contributing to a successful AVS procedure were identified. These components were categorised according to actions required pre-AVS (technical skills development, protocol standardisation, adrenal venous mapping and patient preparation), during AVS (procedural support, rapid cortisol assays and collaboration with pathology) and post-AVS (consensus based criteria and team based approach to AVS outcome interpretation). A taxonomy of strategies effective in implementing change was utilised to support and inform the development of recommendations for scale-up. Using an implementation science approach, the findings of this review and analysis provide practical insights and considerations to facilitate AVS service delivery design. Extracting implementation science information from clinical research has provided a mechanism that accelerates the translation of evidence into practice where implementation research is not yet available. It represents an approach to inform future implementation research in a directed and focussed manner.

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Effect of risedronate on bone loss after hematopoietic stems cell transplantation: A Prospective, Double-blinded, Randomized Controlled Trial

Jin Yu[1], Jeonghoon Ha[1]

1. Seoul Saint Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, South Korea

Publish consent withheld

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Pretreatment with phenoxybenzamine or doxazosin - which better prevents hypertensive surges during laparoscopic adrenalectomy for pheochromocytoma?

Karolina Zawadzka[1, 2], Piotr Małczak[1], Michał Wysocki[3], Piotr Major[1], Michał Pędziwiatr[1], Magdalena Pisarska-Adamczyk[4]

1. Jagiellonian University Medical College, 2nd Department of General Surgery, Kraków, Poland

2. Jagiellonian University Medical College, Doctoral School of Medical and Health Sciences, Kraków, Poland

3. Ludwik Rydygier Memorial Specialized Hospital, Department of General Surgery and Surgical Oncology, Kraków, Poland

4. Jagiellonian University Medical College, Department of Medical Education, Kraków, Poland

Aims: Selective or non-selective alpha-blockers are currently used to prevent intraoperative hypertensive surges during adrenalectomy of pheochromocytomas and paragangliomas (PPGLs). However, the effect of these two types of drugs on the effective prevention of hypertensive surges remains ambiguous. The aim of our study was to compare the effectiveness of pretreatment with phenoxybenzamine (PXB) and doxazosin (DOX) in the context of preventing hypertension during laparoscopic adrenalectomy of phaeochromocytoma.

Methods: From 124 consecutive patients who underwent laparoscopic adrenalectomy of phaeochromocytomas in our clinic between 2003 and 2022, we selected 60 patients pretreated with phenoxybenzamine alone and 30 treated with doxazosin and retrospectively collected their data.

Results: There were no statistically significant differences between the PXB and DOX groups in terms of sex, age, BMI, comorbidities, and pheochromocytoma size. Preoperative systolic blood pressure was higher in doxazosin-treated patients (median 134.5, IQR 20 mm Hg vs median 125, IQR 30 mm Hg in PXB group, p=0.045). There was no difference between groups in diastolic blood pressure before surgery and the first blood pressure measured during surgery. The percentage of patients who experienced hypertension during the procedure did not differ between the PXB and DOX groups: episodes of blood pressure above 160 mmHg in 61.67% vs. 66.67% of patients (p = 0.64), and blood pressure above 200 mm Hg in 23.33% vs. 26.67% of patients (p = 0.73). However, in patients who experienced intraoperative hypertensive episodes, the duration of the BP episodes >200 mmHg was significantly higher in the DOX group: median 7.5 minutes, IQR than in the PXB group (median 7.5, IQR 5 minutes versus median 22.5, IQR 30 minutes, p=0.02).

Conclusion: Patients pretreated with doxazosin had higher systolic blood pressure before surgery. Furthermore, in the doxazosin-treated patients, intraoperative hypertensive episodes above >200 mg lasted significantly longer than in the phenoxybenzamine group.

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A case of pseudoaldosteronism due to naturopathic supplements

Xian Wei Ang[1], Laura Edward[2], David Roberts[2], Simon Ryder[1]

1. Logan Endocrine and Diabetes Services, Logan Hospital, Logan, Queensland, Australia

2. Department of Medicine, Logan Hospital, Logan, Queensland, Australia

Introduction

Pseudoaldosteronism is rare condition of apparent mineralocorticoid excess with hypokalaemia and hypertension, with low renin and aldosterone, attributable to excess liquorice consumption(1). We present a case of pseudoaldosteronism due to commercially available herbal supplement taken at recommended dose.

Case detail

A 71-year-old man was referred to hospital for severe hypokalemia (2.7mmol/L), hypertensive urgency (189/94mmHg), associated with palpitations and tension headache. Medical history included restless legs syndrome, obstructive sleep apnea, benign prostate hyperplasia, without known hypertension. Regular medications included herbal supplements prescribed by his naturopathic practitioner for "adrenal fatigue". This included *Glycyrrhiza glabra* (liquorice), equivalent to 100mg glycyrrhizic acid consumed daily. His herbal supplements were ceased and treated with oral and intravenous potassium supplementation. Antihypertensive medication was considered, but blood pressure improved without further intervention. Biochemistry revealed suppressed renin and aldosterone, which normalised when retested at 6 weeks (table 1). Hypokalaemia resolved without further potassium supplementation on discharge. Serum cortisol:cortisone ratio reduced from 16:1 to 7:1. No adrenal lesion was noted on computed tomography.

Table 1 Pathology results during initial admission and subsequent follow up on week 6.

Test	Initial admission	Week 6 follow up	Reference range
Potassium	2.9mmol/L	4.4mmol/L	3.5-5.2mmol/L
Adrenocorticotrophic hormone	27ng/L	19ng/L	10-50ng/L
Cortisol (LC-MS/MS)	872nmol/L	402nmol/L	140-640nmol/L
Cortisone	53nmol/L	56nmol/L	Not available
Aldosterone (LC-MS/MS)	8pmol/L	38pmol/L	0-400pmol/L
Renin (mass)	1.1mU/L	8.8mU/L	2-29mU/L
Aldosterone-Renin Ratio	7.3	4.3	>55

* All hormone values are early morning samples (between 0600-0800).

Discussion

Pseudoaldosteronism is due to inhibition of 11-hydroxysteroid dehydrogenase type-2 by glycyrrhizic and glycyrrhetinic acid, the active ingredients of liquorice root(1). Liquorice is regulated food additive in United States but not in Australia, with challenging risk prediction, given high number of products, variable content, and lack of routine surveillance for adverse effects(2,3). There is high variability in individual susceptibility, with no established safe dose(4,5). There are also 10-fold variability of glycyrrhizic acid content between plants cultivated in identical conditions(6). Additionally, "adrenal fatigue", "adrenal burnout", "tired adrenals" are terms not recognised by endocrinology societies, although some practitioners continue to advocate for recognition and treatment(7).

Conclusion

This case highlights potential dangers of liquorice-containing products and gaps remain in regulatory requirements. Clinicians should undertake careful medication reconciliation to identify undeclared liquorice consumption as potential cause of hypertension and hypokalaemia.

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Transforming Maternal Health care in rural communities: Leveraging existing resources to strengthen health care systems and Foster Sustainable Solutions for Gestational Diabetes Mellitus

Radhika Dr Arunkumar[1]

1. Baker Heart and Diabetes Institute, Melbourne, VICTORIA, Australia

Gestational Diabetes Mellitus (GDM) poses a substantial health risk to both mothers and the fetus without timely management. Women from rural areas are particularly vulnerable to the risks associated with GDM due to socio-ecological barriers including limited access to health care facilities including transportation, language, communication, lack of awareness, education and inadequate postpartum support. In this study we aim to focus on utilising the existing GDM-related healthcare infrastructure in rural Australia, by a) understanding the potential of digital health support within rural service infrastructure, and b) from the perspective of mothers, partners, and care givers, what is desired in terms of community-based interventions. We will be developing a mixed-methods approach, conducting a survey with expecting mothers and those who have given birth in the last 12 months in rural hospitals of Victoria and Queensland. We aim to firstly, to publish a systematic review on the barriers and enablers in GDM-related intervention in rural population of Australian which will be the first systematic review on the rural co hort; secondly, utilise the survey responses to identify the barriers and enablers to implement better eHealth facilities; thirdly, to educate people about early intervention and management by providing comprehensive health education and resources to make informed decision during pregnancy, childbirth and diabetes management. The second phase of the study will engage industry partners by fostering collaborations, involving clinicians and experts to establish mobile clinics equipped with telemedicine capabilities, making quality prenatal care accessible in remote regions. Involvement of stakeholders will facilitate initiatives using technology to disseminate information about GDM, develop shared goals to bridge gaps ensuring comprehensive maternal health care delivery in remote region supporting generations to come.

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The Proselyte Pituitary: A tale of a pituitary macroadenoma, or so we thought!

Daniel Bennett[1], Kyne Hanlon[1], Andra Desra[2], Jennifer Wong[3, 1], Peter Fuller[4, 5, 6]

1. Endocrinology and Diabetes, Monash Health, Melbourne, VIC, Australia

2. Clinical Biochemistry, Monash Health, Melbourne, Victoria, Australia

3. Monash University, Melbourne, Victoria, Australia

4. Endocrinology, Monash Health, Melbourne, Victoria, Australia

5. Hudson Institute of Medical Research, Melbourne, VIC, Australia

6. Monash Health, Melbourne, VIC, Australia

We describe a case of a 48-year-old man who has undergone 4 pituitary surgeries and two courses of radiotherapy for an initially non-functioning but aggressive pituitary adenoma (PA) over a 9-year period. He underwent both a malignant and secretory transformation with pituitary carcinoma (PC) and Cushing's Disease (CD) after approximately 8 years. He required anti-steroidogenesis therapy with metyrapone as well as bilateral adrenalectomy following sequential admissions to hospital for acute complications of malignant hypertension. He has had a partial response to temozolomide and is planned for Peptide Receptor Radionuclide Therapy (PRRT). His condition is complicated by complete blindness on the right as well as pan-hypopituitarism and primary adrenal insufficiency and he faces significant functional and psychological disability. Monitoring for the development of Nelson's Syndrome (NS) is of great concern to this man given his significant vision loss.

The rarity of PC and lack of prospective data has made the development of clinical guidelines challenging. In 2018 the European Society of Endocrinology published clinical practice guidelines for the diagnosis and management of aggressive PAs and PCs (1). They include recommendations for pituitary surgery, radiotherapy, endocrine and chemotherapies. Temozolomide is recommended as first line treatment in appropriate cases, with approximately 50% of patients responding. Case series data has shown efficacy for immunotherapy, targeted therapies and PRRT (1, 2).

In 2022 a consensus clinical management guideline was published in Lancet Endocrinology & Diabetes for the diagnosis and management of Cushing's' disease including advice on medical therapies (3). Metyrapone is suggested for use in situations where rapid lowing of cortisol levels is required to treat acute severe complications Cushing's' Disease.

Summary

PC is a rare disease which is often functional and can be challenging to treat, requiring a multidisciplinary approach. Options are limited by lack of prospective data but mortality has improved over time.

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Severe hypercalcaemia in pregnancy - use of calcitonin as bridging therapy to parathyroidectomy.

Maria Bilal[1], Krupali Bulsari[1]

1. Macarthur Diabetes, Endocrinology and Metabolism Service, Campbelltown Hospital , Campbelltown, NSW, Australia

We discuss the case of a 41-year-old female gravida 6 para 5 referred to a tertiary hospital at 11 weeks gestation with severe hypercalcaemia. Presenting symptoms included nausea, polydipsia, chest pain and constipation. Past medical history was unremarkable but family history was positive for breast cancer. Her only regular medication was Vitamin D.

Pathology demonstrated a corrected Calcium of 4.18mmol/L and a Phosphate of 0.63 mmol/L. A parathyroid hormone level of 28.4pmol/L confirmed the diagnosis of primary hyperparathyroidism. 4D CT with body shielding found a 34x25mm left inferior parathyroid mass.

Management was initiated with aggressive intravenous fluids, obstetric and cardiology reviews. Despite 72 hours of fluid resuscitation, the corrected calcium remained above 3.5mmol/L and the patient developed fluid overload and hypertension. She received intravenous calcitonin infusions for 3 days - 600mg in 500mL saline over 6 hours. The corrected calcium dropped from 3.52mmol/L to 2.99mmol/L following 24 hours of the calcitonin infusion. The nadir level was 2.81mmol/L, two days after the first calcitonin dose. It then rose to 3.26 mmol/L requiring a further infusion.

She underwent a parathyroidectomy at 12 weeks gestation to remove a large left inferior parathyroid adenoma. She was monitored for hungry bone syndrome and was stable on oral Caltrate and Vitamin D. The foetus was small for gestational age (5^{th} centile). She had induction of labour and vaginal delivery at 39 weeks gestation.

This case demonstrates the use of calcitonin as a bridge to parathyroidectomy where there is hypercalcaemia refractory to intravenous fluids. Whilst effectiveness of calcitonin is limited by tachyphylaxis, it is advantageous as it does not cross the placenta[1] and provides time until definitive management can occur. In contrast Cinacalcet, which is the preferred agent, is Category C in pregnancy[2]. Our patient wanted to avoid Cinacalcet and hence calcitonin was used.

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Cauda equina neuroendocrine tumours - not your standard paraganglioma

Laura Careless[1], Benjamin Sly[1]

1. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Case 1 - 63year old gentleman who presented with cauda equina syndrome. MRI revealed an intrathecal mass lesion displacing the cauda equina nerve roots. He underwent surgical resection in November 2021 with histology showing neuroendocrine cells with immunohistochemistry staining consistent with a spinal paraganglioma. Succinate Dehydrogenase (SDH) was retained. Plasma metanephrines post-operatively were normal, with no symptoms of catecholamine excess. Genetic testing revealed no pathogenic variants. DOTATATE-PET scan postoperatively showed no additional sites of avidity.

Case 2 - 55 year old lady who presented with increasing lower back and right leg pain. MRI spine showed an L4/5 intradural extramedullary lesion, she underwent spinal surgery in February 2023 with complete resection. Histology showed features consistent with a NET. Cells stained positive for keratin, synaptophysin and chromogranin. Ki67 proliferation index was <1%. SDHA and SDHB staining was retained. Post-operative plasma metanephrines were normal, no symptoms of catecholamine excess were noted.Specific genetic testing was negative. DOTATATE-PET scan post operatively was also unremarkable.

Paragangliomas (PGL) are extra-adrenal neuroendocrine tumours arising from neural crest cells migrating to ganglia of the autonomic nervous system and can be either sympathetic or parasympathetic in origin. Sympathetic PGLs secrete catecholamines whereas parasympathetic lesions are non-functional[1,2]. Approximately 40% of patients with PGLs have a hereditary disease-specific germline mutation. In the remaining ~60% that occur sporadically, at least 30% have a somatic mutation[3].

Primary CNS NETs are rare and most commonly occur in the cauda equina region[4,5]. Cauda equina NETs are no longer classified as cauda equina PGLs because they are morphologically, molecularly and histogenetically distinct from PGLs[1,6]. The main distinguishing feature is having an epithelial as well as neuronal component[1]. Unlike PGLs, cauda equina NETs are almost always hormonally silent, cytokeratin positive, GATA3 negative and HOXB13 positive[1,7]. They aren't associated with hereditary germline mutations and are generally indolent[6,7].

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Primary adrenal melanoma masquerading as adrenocortical carcinoma

Rebekah R Chew[1], Esther Quick[1], Jui T Ho[1], Morton G Burt[1]

1. Flinders Medical Centre, Adelaide, SA, Australia

Introduction:

Primary Adrenal Melanoma (PAM) is an extremely rare malignancy, with fewer than twenty-five cases described. The symptoms are non-specific and it may present as an adrenal incidentaloma. PAM is diagnosed by histopathology and exclusion of primary melanoma from other sites. Although adrenal metastases from skin melanoma are more common, PAM is a differential diagnosis in a unilateral adrenal mass that appears malignant if there is no history of malignancy or skin lesion.

Case:

We present a 69 year old lady with an incidental right adrenal mass measuring 85x60x72 mm (density of 34 Hounsfield Units) with inferior vena cava extension and bilateral pulmonary lesions. There were no clinical or biochemical features of hormone

excess (*Table 1*). FDG-PET showed intense FDG avidity of the adrenal mass and pulmonary lesions. Based on imaging characteristics and in the absence of extra-adrenal malignancy, the presumptive diagnosis was a non-functioning adrenocortical carcinoma. She unfortunately developed new onset haemoptysis and underwent bronchoscopy to localise the source of bleeding. The pulmonary lesions were inaccessible for bronchoscopic biopsy. There were unforeseen complications post-procedure with decreased conscious state, necessitating intubation and management in the Intensive Care Unit. CT imaging post-procedure showed new pulmonary emboli and a 25% increase in the volume of the adrenal lesion. MRI showed diffuse hypoxic-ischaemic brain injury. At the family's request, she underwent CT-guided adrenal core biopsy which revealed the unexpected finding of melanoma. There were no skin lesions identified. Due to poor neurological recovery, the decision was made for palliative care following extensive discussion with her family.

Conclusion:

Adrenal biopsy is not routinely recommended for diagnosis unless there is evidence of metastatic disease that precludes surgery and histopathology is required to guide oncological treatment. In this case, a diagnosis of melanoma would have broadened therapeutic options if the patient's clinical state had improved.

Investigation (units)	Result	Reference Range
1 mg dexamethasone suppression test – cortisol (nmol/L)	48	< 50
24 hour urinary free cortisol (nmol/24h)	14	50 – 150
Plasma metanephrine level (pmol/L)	< 100	< 500
Plasma normetanephrine level (pmol/L)	780	< 900
3-Methoxy-Tyramine level (pmol/L)	< 100	< 150
Dehydroepiandrosterone Sulphate (DHEAS) level (umol/L)	0.3	1.8 – 9.2
Testosterone Level (nmol/L)	< 0.10	0.00 – 1.49

Table 1. Hormonal evaluation of right adrenal mass.

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Not just an insulinoma

Ritoo Chowdhury[1], Sumathy Perampalam[1]

1. The Canberra Hospital, Campbell, ACT, Australia

A 20-year-old male presented to the Emergency department following a hypoglycaemic seizure. He had a background of ADHD and a recent diagnosis of epilepsy. He was a smoker and consumed 4 standard drinks daily. On examination he was confused with a capillary glucose level of 2.6mmol/L, the confusion improved following intravenous dextrose. The following morning capillary glucose level measured 2.4mmol/L. Electrolytes, liver and renal function were normal and early morning cortisol was 590nmol/L.

A 72 hour fast was commenced and terminated at twelve hours when serum glucose measured 2.1mmol/L, c-peptide 0.9nmol/L (ref 0.1-1.5), insulin level 4mU/L (ref<27mU/L), and proinsulin level 94.4pmol/L (ref <13.3) suggestive of an insulinoma. Insulin antibodies were not detected. CT abdomen revealed a pancreatic tail lesion measuring 36x30x25mm and a left adrenal nodule measuring 33x22mm, 36HU. Biochemical screening confirmed a non-functioning adrenal lesion. The pancreatic lesion exhibited marked DOTATATE activity. He underwent a distal pancreatectomy and left adrenalectomy. Histopathology confirmed a 29mm pancreatic neuroendocrine tumour and a 46mm adrenal cortical neoplasm of uncertain malignant potential. The genetic screening for MEN1, CDKN1B and TP53 were negative. He will continue to have six monthly adrenal MRI and DOTATATE PET for two years and monitoring for biochemical recurrence and screening for primary hyperparathyroidism.

Insulinomas are rare neuroendocrine tumours with an incidence of four cases per million individuals per year (1). Although genetic screening for MEN-1 syndrome was negative, 10-30% of patients have no mutation in the MEN-1 gene (2). Adrenal oncocytic neoplasms (AONs) are extremely rare (3). In a systematic review of 140 cases of AONs 35% were benign, 41% borderline and 24% malignant. The five-year survival being 100%, 88% and 47% respectively (4). Currently there are no standard post-operative surveillance for AONs, however it has been suggested that close follow up for a minimum of five years is required.

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Risk factors associated with osmotic demyelination syndrome: beyond rapid correction of hyponatraemia

Kay Hau Aaron Choy[1], K. Somila Silva[2]

1. Department of Endocrinology and Diabetes, Barwon Health, Geelong, Victoria, Australia

2. Department of General Medicine, Barwon Health, Geelong, Victoria, Australia

Introduction: Osmotic demyelination syndrome (ODS) is an uncommon neurological disorder that occurs secondary to rapid plasma osmotic shifts. It is a recognised complication of overcorrection of hyponatraemia.

Methods: We present a case of ODS in a patient with severe hyponatraemia and other predisposing factors.

Results: A 60-year-old man with alcoholic liver disease and malnutrition was hospitalised with delirium. Assessment revealed hypervolaemic hyponatraemia (serum sodium 117mmol/L, range: 135-145), mild hypokalaemia, Wernicke encephalopathy and deranged liver function. Computed tomography of the brain (CTB) was unremarkable. With close monitoring of serum sodium (SNa), fluid restriction was commenced, leading to an increase in SNa to 123mmol/L 24 hours later, 128mmol/L 48 hours later and 132mmol/L 72 hours later. SNa normalised (136mmol/L) on day 7 of admission. He subsequently underwent inpatient rehabilitation for 2 weeks before he self-discharged against medical advice. He represented a week later with functional and cognitive decline. Repeat SNa was normal at 138 mmol/L. Repeat CTB revealed a new 15 mm hypodense focus in the pons. Further evaluation with a magnetic resonance imaging (MRI) demonstrated a region of T2 and fluid-attenuated inversion recovery (FLAIR) hyperintensity and low T1 signal intensity within the central pons with restricted diffusion, in keeping with osmotic demyelination syndrome. At 3-month follow-up, there was minimal improvement in his functional status and cognition. Repeat MRI showed ongoing central pontine abnormality with new surrounding cavitation, indicating coagulative necrosis.

Conclusion: Mechanisms of ODS have classically focused on sudden osmotic shift in the context of overcorrection of hyponatraemia. However, it is important to be vigilant for other conditions that can be associated with ODS, independent of SNa changes, such as those seen in this case: alcoholism, liver disease, hypokalaemia and malnutrition. Judicious correction of hyponatraemia is warranted in those with comorbid factors susceptible to ODS.

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The Eyes, The Heart, and The Adrenal, How The Optics Matter

Raymond Dharmaputra[1]

1. Endocrinology, Gold Coast Health Service, Southport, QLD, Australia

We present the case of a 40-year-old man with multiple hypertensive target organ damage due to delayed diagnosis of phaeochromocytoma.

S, a 40-year-old toy maker presented to ED with blood pressure of 211/127 mmHg and pulmonary oedema. He was transferred to ICU for treatment of malignant hypertension. Bed-side ECG and transthoracic echo demonstrated left ventricular hypertrophy and dilated aortic root with aortic regurgitation consistent with hypertensive cardiomyopathy. His medical history includes chronic resistant hypertension, obesity, and idiopathic intracranial hypertension(IIH). Following rapid blood pressure control, he was discharged on prazosin 0.5mg BD, metoprolol 50mg bd, and perindopril 5mg daily.

S attended the ophthalmology clinic with deteriorating vision and was readmitted for management of IIH. He remained hypertensive despite of titration of his anti-hypertensive agents. CT-scan detected a 41mm solid right adrenal mass and MRI-brain revealed central posterior reversible encephalopathy syndrome(PRES). Plasma normetadrenaline was elevated at 20000 pmol/L and urinary noradrenaline was measured at 21800 nmol/24 hr. Following commencement of intensive alpha-blockade, right adrenalectomy was performed. Histology confirmed phaeochromocytoma with GAPP score of 3. His blood pressure rapidly normalised and he was discharged without any anti-hypertensive. Plasma and urinary free metanephrines normalised following surgery and his vision subsequently improved.

The prevalence of phaeochromocytoma in individuals with adrenal mass and hypertension is as high as 14%[8]. Positive imaging paired with elevated plasma free metanephrines has a sensitivity and specificity of close to 100%[2]. This raised the question whether early biochemical assessment should be considered despite of acute illness. Diagnostic pitfalls in this case include ascertainment bias of obesity being the sole explanation of the patient's clinical presentations leading to cognitive anchoring on the initial diagnosis; and the interrupted diagnostic momentum due to fragmentation of patient care affecting clinical follow up.

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Phaeochromocytoma crisis presenting with haemodynamic instability and multi-organ failure

Frank Gao[1, 2], Amin Sharifi[3], Jenny Ng[3]

1. Austin Health, Heidelberg Heights, VIC, Australia

2. University of Melbourne, Melbourne, VIC, Australia

3. Eastern Health, Box Hill, VIC, Australia

A 56-year-old woman from home alone presented to the emergency department with vomiting and chest tightness. On arrival to hospital, she was found to have significant biochemical derangement with pH <6.8, potassium 8.6 mmol/L, lactate 12 mmol/L, glucose 42 mmol/L, ketones 4.8 mmol/L and serum osmolality 356 mmol/L. ECG demonstrated ventricular tachycardia and bedside echocardiogram showed severe left ventricular dysfunction. Systolic blood pressure was initially >200 mmHg and she required intubation for acute pulmonary oedema and renal replacement therapy for acute kidney injury. Following intubation, her blood pressure dropped significantly requiring inotropic support, however she had periods of significant blood pressure spikes with systolic blood pressure intermittently >200 mmHg treated with glyceryl trinitrate and hydralazine, and required sedation with dexmedetomidine. During an attempted tracheostomy and bronchoscopy under sedation, her systolic blood pressure ranged from 60 to 190 mmHg and the procedure had to be aborted.

CT chest/abdomen/pelvis performed in the setting of fever and undifferentiated cause for multi-organ failure demonstrated a 3.5cm heterogeneously enhancing left adrenal mass, which was inconsistent with a simple adenoma on MRI. Further investigations demonstrated plasma normetanephrine 15506 pmol/L (<900), metanephrine 6126 pmol/L (<500), and 3-methoxytyramine 1007 pmol/L (<110). She was diagnosed with a phaeochromocytoma, however management was complex due to intermittent hypotension even prior to commencement of alpha-blockade. She was commenced on low-dose prazosin, which was changed to phenoxybenzamine once her clinical status had stabilised, and later commenced on beta-blockade with metoprolol. Following adequate alpha-blockade, she underwent elective surgical excision of her phaeochromocytoma without complication.

Phaeochromocytoma crisis is a rare presentation of phaeochromocytoma, characterised by haemodynamic instability (including either transient or sustained hypotension) and end-organ dysfunction. Management is complex due to difficulties with alpha-blockade if hypotension is a feature. Surgical timing and the role of emergency adrenalectomy is controversial.

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A case of severe myositis: clinicopathological challenges in the differentiation between statin induced myopathy and immune-mediated necrotising myositis

Han Goh[1], Edward Quan[1], Felicity Stringer[1], Caroline Jung[2, 3]

1. St Vincent's Hospital, Melbourne, VIC, Australia

2. University of Melbourne, Melbourne, VIC, Australia

3. Department of Endocrinology, St Vincent's Hospital, Melbourne, VIC, Australia

A 68 year old man presented with 10 days of progressive lower limb myalgia and weakness. Past medical history was significant for ischaemic heart disease, type 2 diabetes mellitus and stage 4 chronic kidney disease, and with notable medication history of rosuvastatin 40mg nocte for the last 5 years.

He was found to have a CK of 33775 U/L, and severe acute on chronic kidney injury, with an eGFR of 6mL/min/1.73m^2, from a baseline of 31mL/min/1.73m^2. A muscle biopsy of the right vastus lateralis showed features initially believed to be consistent with immune-mediated necrotising myositis (IMNM), with immunohistochemistry for C5b9 showing prominent granular membranous reactivity.

Based on this diagnosis, the patient was immunosuppressed with a combination of intravenous immunoglobulin, high dose steroids and rituximab. Haemodialysis was also commenced in the setting of end stage renal failure.

The myositis antibody panel was requested, including 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibody which returned negative several weeks into the admission. This, alongside minimal clinical improvement with immunosuppression, prompted review of the initial diagnosis.

A review of the literature suggested that the finding of granular membrane deposits of C5b9 on non-necrotic muscle fibres is not specific for IMNM but can also be seen in regenerating fibres in other conditions [1-3]. Overall, the clinical presentation was felt to be more in keeping with a toxic necrotising myopathy and rhabdomyolysis, caused by a combination of statin use and long standing type 2 diabetes mellitus, with recovery impaired in the setting of severe renal failure.

The patient was therefore weaned off immunosuppression, and was managed conservatively with a focus on rehabilitation and optimisation of his comorbidities. The diagnosis of IMNM and subsequent immunosuppression should be made in the right clinical context, given non-specific muscle biopsy features and slow turnaround time of HMGCR antibody result.

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Metanephrine mirage: distinguishing the phaeocopies

Sylvia Ye[1], Joanna Y Gong[1], Debbie Gordon[1, 2], Bill Fleming[3], Jason Tan[3], Toby Hulf[3], Thiep Kuany[3], Maryam Shamassi[4], Christopher J Yates[1, 5], Dev Kevat[1, 6]

1. Department of Endocrinology and Diabetes, Western Health, Melbourne, Victoria, Australia

2. Department of Endocrinology and Diabetes, Eastern Health, Melbourne, Victoria, Australia

3. Department of Endocrine Surgery, Western Health, Melbourne, Victoria, Australia

4. Department of Anatomical Pathology, Dorevitch Pathology, Western Health, Melbourne, Victoria, Australia

5. Department of Diabetes and Endocrinology, Melbourne Health, Melbourne, Victoria, Australia

6. Department of Endocrinology and Diabetes, Monash Health, Melbourne, Victoria, Australia

Neuroblastomas (NB) are a rare cause of adrenal malignancy in adults that may present as an incidentaloma. We report the case of an adult catecholamine-secreting adrenal NB, one of only 6 such reported cases.[1]

A 63-year-old male was urgently reviewed at the endocrinology outpatient clinic for a right 12cm adrenal incidentaloma, incidentally found on a CT chest performed to investigate weight loss. He was a current heavy smoker (59 pack-year history), with chronic obstructive pulmonary disease and schizophrenia.

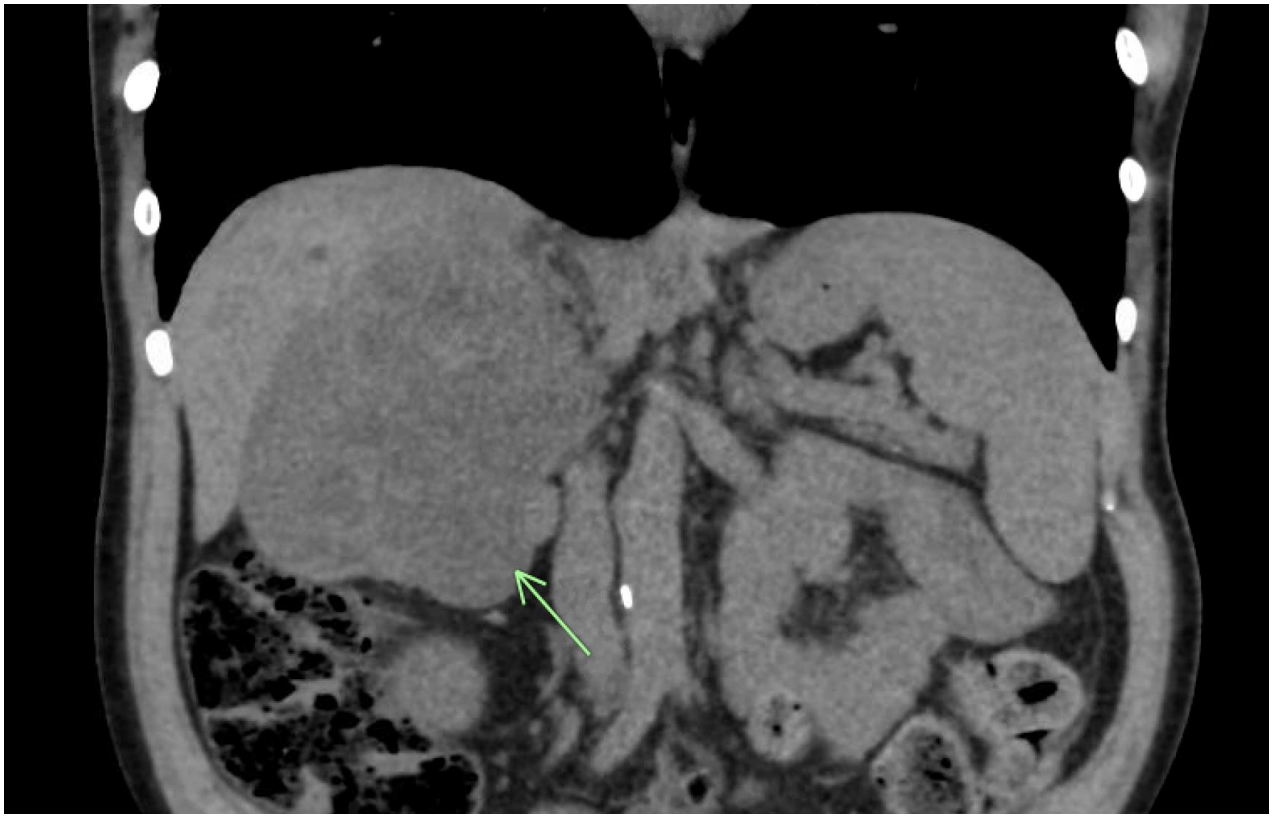
The patient had asymptomatic hypertension (systolic blood pressure 134−163mmHg) and plasma normetanephrine and 3-methoxytyramine levels 10 times the upper limit of normal at 9272pmol/L (<900) and 1023pmol/L (<110) respectively, while metanephrine was unremarkable. Urine biochemistry was concordant. CT revealed an adrenal mass measuring 12.2cm x 9.7cm x 10.7cm with heterogeneous post-contrast enhancement with enhancing solid areas, non-enhancing necrotic and cystic areas. The adrenal mass was inseparable from the liver on imaging (*Figure 1*) and thus suspected to be an invasive malignant phaeochromocytoma.

The patient underwent a right adrenalectomy and segment 6/7 liver resection (*Figures 2* and *3*). Post-operatively, he recovered well.

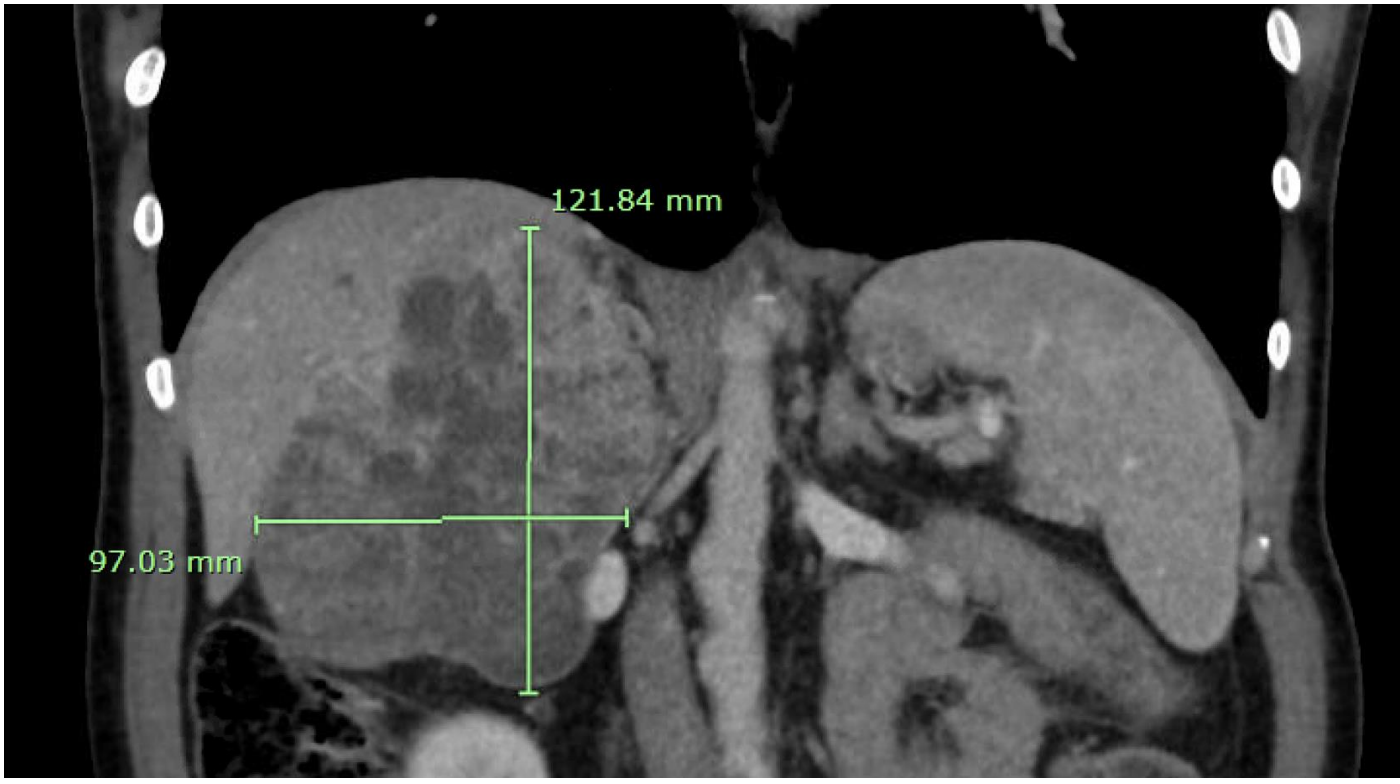
Neuroblastic tumours arise from sympathetic ganglion cells, comprising of neuroblastomas (immature, malignant), ganglioneuroblastomas (intermediate) and ganglioneuromas (mature). NB may be misdiagnosed as phaeochromocytoma when they secrete catecholamines and may have similar radiological features.[2]

The initial differentials for this case included phaeochromocytoma and composite phaeochromocytoma. There are no established criteria to distinguish catecholamine-secreting NB from phaeochromocytoma and paraganglioma (PPGL). Histological diagnosis was critical given the management of NB differs from that of PPGL. A critical eye is required for the accurate diagnosis and management of malignant adrenal incidentalomas.

A)



B)



C)

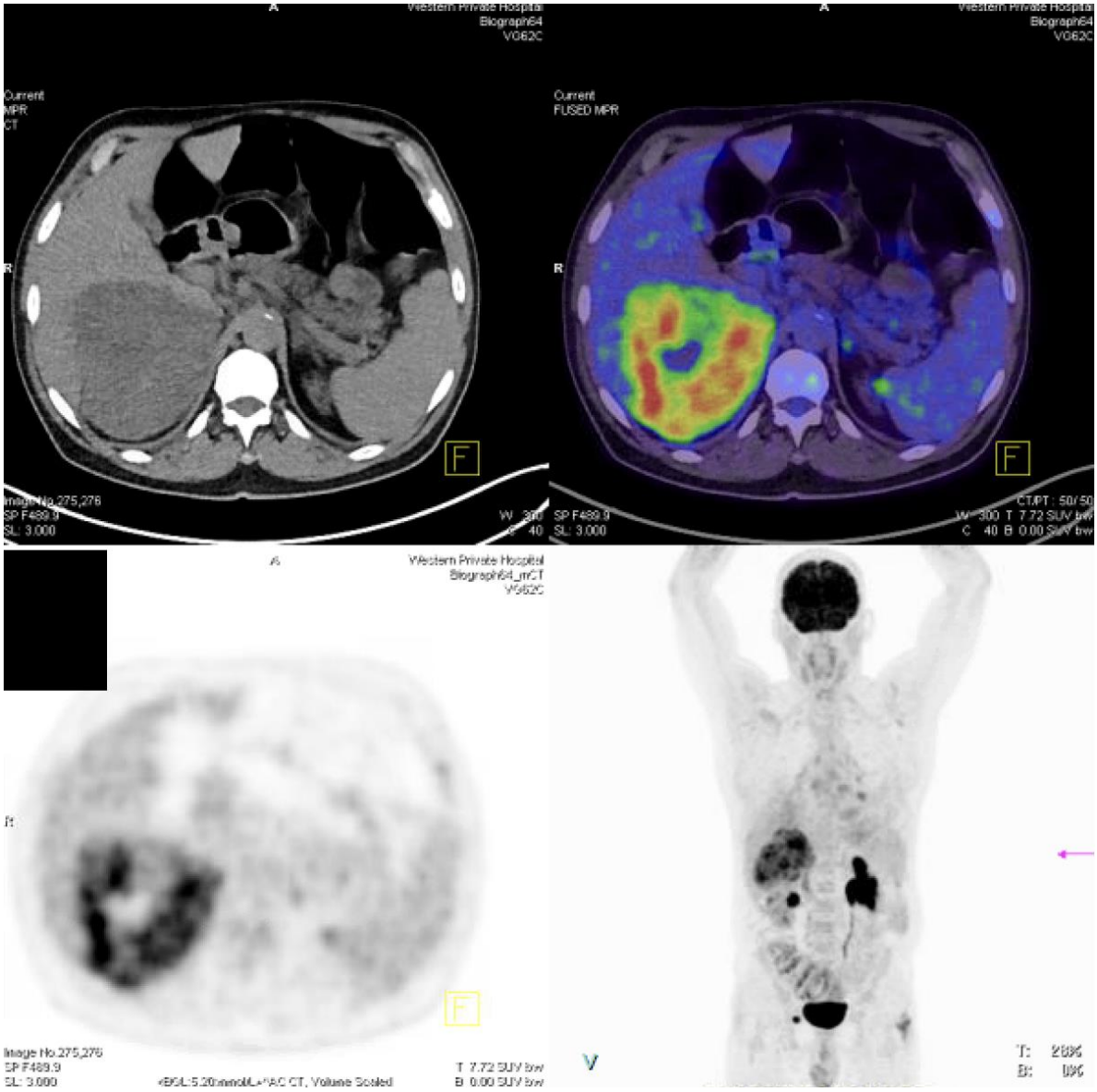


Figure 1. A) Non-contrast adrenal CT, **B)** Contrast adrenal CT, **C)** GaTate PET-CT and FDG-PET.

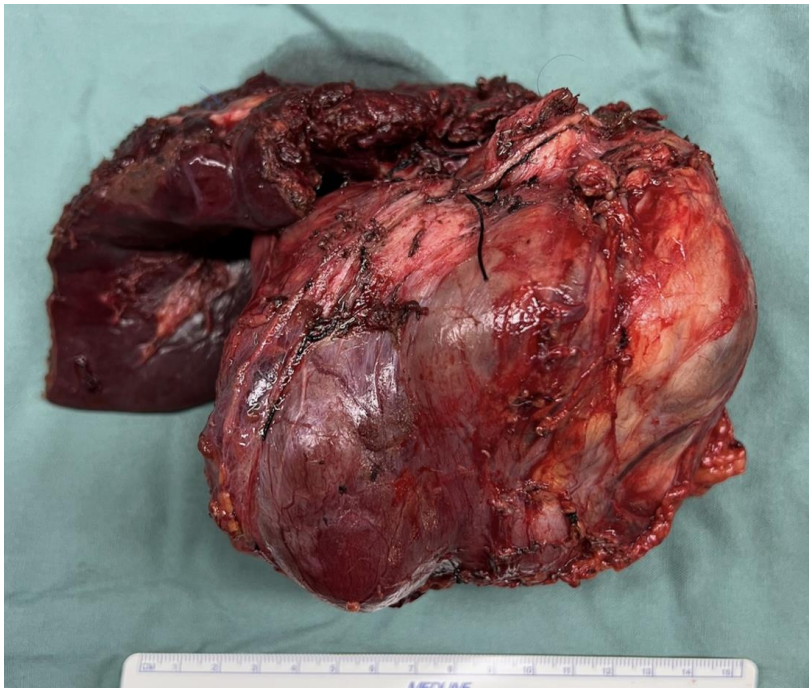


Figure 2. Resected right adrenal mass.

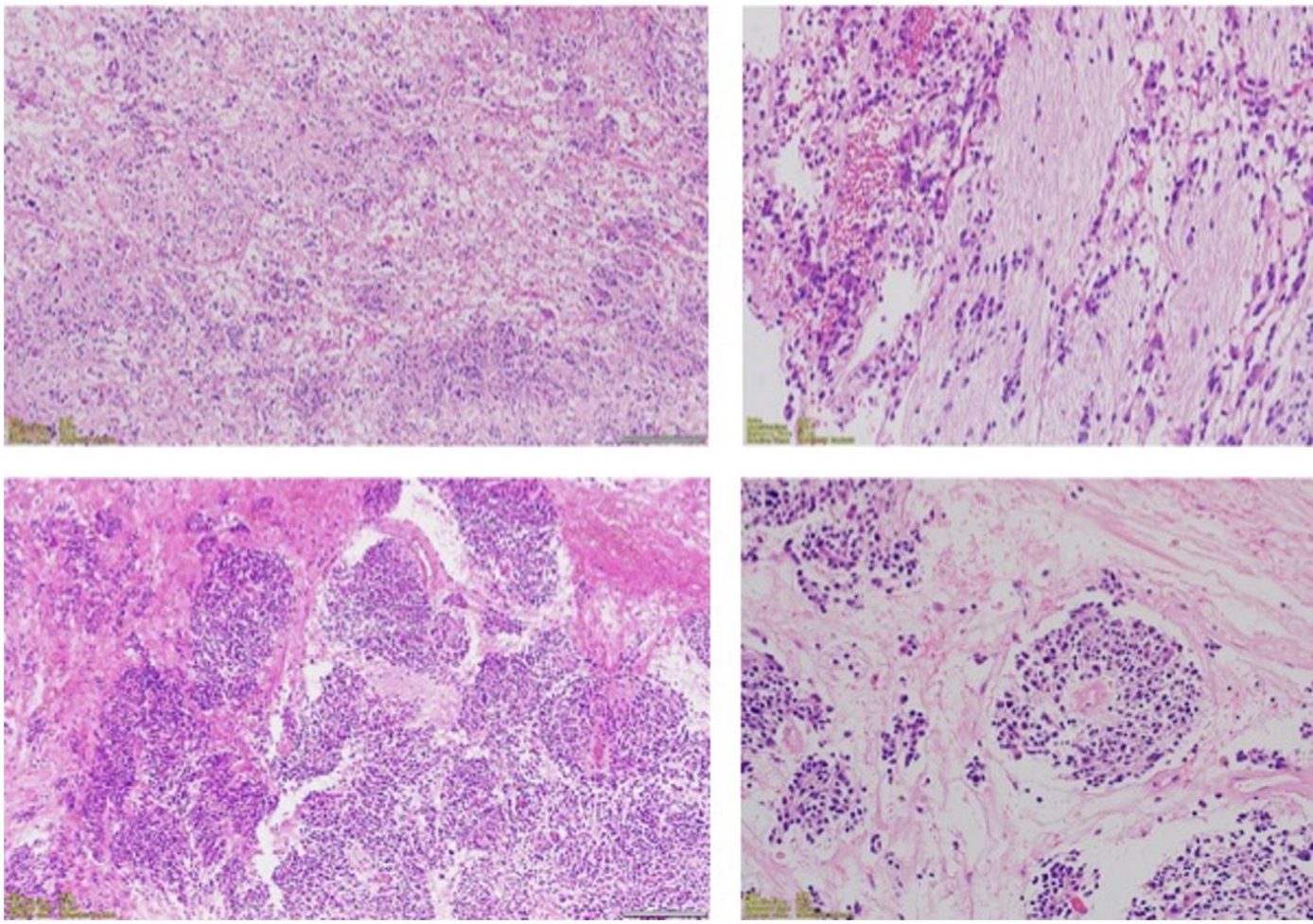


Figure 3. Histology of the resected neuroblastoma.

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A duelling case of male hypogonadism

Mauli Govinna[1], Carolyn Allan[1, 2], Stella Sarlos[1, 2], Rinky Giri[1]

1. Department of Endocrinology and Diabetes, Monash Health , Clayton, VIC, Australia

2. Hudson Institute of Medical Research, Clayton, VIC

Mr KC, a 14-year-old Year 9 student, presented to the Emergency department with a six-day history of new onset headaches and transient visual blurring. He had a past medical history of renal stones (with normal genitourinary tract anatomy on imaging) and anxiety. .

An MRI scan showed a 2.1cm pituitary lesion with local mass effect and minimal compression of the optic chiasm. The pre-operative pituitary panel showed elevated gonadotrophin levels, FSH 29.8 IU/L (1.2 – 5.2 IU/L) and LH 8.8 IU/L (2.0 – 8.0 IU/L), with a testosterone level of 10.9 nmol/L (0.7 – 17.6 nmol/L). Remainder of anterior pituitary function was normal.

On examination, he was tanner stage P4 G4, testicular volumes 5-6ml (right) and 6-8ml (left) with expected volumes of 15-20ml at this Tanner stage[1]. There was no history of cryptorchidism. Height was 175cm (75th centile) and weight 60.1kg (63rd centile). He started undergoing puberty at the age of 12.

He underwent transsphenoidal resection (TSS) of the pituitary lesion. Intraoperatively, appearances were suggestive of a craniopharyngioma adherent to the pituitary stalk, necessitating transection of stalk to achieve complete resection. Post-operatively, the patient developed AVP-deficiency, hypocortisolism and secondary hypothyroidism requiring replacement with desmopressin, hydrocortisone and levothyroxine. The histopathology of the lesion was suggestive of a Rathke's cleft cyst.

Karyotype testing confirmed a 47 XXY genotype, consistent with Klinefelter syndrome. Repeat assessment of gonadotrophin levels revealed low FSH and LH levels with an undetectable testosterone level, consistent with post-TSS hypogonadotrophic hypogonadism superimposed on a background of primary hypogonadism. He was commenced on testosterone replacement therapy. Our patient's case highlights the importance of careful history taking and physical examination, particularly a thorough assessment of pubertal development and testicular examination. The presence of small testes in conjunction with evidence of hypergonadotrophic hypogonadism on hormonal assessment, should trigger further assessment with karyotype[2].

Figure 1 – MRI Brain demonstrating a large 2.1cm complex cystic pituitary lesions with local mass effect

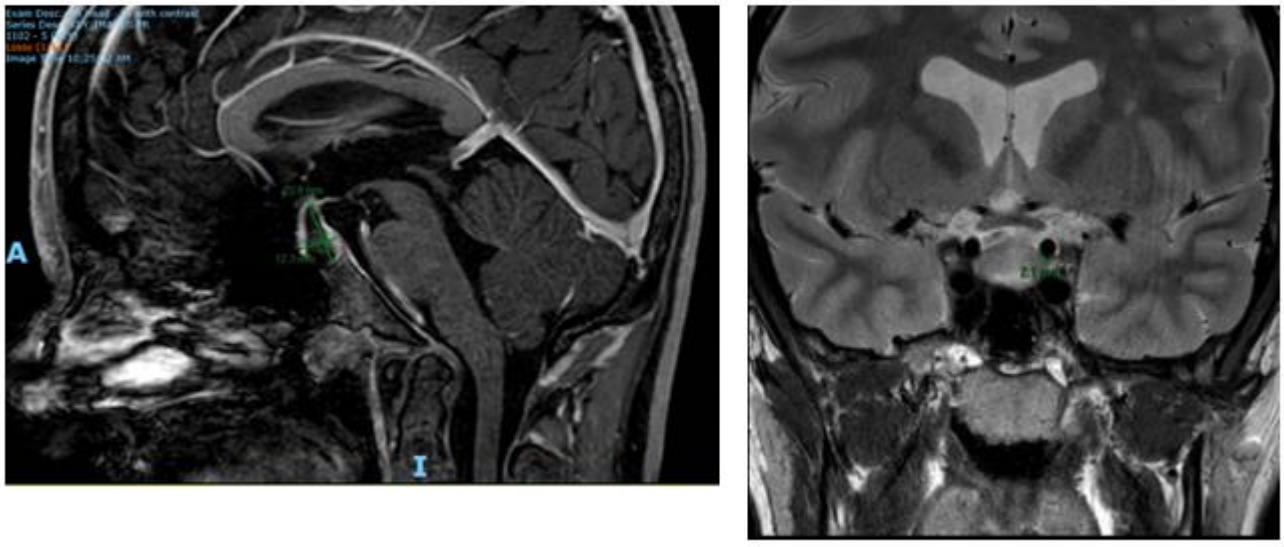
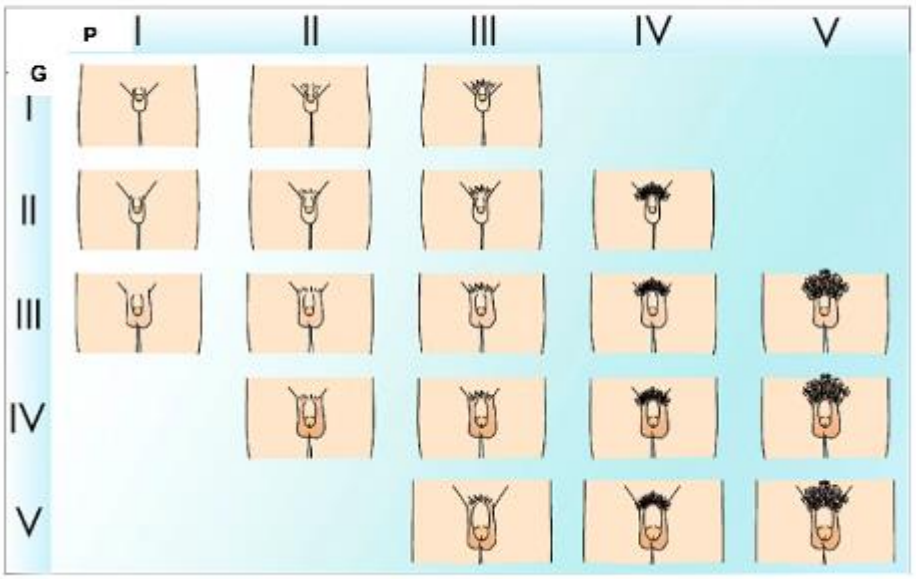


Table 1 – Pituitary panel results

	Pre-operative (Sept 2021)	3 months post-operation (Dec 2021)	6 months post-operation (March 2022)	Reference range
FSH (IU/L)	29.8	-	0.7	1.2 – 5.2
LH (IU/L)	8.8	0.3	0.3	2.0 – 8.0
Testosterone (nmol/L)	10.9	<0.1	<0.1	0.7 – 17.6
TSH (mU/L)	1.06	<0.01	<0.01	0.40 – 4.80
T4 (pmol/L)	11.2	14.6	14.4	8.8 – 17.7
T3 (pmol/L)	5.4	-	-	4.0 – 7.0
IGF-1 (nmol/L)	52.7	-	-	31.3 – 130.5
Cortisol (nmol/L)	419	-	-	185-625
ACTH (pmol/L)	7	-	-	<10
Prolactin (mU/L)	198	-	-	

Figure 2 – Tanner staging in males: P (pubic hair) and G (Genital development)



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Hyponatraemia with altered conscious state precipitated by colonoscopy preparation

Alpha Bi[1], Mauli Govinna[1], Daniel Bennett[1], Howard O'Brien[1], Emmy Sawires[1], Shireen Tabatabai[2], Georgia Soldatos[3, 1], Carolyn Allan[3, 4, 1]

1. Department of Endocrinology, Monash Health, Clayton, Victoria, Australia

2. Department of Gastroenterology, Monash Health, Clayton, Victoria, Australia

3. School of Clinical Sciences at Monash Health, and Faculty of Medicine, Nursing and Health Sciences , Monash University, Clayton, Victoria, Australia

4. Hudson Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, Victoria, Australia

A 67-year-old woman presented to a regional Emergency Department with rapidly increasing confusion, unsteady gait, and vomiting (x2) after commencing bowel preparation (Prepkit C® polyethylene glycol (PEG) x 1, sodium picosulfate (NaP) x 2) for colonoscopy.

Presentation: GCS 8 (Eye 4, Verbal 2, Motor 2), BP 120/80, PR 78; assessed as mildly hypovolemic. Investigations: Na 118 mmol/L; K 3.6 mmol/L; BIC 21 mol/L; Cl 89 mmol/L; glu 7.9 mmol/L; Cr 76 umol/L; Se Osm (calc) 248 mosm/kg; urine SG 1.010 [Corrected Ca 2.35 mmol/L, cortisol 416 nmol/L, TSH 0.61mIU/L]. Perfusion CT brain: possible seizure activity. Management: 250 mls of 0.9% saline over 2 hours before transfer to a tertiary hospital ED when assessed as euvolaemic. At that time GCS 10 (E4, V1, M5) Na 119 mmol/L; Se Osm 248 mosm/kg; U Osm 373 mmosm/kg, uNa38 mmol/L. MRI: normal; EEG: moderate encephalopathy. 500 mls/day fluid restriction was initiated. Na increased to 125 mmol/L after 24 hours with UO 4890 mls and CGS 14 (E5, V4, M5). After 48 and 72 hours, Na levels 133 and 135 mmol/L with return to baseline clinical function.

Na was 139 mmol/L 8 weeks prior. Medications were pantoprazole and propranolol. As per colonoscopy protocol instructions, water intake was estimated to be 4.5L over the preceding 8 hours. Only 1 sachet of NaP was consumed.

A diagnosis of acute water intoxication with hyponatraemic encephalopathy was made.

Our patient was prescribed standard combination hyperosmotic (NaP) and isosmotic (PEG) bowel preparation. Severe hyponatraemia is a rare adverse effect, reported more commonly with NaP (0.09%) than PEG (0.04%) (1), usually with co-morbidities and/or medications known to precipitate hyponatraemia (2).

Besides age, our patient had no risk factors for hyponatraemia and consumed the recommended volume of water. Patients preparing for colonoscopy should be counselled about this rare but potentially life-threatening complication.

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From Fractures to Myeloma: A Journey Through ACTH-Dependent Cushing's Syndrome

Saba Gul[1], Elizabeth Wootton [1], Amy Harding [1], Jessica Triay[1]

1. Bendigo Hospital , Melbourne , Victoria , Australia

A 44-year-old man, with a history of smoking, hypertension and chronic obstructive airway disease was noted to have proximal myopathy, central adiposity, skin atrophy and bruising. He had recently commenced denosumab for multiple atraumatic rib fractures. Screening investigations identified a persistent leucocytosis (neutrophils $14.0x10^9$/L, monocytes $2.0x10^9$/L), mild PTH-independent hypercalcaemia (corrected calcium 2.82 mmol/L, PTH 1.4pmol/L), vitamin D deficiency (23 nmol/L) and elevated serum cortisol (2803 nmol/L) with hypokalaemia (2.5 mmol/L). An initial myeloma screen was unremarkable. ACTH-dependent Cushing's syndrome was confirmed with a 1mg dexamethasone suppression test demonstrating elevated cortisol (1082 nmol/L) and ACTH (108 pg/ml). Metyrapone, colecalciferol and antihypertensive therapies were initiated. Petrosal venous sinus sampling was consistent with ectopic ACTH production; however, no focal lesion was identified, despite multimodal imaging (pituitary MRI, high-resolution CT chest, serial CT-chest-abdomen-pelvis, FDG-PET, DOTATE-PET). The patient underwent an uncomplicated bilateral adrenalectomy and commenced adrenal steroid replacement (hydrocortisone 10mg twice daily, fludrocortisone 100mcg daily). The development of osteonecrosis of the jaw necessitated denosumab cessation. Over the two years following adrenalectomy, the patient developed weight loss, worsening renal function and declining bone density. A repeat myeloma screen was positive (IgA paraprotein 20g/L, kappa/lambda free light chain ratio 2.21) and bone marrow biopsy demonstrated 70% plasma cells. Chemotherapy with bortezomib and dexamethasone has commenced.

ACTH-dependent Cushing's syndrome arises from pituitary or neuroendocrine tumours, typically of the lung, thymus, pancreas or appendix (1). The interplay between Cushing's syndrome and multiple myeloma is unknown. As dexamethasone is a cornerstone of myeloma management, untreated Cushing's syndrome may mask overt disease (2). It is possible that ACTH may also drive myeloma development, warranting further research. This case highlights difficulties in the diagnosis and management of ectopic ACTH syndrome and the importance of reevaluation in situations of unexplained clinical decline.

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Difficulty in Controlling Bone Turnover and Managing Osteoporosis After Roux-end-Y Gastric Bypass

Michael Hancock[1], Bronwyn Stuckey[1, 2, 3]

1. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth , WA, Australia

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

3. School of Medicine , University of Western Australia, Perth , WA, Australia

Case Report

Mrs SN, a 58-year-old female, underwent Roux-en-Y gastric bypass in 2018, with substantial weight loss, 120 Kg (BMI 46) to 66.8 Kg (BMI 25). Secondary hyperparathyroidism with elevated bone turnover markers (BTMs) and vitamin D deficiency was observed (table 1), and osteopaenia 3 years post-op (figures 1-3).

Oral bisphosphonate (risedronate) caused symptomatic hypocalcaemia and was ceased. MHT (Estradot) and vitamin D supplementation were commenced and titrated, but ineffective as BTMs remain markedly elevated with progressive reduction in bone mineral density (BMD), now within osteoporosis range, 5 years post-op.

Discussion

Post-bariatric surgery, osteoporosis and secondary hyperparathyroidism are common complications, seen in 27% and 54% at 10 years respectively[1-3]. Weight loss greater than 30% excess weight loss (EWL) increases this risk, particularly approaching or exceeding BMI 25, 100% EWL, as with Mrs SN2. Bone microarchitecture is altered, particularly sites with greater periosteal bone[4].

Improving secondary hyperparathyroidism with vitamin D and calcium supplementation may not alter progressive renal calcium conservation, BTMs and altered bone microarchitecture[5]. Normalising and supressing BTMs may be a more suitable therapeutic target, however bisphosphonate induced hypocalcaemia, and medication malabsorption can complicate this. MHT may be beneficial in post-menopausal women, but minimal published data exists. Transdermal routes or oestrogen implants may be required as trialled in Mrs SN, with sub-therapeutic oestrogen levels impacting efficacy.

Interpreting BMD is challenging, with reduced fat mass potentially lowering BMD. Degenerative osteoarthritis is common in bariatric patients, potentially increased BMD, also impacting interpretation. Mrs SN had significant osteoarthritis affected her lumbar spine, evidenced by discrepancies between spine and hip measurements.

Conclusions

1. Roux-en-Y gastric bypass achieves significant weight loss, but risks osteoporosis and malnutrition.
2. Suppression of BTMs is an important therapeutic target, however determining the optimal approach requires consideration of gender, menopausal status, and nutritional deficiencies.
3. Physiological changes post-operatively, influence BMD interpretation.

	October 2020	September 2021	March 2022	March 2023
Calcium – corrected (2.10 – 2.60 mmol/L)	2.17	2.10	2.20	2.14
Ionised Calcium – corrected (1.12 – 1.32 mmol/L)	1.19	1.22	1.22	1.18
PTH (1.6 – 9.0 pmol/L)	18	19	16	17
Vitamin D (> 50 nmol/L)	34	34	86	87
Calcium Creatinine Ratio Urine (0.10 – 0.58 mmol/mmol creat)	-	0.08	0.05	0.11
Calcium excretion Urine (5 – 27 umol/L GF)	5	4	2	5
NTX Creatinine Ratio (< 50 nmol BCE/mmol creat)	195	341	166	283
P1NP (15 – 90 ug/L)	136			162
ALP (30 – 110 U/L)	134	110	129	117
Ferritin (30 – 400 ug/L)	86	69	55	66
Vitamin E (18 – 46 umol/L)		14	18	10
Copper (11 – 23 umol/L)		9		11
Zinc (9 – 16 umol/L)		8	9	9
Medical Management	Calcium 1200mg Vitamin D 1000 IU Estradot 25mcg (Feb) Estradot 37.5 mcg (Nov)	Calcium 1200mg Vitamin D 1000 IU Estradot 50 mcg (Nov)	Calcium 1200mg Vitamin D 1000 IU Estradot 75mcg (Mar)	Calcium 1200mg Calcitriol 1.5mcg/day Estradot 50mcg Estrogel 4x actuations Creon 25,000 TDS. Cholestyramine TDS.

Table 1: Persistent secondary hyperparathyroidism with elevated bone turnover markers and renal calcium conservation, despite escalating medical therapies.

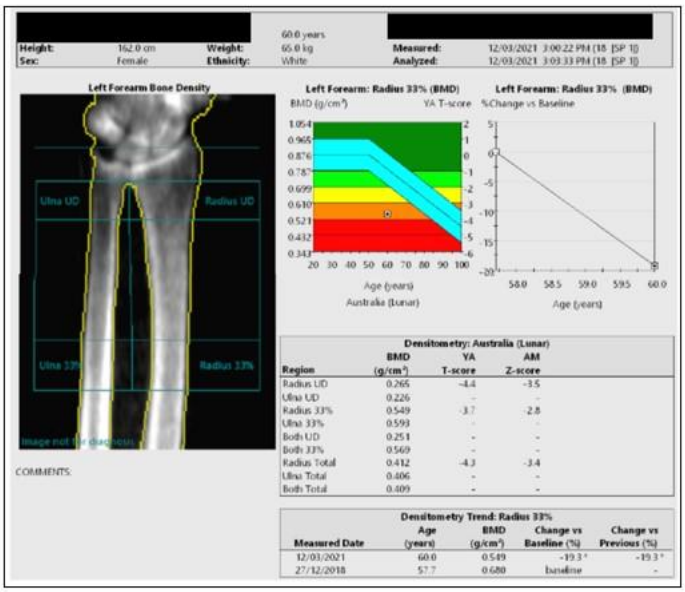

Figure 1: Bone densitometry report for left forearm. Significant reduction in bone density observed between 2018 and 2021.

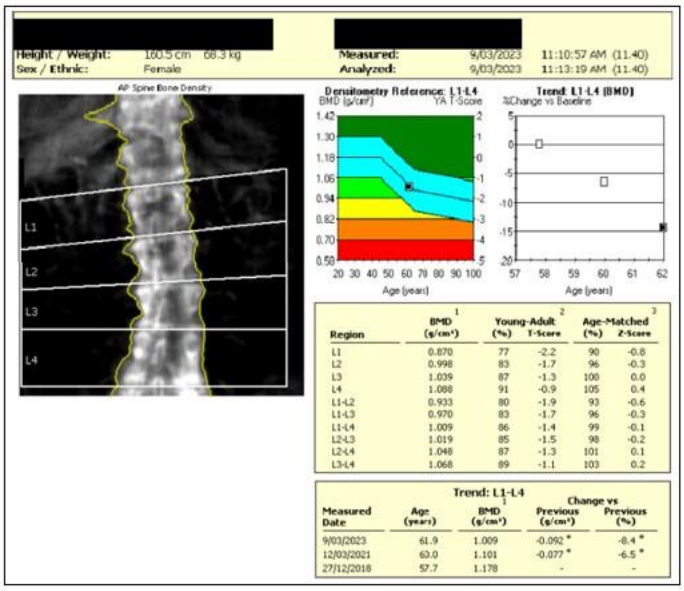

Figure 2: Bone densitometry report for lumbar spine. Significant reduction in bone density observed between 2018, 2021 and 2023.

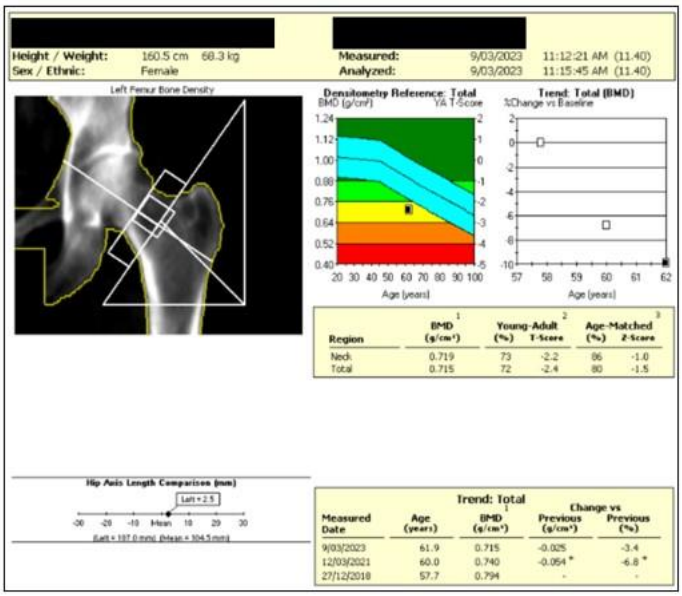

Figure 3: Bone densitometry report for left total hip and femoral neck. Significant reduction in bone density at total hip observed between 2018 and 2021, and a reduction without reaching significance between 2021 and 2023.

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SDHB and the pituitary: a tale beginning with one sibling

Annabelle Hayes[1], Venessa Tsang [2, 3], Roderick Clifton-Bligh[2, 3]

1. Department of Endocrinology, St Vincent's Hospital Sydney, Darlinghurst, NSW, Australia

2. Department of Endocrinology, Royal North Shore Hospital , Sydney, NSW, Australia

3. University of Sydney, Sydney, NSW, Australia

Background: SDHx mutations are estimated to represent 20% of paraganglioma/phaeochromocytoma (PPGL) [1]. Their role in the 3PA syndrome comprising pituitary adenoma, paraganglioma and phaeochromocytoma has been more recently described and remains rare [2].

To date 38 pituitary tumours have been described in patients with confirmed germline SDHA, SDHB, SDHC, SDHD and SDHAF2 mutations [3]. Given the high frequency of pituitary tumours (occurring in up to 1 in 1000), their occurrence in the presence of SDHx mutations does not demonstrate causality. However, links have been established with immunohistochemistry staining, tumour genetic studies and ^{1}H-MRS. SDHx associated pituitary tumours demonstrate aggressive behaviour and prolactinoma subtypes are often resistant to medical therapy [4].

Case: A 29-year-old man was found to carry an SDHB gene mutation via cascade genetic testing in 2015. His father had died secondary to metastatic PPGL. His sister, also an SDHB carrier, had undergone surgical resection of a non-functioning head and neck PPGL.

At diagnosis, urinary metanephrine, normetanephrine and dopamine levels were within normal range. Imaging demonstrated a suspicious mediastinal lesion which required two thoracotomies to resect. The tumour was histologically consistent with a paraganglioma with loss of SDBH immunohistochemical staining.

Routine MRI follow up for PPGL continued spanning from the base of skull to pelvis but did not image the pituitary. New headaches, weight gain and symptomatic hypogonadism led to the diagnosis of 18mm pituitary tumour in 2020 with hyperprolactinaemia. Serum prolactin responded well to dopamine agonist therapy but apoplexy and further tumour growth has prompted surgical intervention.

His sister is also currently being investigated for a pituitary tumour.

Conclusion: SDHx associated pituitary tumours represent a minority of all pituitary tumours but may provide insight into a subset of tumourogenesis with a more aggressive clinical course. Their contribution to the 3PA syndrome is increasingly recognized

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Oxalate nephropathy after rapid pasireotide response

Annabelle G Hayes[2, 1], Mark Penny[3], Jerry R Greenfield[2, 1, 4]

1. Garvan Institute of Medical Research, Sydney, NSW, Australia

2. Department of Endocrinology, St Vincent's Hospital Sydney, Darlinghurst, NSW, Australia

3. Department of Nephrology, St Vincent's Hospital Sydney, Darlinghurst, NSW, Australia

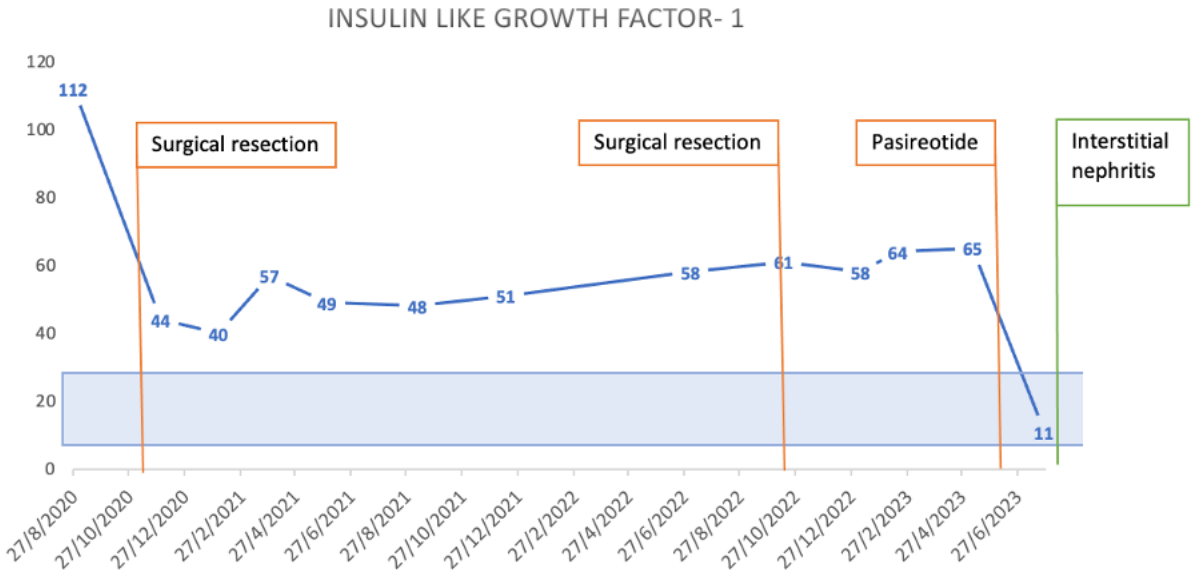
4. University of New South Wales, Sydney, New South Wales, Australia

Background: Pasireotide is a second generation, pan-somatostatin analogue with higher receptor affinity, broader action and longer half-life than octreotide and lanreotide. While it is known to impair insulin secretion, it also results in pancreatic exocrine dysfunction, hypothesised to result from high affinity binding of somatostatin receptors 1, 2, 3 and 5 [1,2]. We report a case of interstital nephritis, likely secondary to oxalate nephropathy after commencing pasireotide.

Case: A 73-year-old woman was diagnosed with acromegaly in 2020 in the setting of newly-diagnosed type 2 diabetes. She had persistent IGF-1 elevation despite two surgical resections of an invasive mammosomatotroph pituitary tumour, cabergoline and maximal dose lanreotide treatment (figure 1). The tumour expressed SSTR5, but not SSTR2, predicting good response from pasireotide, which was commenced at 40mg 4 weekly in place of lanreotide. IGF-1 rapidly normalised. She rapidly

developed significant nausea and lethargy, soon followed by an acute kidney injury (serum creatinine 425µmol/L, baseline 100µmol/L). Serum cortisol was 374nmol/L. There was no haematuria and low-level proteinuria. Renal imaging was unremarkable and glomerulonephritis screen was negative. She was treated presumptively for acute drug-induced interstitial nephritis with pulsed methylprednisolone. Subsequent renal biopsy showed acute-on-chronic interstitial nephritis with numerous oxalate crystals, raising the possibility of secondary hyperoxaluria contributing to interstitial injury. Increased faecal fat globules were noted on fat stain (3+). Shortly after drug withdrawal, urine oxalate excretion was high-normal (0.498mmol/day, RR <0.500). She continues prednisolone 37.5mg with down-trending creatinine.

Conclusion: We hypothesise that development of interstitial nephritis after three doses of pasireotide was secondary to oxalate nephropathy due to pancreatic exocrine insufficiency, or direct drug induced interstitial nephritis. A previous case of oxalate nephritis has been described with octreotide therapy, albeit with other complicating factors [3]. Hyperoxaluria should be considered in patients who develop renal dysfunction in the setting of pasireotide therapy.



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Diagnostic challenges in a case of refractory hypercalcemia

Thaw (Thaw Dar) Htet[1], Jeremy Knott[2], Myron Lee[3], Andrea Rita Horvath[4]

1. Endocrine Department, St George Hospital, Kogarah, NSW

2. Endocrine Department, St Geroge Hospital, Kogarah, NSW

3. Department of Chemical pathology, NSW Health Pathology, Sydney, NSW

4. Department of Chemical Pathology, NSW Health Pathology, Sydney, NSW

We present a complex diagnostic and management case of a 60-year-old female who presented with severe hypercalcemia, refractory to conventional therapy, on a background of chronic stage IV diabetic nephropathy (Cr247 umol/L). Our patient presented with asymptomatic PTH-independent hypercalcemia (PTH 1.2pmol/L, CrCa 3.32mmol/L) with normal 25-hydroxy vitamin D (68nmol/L), mildly elevated 1,25-dihydroxyvitamin D (206 pmol/L), reduced ACE (<12U/L) and normal PTHrP. While undergoing further investigations to ascertain the cause, her calcium level remained persistently elevated above 3.0mmol/L for over five weeks despite intravenous fluids and multiple doses of pamidronate, denosumab and calcitonin.

The patient subsequently underwent a PET scan which revealed FDG avid lymphadenopathy and splenic uptake. A splenic biopsy was diagnostic of sarcoid-like well-formed, diffuse granulomas. Despite commencement of high-dose prednisolone therapy, her hypercalcaemia persisted. Moreover, judicious use of required intravenous fluid therapy was complicated by fluid overload on her background of chronic renal disease and had to be limited. Given her hypercalcemia was not responsive to steroid therapy, with a now normalised repeat 1,25-dihydroxyvitamin D (161pmol/L), the diagnosis of sarcoid-induced hypercalcemia was questioned. Ketoconazole was trialled as second-line with nil initial improvement in serum calcium levels until two weeks. After five weeks of ketoconazole and seven weeks of glucocorticoids calcium levels have normalised (2.20mmol/L).

This case illustrates the diagnostic and therapeutic challenges associated with asymptomatic hypercalcaemia attributed to systemic sarcoidosis on a background of chronic renal impairment. It demonstrates that hypercalcemia can occur in granuloma-forming disorders such as sarcoidosis and in the setting of only mildly elevated 1,25-dihydroxyvitamin levels. Contributing factors for refractory hypercalcaemia may include intravascular depletion and decreased calcium excretion, particularly in renal insufficiency. Therapy of choice includes hydration, glucocorticoids and second-line ketoconazole. It underscores the

importance of considering systemic sarcoidosis as a potential aetiology in cases of acute PTH-independent hypercalcemia resistant to initial therapy.

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A surprising case of delayed drug hypersensitivity to Teriparatide

Annabel Jones[1], David Buxton[1], Shoshana Sztal-Mazer[1, 2]

1. Department of Endocrinology and Diabetes, Alfred, Melbourne, Victoria, Australia

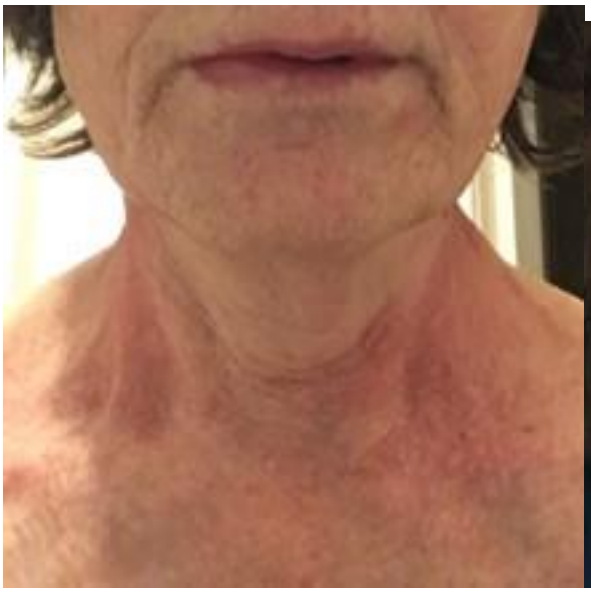
2. School of Public Health and Preventative Medicine, Monash University, Melbourne, VIC, Australia

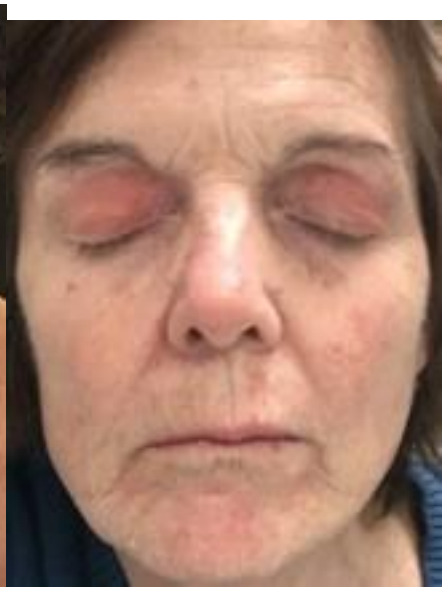
A 63-year-old lady commenced teriparatide in the setting of severe osteoporosis (T Score -3.1 at femoral neck) with three atraumatic fractures on IV bisphosphonate therapy. After the second dose, our patient reported a vague non-localised sensation of whole body itch. After the third dose, our patient reported intense pruritis and patchy whole body urticariafacial urticaria affecting the eyelids, nasolabial folds, and chin; and mild periorbital and lip angioedema without airway compromise (Figures1-4). The agent was ceased.

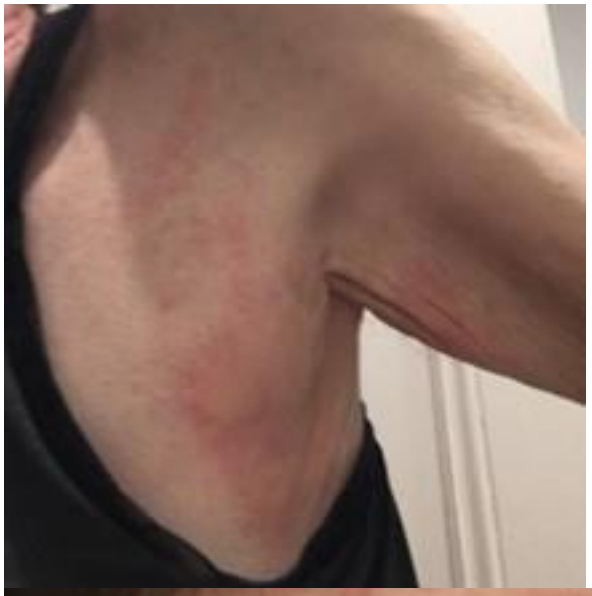
Symptom onset was several hours after administration. The rash was not typical of other causes of a pruritic rash, such as atopic dermatitis, contact dermatitis of bullous pemphigoid. Serum tryptase testing was normal (6.0mcg/L) and delay in presentation precluded biopsy. The immunology team confirmed urticaria possibly attributable to the commencement of teriparatide and suggested that re-challenge with high dose antihistamine and steroid cover could also be trialled, however the patient declined.

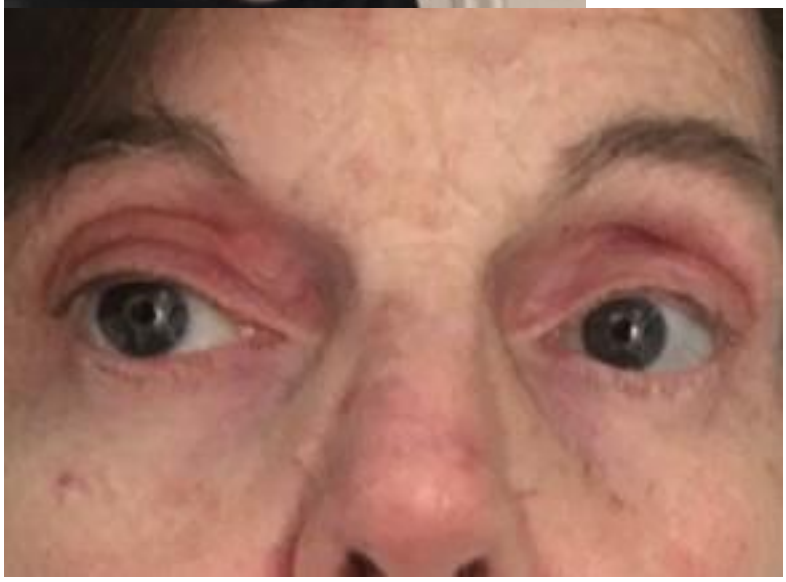
Compared with other treatments for osteoporosis, Teriparatide (recombinant parathyroid hormone) is generally well tolerated, a minority of patients report limited and or minor side effects (1). Nausea, arthralgia, headache and limb pain are the most common reported side effects (2). Additionally, hypercalcaemia (which is usually transient) and hypercalciuria are clinically significant adverse effects related to disruption of calcium homeostasis (3). As with any biologic compound, allergy (manifesting as anaphylaxis, angioedema, urticaria or hypersensitivity) is also a potential concern (4). Based on real world reporting data, the prevalence of allergic reactions to teriparatide injections is estimated to be less than 1 per 1000 patients treated (5). However, despite this, case reports of urticaria attributable to teriparatide are lacking within the literature and in correspondence with colleagues internationally.

We report an unusual case of delayed drug hypersensitivity resulting in urticaria and mild-angio-oedema related to teriparatide, an adverse reaction that has not been reported before.









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Masquerading mass: Clinically silent paraganglioma

Victoria A.L. Kanowski[1], Luke Conway[2, 3]

1. Department of Medicine, Cairns Hospital, Cairns, QLD, Australia

2. Department of Diabetes and Endocrinology, Cairns Hospital, Cairns, QLD, Australia

3. School of Medicine and Dentistry, James Cook University, Cairns, QLD, Australia

Introduction:

Paragangliomas are rare neuroendocrine tumours arising from chromaffin cells of the autonomic nervous system. When originating in the sympathetic nervous system, they have the potential to secrete catecholamines.[1] Although incidence is ~0.8 per 100,000 people, increasing susceptibility is seen with inherited syndromes such as neurofibromatosis type 1 (NF1).[2] We present a case of masquerading mass confirmed to be a paraganglioma in a patient with newly diagnosed NF1.

Case:

A 66 year old First Nations male was referred with elevated ESR (80mm/hr) and several weeks of fatigue. He was otherwise asymptomatic. Apart from elevated blood pressure (160/99mmHg), vital signs were normal (heart rate 70-80 beats/minute).

CT chest/abdomen/pelvis revealed a 39x23x26mm mass behind the inferior vena cava, partial right kidney infarction, enhancing small bowel lesion, oesophageal wall thickening, and diffuse bony abnormality.

Due to the asymptomatic nature of the presentation and the confounding presence of elevated ESR, investigations were broad. Although typical NF1 clinical features including cutaneous lesions were present, paraganglioma was not initially considered. Eventual biochemical testing for neuroendocrine tumour revealed elevated normetadrenaline (5350pmol/L), metadrenaline (540pmol/L) and chromogranin A (236µg/L).

The retroperitoneal lesion was surgically excised and paraganglioma confirmed on histopathology. The small bowel lesion was not DOTATATE avid on PET and remains under surveillance. The ESR was presumed secondary to an underlying unrelated inflammatory condition.

Discussion:

Patients with NF1 can have hypertension related to paraganglioma or renovascular disease. They are at ~5% risk of developing extra-adrenal paraganglioma.[3] If left unchecked, the secretion of excessive catecholamines can lead to life-threatening hypertension, cardiac arrhythmias, and sudden death.[4]

Conclusion:

Given presentation can be asymptomatic, there is a need for increased recognition and screening of paraganglioma in high-risk populations to avoid potentially life-threatening complications. For patients with other clinical features suggestive of neurofibromatosis, surveillance for paraganglioma should be considered.

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Severe osteoporosis in a female with type 1 diabetes and pancreatic exocrinopathy with chronic malabsorption and vitamin D deficiency: case report

Jeremy A Knott[1], Terrence Diamond[1]

1. Endocrinology, St George Hospital, Sydney, NSW, Australia

The prevalence and severity of osteoporosis in patients with type 1 diabetes (T1DM) is increased compared to non-diabetic patients, attributed mainly to insulin deficiency and its positive effects on osteoblast proliferation. The association of T1DM with other autoimmune malabsorptive conditions, such as coeliac disease, predisposing to osteoporosis is not uncommon. However, the co-existence of pancreatic exocrinopathy and T1DM leading to osteoporosis is exceptionally rare. Here we present a case of a 29-year-old female with a 10-year history of suboptimally controlled T1DM presenting with significant weight loss and osteofragility fractures who was found to have chronic malabsorption, prolonged vitamin D deficiency and probable osteoporosis/osteomalacia due to severe pancreatic exocrinopathy.

The patient initially presented with an atraumatic left tibial fracture. The patient had suboptimally controlled T1DM with an elevated HbA1c (10.1-14.7%), proliferative retinopathy, polyneuropathy, nephropathy and autonomic neuropathy. Physical

examination revealed reduced muscle strength and body composition confirmed very low skeletal muscle mass (19.2kg) but an elevated fat mass (25.5kg). She had a 4-year history of amenorrhea due to weight-related hypothalamic hypogonadism (low FSH <0.1mu/L and estradiol <70pmol/L). Spinal x-rays demonstrated osteofragility fractures. DXA confirmed a low peak bone mass/osteoporosis with significantly reduced total hip BMD of 0.60g/cm² (t-score of -3.4). Malabsorption was documented with low serum 25-vitamin D (50nmol/L), ferritin (8µmol/L), vitamin B12 (12pmol/L) and faecal elastase (42mcg/G). Coeliac serology was negative.

Therapy was initiated with oral cholecalciferol 5000IU daily, calcium citrate 500mg TDS and Creon pancreatic enzyme capsules 75,000IU TDS. Intensive diabetic management was achieved using a Medtronic-770G Smartguard insulin pump and dietary adjustments. The benefits of parenteral bisphosphonates were considered.

This case highlights the complex nature of osteofragility fractures in a young individual with T1DM and highlights the need for a comprehensive investigation of potential contributing factors. A multifactorial approach to management is vital to ensure overall long-term well-being.

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Diagnostic challenges in a case of refractory hypercalcemia: case report

Jeremy A Knott[1], Myron Lee[2], Andrea Rita Horvath[2], Thaw Htet[1]

1. Endocrinology, St George Hospital, Sydney, NSW, Australia

2. Department of Chemical Pathology, NSW Health Pathology, Sydney, NSW, Australia

We present a complex diagnostic and management case of a 60-year-old female who presented with severe hypercalcemia, refractory to conventional therapy, on a background of chronic stage IV diabetic nephropathy (Cr247 umol/L). Our patient presented with asymptomatic PTH-independent hypercalcemia (PTH 1.2pmol/L, CrCa 3.32mmol/L) with normal 25-hydroxy vitamin D (68nmol/L), mildly elevated 1,25-dihydroxyvitamin D (206 pmol/L), reduced ACE (<12U/L) and normal PTHrP. While undergoing further investigations to ascertain the cause, her calcium level remained persistently elevated above 3.0mmol/L for over five weeks despite intravenous fluids and multiple doses of pamidronate, denosumab and calcitonin.

The patient subsequently underwent a PET scan which revealed FDG avid lymphadenopathy and splenic uptake. A splenic biopsy was diagnostic of sarcoid-like well-formed, diffuse granulomas. Despite commencement of high-dose prednisolone therapy, her hypercalcaemia persisted. Moreover, judicious use of required intravenous fluid therapy was complicated by fluid overload on her background of chronic renal disease and had to be limited. Given her hypercalcemia was not responsive to steroid therapy, with a now normalised repeat 1,25-dihydroxyvitamin D (161pmol/L), the diagnosis of sarcoid-induced hypercalcemia was questioned. Ketoconazole was trialled as second-line with nil initial improvement in serum calcium levels until two weeks. Currently, after five weeks of ketoconazole and seven weeks of glucocorticoids calcium levels have normalised (2.20mmol/L).

This case illustrates the diagnostic and therapeutic challenges associated with asymptomatic hypercalcaemia attributed to systemic sarcoidosis on a background of chronic renal impairment. It demonstrates that hypercalcemia can occur in granuloma-forming disorders such as sarcoidosis and in the setting of only mildly elevated 1,25-dihydroxyvitamin levels. Contributing factors for refractory hypercalcaemia may include intravascular depletion and decreased calcium excretion, particularly in renal insufficiency. Therapy of choice includes hydration, glucocorticoids and second-line ketoconazole. It underscores the importance of considering systemic sarcoidosis as a potential aetiology in cases of acute PTH-independent hypercalcemia resistant to initial therapy.

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Severe overt hypothyroidism-induced rhabdomyolysis complicated by acute renal impairment: a case report

Jeremy A Knott[1], Thaw Htet[1]

1. Endocrinology, St George Hospital, Sydney, NSW, Australia

Thyroid disorders are prevalent in the general population affecting approximately 5-10%, with a higher incidence of hypothyroidism compared to hyperthyroidism. Clinical symptoms of hypothyroidism are often non-specific including muscular manifestations, which can vary from myalgia, fatigue, and cramping, occurring in up to 70% of cases. However, rhabdomyolysis, the rapid breakdown of skeletal muscle, is a rare manifestation, often triggered by a precipitating factor. We present a case of a 25-year-old man with a known history of Hashimoto's thyroiditis who presented with symptoms of rhabdomyolysis complicated by renal impairment secondary to severe overt hypothyroidism in the context of medication non-compliance. Our patient presented with symptoms of generalised myalgia, cramping and generalised muscle fatigue. Physical examination revealed generalised muscle tenderness with intermittent cramping. Laboratory investigations were consistent with severe overt hypothyroidism with TSH 531.4mIU/L and free-T4 0.9pmol/L. Creatine kinase (CK) levels were elevated 1052U/L with associated acute renal impairment creatinine 129umol/L. There were no other identifiable precipitating factors for rhabdomyolysis and renal impairment. Our patient was managed with prompt recommencement of thyroxine therapy and intravenous hydration. Over the course of hospitalisation, the patient's myalgias gradually improved, with improvement in CK levels and renal function. Our case highlights the potential consequences of prolonged non-compliance with thyroxine replacement therapy in patients with Hashimoto's thyroiditis, highlighting a rare but significant complication of rhabdomyolysis and associated renal impairment. Clinicians should remain vigilant in monitoring patients' adherence to prescribed medications and be aware of the possible complications arising from non-compliance. Early recognition and prompt management of such cases can lead to successful recovery and prevent long-term sequelae.

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Parathyroid cyst: an uncommon cause of primary hyperparathyroidism

Sneha Krishna[1], Adam Morton[1]

1. Mater Hospital Brisbane, South Brisbane, QLD, Australia

Introduction

Parathyroid cysts (PTC) represent <0.5% of parathyroid gland pathology and 1-5% of neck masses.[1] A case of a large functioning PTC is presented.

Case

A 41-year-old man presented with recurrent non-obstructive ureteric calculus and symptomatic hypercalcaemia.

Biochemistry revealed corrected calcium 3.42mmol/L (2.10-2.60), ionised calcium 1.89mmol/L (1.13-1.3), PTH 49pmol/L (2.0-9.5), serum creatinine 163umol/L (60-110), 25-OH vitamin D 67nmol/L (50-150) and 24-hour urine calcium 5.0mmol/24hr (1.2-7.5) consistent with primary hyperparathyroidism. Neck ultrasonography and 4D-CT showed non-enhancing 71x37x33mm cystic lesion inseparable from the inferior margin of the left thyroid lobe, reported as being consistent with a colloid thyroid nodule. Cyst aspirate yielded 50mls of fluid with PTH 167450pmol/L consistent with parathyroid cyst adenoma (PTCA). Sestamibi study post-aspiration revealed a discordant focus of activity adjacent to the inferior pole of left thyroid lobe. No pathogenic variants were detected in CDC73, CDKN1B, MEN1 or RET genes.

He was treated with intravenous fluids and pamidronate 60mg with normalisation of renal function and corrected calcium to 2.39mmol/L. Hypercalcaemia recurred three weeks later requiring further pamidronate. A 6.9g partially cystic fibroadipose tissue measuring 60x30x10mm was surgically removed with ~40% of tissue remaining in situ. Histopathology was consistent with benign parathyroid adenoma with cystic changes.

Discussion

PTCs may present as an incidental neck mass (41.7%), with compressive symptoms (20.6%), or with symptomatic hypercalcaemia (6.5%). PTCs are functional (PTCA) with hypercalcaemia in 17.5% of cases, and nephrolithiasis in 2.8%.[1] PTCAs account for 1-2% of cases of primary hyperparathyroidism.[2]

Functioning and non-functioning parathyroid carcinoma has been described within PTCs.[3] Surgical excision is recommended where PTCs are functional/hypercalcaemic, symptomatic, with uncertainty of diagnosis/suspicion of malignancy, with mediastinal disease or with recurrence following fine-needle aspiration. Intraoperative parathyroid hormone levels may be unreliable due to prolonged decay presumed due to microscopic leakage of cyst fluid with subsequent absorption by surrounding tissue.[4]

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A peculiar presentation of paralysis

Sneha Krishna[1], Liza Phillips[1], Stephanie Teasdale[1]

1. Mater Hospital Brisbane, South Brisbane, QLD, Australia

Introduction

Thyrotoxic periodic paralysis (TPP) is a rare cause of acute quadriparesis affecting 0.1-0.2% of thyrotoxic patients. It is a sporadic cause of hypokalaemic periodic paralysis, which has an incidence of 1 in 100,000.[1] We present a case of TPP in an Italian gentleman.

Case

A 43-year-old man of Italian descent presents with severe hypokalaemia of 1.7 mmol/L and acute proximal quadriparesis several hours post ingestion of a large carbohydrate-heavy meal. There were no sensory, speech, or visual deficits on examination. There were three prior episodes with resolution of symptoms within 48 hours without medical intervention. Investigations revealed a new diagnosis of T3 thyrotoxicosis secondary to Graves' disease (TSH <0.01 mU/L, free T4 18.5 pmol/L, free T3 7.7 pmol/L, TSH receptor antibodies 1.4 IU/L). He was appropriately treated with intravenous potassium, carbimazole 10mg daily, and propranolol PRN.

Discussion

TPP is a rare neuromuscular disorder affecting 1-2% of thyrotoxic East Asian men between age 20 to 40 and is much less common in Western populations.[1] It is characterised by intermittent painless proximal muscle paralysis and hypokalaemia, often triggered after carbohydrate-rich meals or strenuous exercise. Whilst many cases of hypokalaemic periodic paralysis are hereditary, TPP is sporadic and can be triggered by any cause of hyperthyroidism including levothyroxine excess.[2]

The pathogenesis is not well understood, as many have mildly elevated serum thyroid hormone levels. Thyroid hormone increases sodium-potassium ATPase pump activity, causing a rapid shift of potassium intracellularly and hyperpolarisation of skeletal muscle membrane.[3] Insulin can act synergistically with thyroid hormone as it also activates the sodium-potassium ATPase pump. Pre-existing insulin resistance combined with a carbohydrate-heavy meal can trigger a TPP attack. Rapid

intravenous potassium replacement during acute attack is warranted to prevent arrhythmia. Non-selective beta-blockade can prevent recurrence until euthyroidism is achieved.[3]

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Familial partial glucocorticoid resistance syndrome

Sneha Krishna[1], Adam Morton[1]

1. Mater Hospital Brisbane, South Brisbane, QLD, Australia

A 27-year-old male was referred with fatigue, arthralgia, and hypercortisolaemia. Morning serum cortisol levels were 933 and 952nmol/L (RR 140-640) on Siemens immunoassay and failed to suppress following 1mg dexamethasone (serum cortisol 518nmol/L; RR < 50). He denied symptoms of Cushing syndrome, alcohol excess, anxiety or depression, consumption of herbal or complimentary products, and was not aware of any family history of hirsutism, hypertension, or hypokalaemia. There were no features of Cushing syndrome on examination. He was normotensive with BP 110/70mmHg.

Further investigations revealed serum ACTH of 34ng/L (RR 10-50), 24-hour-urine free cortisol 280nmol/day (RR 10-165), urine cortisol:cortisone <1.0, and absence of assay interference when cortisol was checked by high performance liquid chromatography. Bone mineral densitometry, aldosterone, renin and dehydroepiandrosterone were normal. Early morning and late-night salivary cortisol confirmed intact diurnal rhythm of cortisol production.

It was discovered that the patient's sister had been investigated for hirsutism and aggressive behaviour at age 17. Her serum cortisol levels were 843 and 826nmol/L fasting, and 201 nmol/L following 1mg overnight dexamethasone.

A diagnosis of familial partial glucocorticoid resistance syndrome was made. Genetic testing is pending.

Discussion

Familial glucocorticoid resistance was first described in 1976 as hypercortisolism without overt Cushing syndrome stigmata [1,2] It is caused by loss-of-function mutations of the glucocorticoid receptor, often in the *NR3C1* gene, resulting in hypothalamic-pituitary axis hyperactivation via increased ACTH.[1,3] It is a heterogenous condition with 33 index cases. It can manifest with hypertension (13/33), hirsutism (13/17), adrenal hyperplasia (13/33), and hypokalaemic alkalosis.[1,4,5,6] Other causes of

hypercortisolaemia are listed in Table 1.[4,7]

Table 1. Causes of hypercortisolaemia

ACTH-dependent	Cushing disease (Pituitary Cushing) Ectopic ACTH syndrome Laboratory assay interference Oestrogen - Pregnancy, Oral contraceptive pill, Oestrogen replacement Glucocorticoid resistance – familial/sporadic Anxiety/depression Alcohol excess Acute illness Obesity Polycystic ovarian syndrome End-stage kidney disease Obstructive sleep apnoea Restrictive eating disorders Illicit drug use
ACTH-independent	Adrenal adenoma/carcinoma/hyperplasia Food-dependent Cushing syndrome Adulteration herbal/complementary therapies with hydrocortisone Apparent mineralocorticoid excess

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Biochemically negative phaeochromocytomas

Sneha Krishna[1], Adam Morton[1]

1. Mater Hospital Brisbane, South Brisbane, QLD, Australia

The prevalence of adrenal incidentaloma >1cm in diameter on CT varies from 1.4-7.3%.[1] Phaeochromocytomas account for 4-7% of adrenal incidentalomas.[2] Plasma free metanephrine measurement by LC-MS/MS is the appropriate biochemical screening test for pheochromocytoma with adrenal incidentalomas, with 99% sensitivity and 89% specificity.[3]

Case

A 46-year-old woman awaiting urogynaecological surgery was found to have a 21mm right adrenal incidentaloma on CT. She was asymptomatic, normotensive, and had no clinical features of Cushing's syndrome or hereditary syndromes associated with phaeochromocytoma. The adrenal lesion was 30HU with 20% washout and hyperintense on T_2-weighted MRI. Seated fasting plasma metanephrines and 3-methoxytyramine by LC-MS/MS were normal on two occasions (Table 1). 1mg overnight dexamethasone suppression test showed incomplete suppression with cortisol 94nmol/L (REF <50) in the setting of obesity. DOTATATE-PET showed avid uptake in the adrenal nodule. Following alpha-blockade with phenoxybenzamine, a 27mm phaeochromocytoma was removed uneventfully. Histology revealed cellular monotony, large nests, and focal marked nuclear pleomorphism, but no spindling, necrosis, or capsular/vascular invasion. Staining for succinate dehydrogenase A (SDHA), SDHB, fumarate hydratase, and S-(2-succinyl) cysteine was negative. Further genetic tests are pending.

Discussion

Biochemically negative phaeochromocytomas are uncommon, with fewer than 40 cases described. Plasma metanephrines should be performed supine, as there are posture-associated increases. False negative testing can also occur with small tumours, early recurrences, and familial phaeochromocytoma syndromes. In patients presenting with characteristic radiologic features of phaeochromocytomas and negative biochemical testing, functional imaging with PET should be considered.[4,5] Genetic mutations were found in 12 of 16 (62.5%) of patients tested - *SDHB* (5); *MEN2A* (4); *VHL* (2), and *SDHD* (1).[3,6,7] Defective catecholamine synthesis due to negligible tyrosine hydroxylase immunoreactivity has been shown in biochemically negative phaeochromocytomas with *SDHB* gene mutations. Alpha-blockade is imperative to prevent haemodynamic instability.[3,4] Follow-up with annual whole-body MRI can be appropriate in absence of guidelines.

Table 1. Seated fasting plasma metanephrines and 3-methoxytyramine

	# 1	# 2	Erect reference interval
Free normetadrenaline (pmol/L)	844	888	120-1300
Free metadrenaline (pmol/L)	312	336	30-540
3-methoxytyramine (pmol/L)	18	13	< 120

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A rare case of mandibular metastasis in a patient with low-grade oncocytic adrenocortical cancer

Shejil Kumar[1, 2], Tegan Millington[1], Roderick Clifton-Bligh[1, 2, 3]

1. Endocrinology Department, Royal North Shore Hospital, Sydney

2. Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia

3. Cancer Genetics Laboratory, Kolling Institute of Medical Research, Sydney

Background: Oncocytic adrenocortical carcinoma (ACC) is an uncommon subtype of ACC which may behave more indolently and for which there is limited data regarding efficacy of systemic treatments. Jaw metastases are exceedingly rare in ACC and only one case has been reported in oncocytic ACC.

Case Description: A 65-year-old female had an incidental left abdominal mass on a CTPA scan after presenting with chest pain. CT abdomen scan confirmed a 170x140x110mm arterially-enhancing left adrenal mass with increased avidity on ^{18}F-FDG-PET/CT scan (SUVmax 15.2). Contralateral adrenal was unremarkable. She had no clinical or biochemical evidence to suggest a functional adrenal lesion. Left adrenalectomy in August 2020 confirmed a completely excised 180mm low-grade oncocytic ACC (Ki67 index 10%). Adjuvant systemic therapy was not recommended at multidisciplinary team discussion. In November 2021, she developed left jaw discomfort and numbness and CT scan revealed a 24x13x34mm lytic left mandibular lesion. Biopsy demonstrated metastatic ACC. An ^{18}F-FDG-PET/CT scan showed low-grade uptake in the mandibular lesion (SUVmax 3.4) and a 9mm pulmonary nodule (SUVmax 3.9). She underwent radiation to the left mandible (35Gy/5#) in April

2022. Repeat ^{18}F-FDG-PET/CT scan three-months later demonstrated the left mandibular lesion was no longer FDG-avid however the pulmonary nodule enlarged (11mm) and she received further 48Gy/4# radiotherapy for suspected ACC metastasis. After endocrinology referral, we commenced mitotane 1000mg BD in October 2022 without chemotherapy. She experienced marked liver enzyme derangement within 3-weeks and after initial dose interruption, mitotane was ceased after <1-month exposure. She remains under surveillance and symptomatically well three-years after initial ACC diagnosis.

Conclusions: Mitotane is recommended in advanced, metastatic or recurrent ACC and is often limited by side effects (e.g. hepatotoxicity). Jaw metastasis should be considered in the differential diagnosis for patients with ACC with local symptoms. Radiotherapy can result in symptomatic improvement and metabolic response.

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Silent prolactinoma in a woman of childbearing age: a case report

Minh V Le[1], James King[2], Spiros Fourlanos[1, 3], Angeline Shen[1]

1. Department of Diabetes & Endocrinology, Royal Melbourne Hospital, Melbourne

2. Department of Neurosurgery, Royal Melbourne Hospital, Melbourne

3. Department of Medicine, The University of Melbourne, Melbourne

Case:

A 29-year-old female presented with a 2-year history of amenorrhoea and headache. MRI-pituitary identified a macroadenoma (24x23x19mm) with optic chiasm compression. Pituitary investigation confirmed hypogonadotropic hypogonadism (Table 1), and low prolactin (PRL). Dilution test was undertaken to exclude underlying 'hook effect'. She subsequently underwent transsphenoidal resection. Surprisingly, tumour immunohistochemistry (IHC) stained positive for PRL, leading to the diagnosis of silent prolactinoma. Patient conceived spontaneously shortly after surgery and delivered a healthy child. Over the next 2 years, there was gradual tumour regrowth on MRI. Cabergoline (CAB) was offered instead of repeated surgery. She responded well to therapy and conceived again spontaneously. Post-partum, CAB was recommenced due to tumour recurrence (9x13x13mm), contacting optic chiasm. Her latest MRI 6 months after treatment showed marked tumour size reduction.

Discussion:

Silent prolactinoma is a rare subtype of PitNETs, where the tumour displays positive IHC staining to PRL without evidence of hyperprolactinaemia. The current 2022 WHO classification of PitNETs endorses the testing of transcription factors (TFs) to differentiate various PitNET subtypes.[1] It is believed that antibodies against TFs are more specific and reproducible than anterior pituitary hormones on IHC. Prolactinomas (secretory or non-secretory) have PIT-1 cell-lineage and typically express dopamine-2 receptors (D2R) on cell surface. Treatment with dopamine agonist such as CAB is effective both at normalising PRL level and reducing tumour size in 85-90% cases.[2]

Hook effect is a phenomenon when high PRL concentration saturates the anti-PRL-antibodies on immunoassay, leaving some PRL molecules uncaptured and unmeasured, therefore under-estimates the true PRL level.[3] It is typically seen in up to 20% of macroprolactinoma.[4] By diluting the sample by 1:100 or using an immunoassay not affected by hook effect, a true PRL level can be obtained. It is important to exclude this at diagnosis as macroprolactinoma can be successfully treated medically instead of surgery.

Table 1. Summary of pituitary function results at diagnosis

Test	Level	Reference Range
FSH	3.9	3.9-8.8 IU/L
LH	1.0	2.1-10.9 IU/L
Oestradiol	71	71 pmol/L
Progesterone	1.2	1-5.0 nmol/L
PRL	61	70-570 mIU/L
Cortisol (8am)	294	240-620 nmol/L
TSH	1.35	0.30-5.00 mU/L
T4	13.4	7.5-21.0 pmol/L
T3	4.6	3.8-6.0 pmol/L
GH	0.7	0-10.0 mU/L
IGF-1	41.7	15.3-43.1 nmol/L

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A Case Report of Multiple Endocrine Neoplasia Type 1 (MEN1): Contemporary lessons from a classical syndrome

Minh V Le[1], Spiros Fourlanos1[1, 2], Christopher J Yates[1, 2], James King[3], Angeline Shen[1]

1. Department of Diabetes & Endocrinology, Royal Melbourne Hospital, Melbourne

2. Department of Medicine, The University of Melbourne, Melbourne

3. Department of Neurosurgery, Royal Melbourne Hospital, Melbourne

Case A 34-year-old female, recently migrated from Eritrea, presented with gastric outlet obstruction. A 1.7x1.3x1.8cm duodenal mass was identified on CT and endoscopic-biopsy revealed a gastroenteropancreatic-neuroendocrine-tumour (GEP-NET). Gastrin was elevated (17,650pmol/L) and lanreotide was commenced (Table 1). GATATE-PET confirmed a duodenal mass with nodal involvement. A Whipple's procedure was performed. Histopathology demonstrated multifocal grade-2 pT3N1M1 duodenal gastrinoma (15/35 positive nodes, Ki67 3.5%) alongside gastrin-negative intrapancreatic lesions. Post-operatively, gastrin normalised despite lanreotide cessation. MEN1 was suspected as she had a 12-year history of macroprolactinoma. Despite longstanding cabergoline 0.5mg/week, her first MRI-pituitary in Australia showed a 20x29x25mm Pituitary-NET with optic chiasm distortion, with prolactin 10,260mIU/L. Uptitration of cabergoline was complicated by CSF leak and she underwent transsphenoidal resection and repair. Hyperprolactinaemia persisted post-operatively (latest 1,053mIU/L) and cabergoline was up-titrated to 2.5mg/week. PTH-dependent hypercalcaemia was identified (corrected-Ca 2.87mmol/L, PTH 33.3pmol/L) and although a left superior parathyroid adenoma was suspected on imaging she underwent subtotal-parathyroidectomy after a

pathological mutation of *MEN1* (c.186dupC) was confirmed, with postoperative hypoparathyroidism. She was referred to genetic counselling and wishes to conceive soon using IVF.

Discussion This case highlights the classic MEN1 triad: hyperparathyroidism, Pituitary-NET and GEP-NET. Multifocal gastrinoma is the most common GEP-NET in MEN1 and GATATE-PET provides highly sensitive functional imaging for GEP-NETs.[1] While generally not curative, surgical debulking is recommended for symptom control.[2,3] Localisation studies may be misleading and subtotal parathyroidectomy plus thymectomy is preferred for hyperparathyroidism due to multi-gland involvement and risk of thymic carcinoid.[4] Pituitary-NETs occur in 20-40% with MEN1.[5,6] The majority are macroadenomas (mostly prolactinomas) and treatment resistance is more common than sporadic disease.[6] Dopamine agonists can lead to CSF leak due to macroprolactinoma shrinkage and persistent hyperprolactinemia is common after transsphenoidal resection. Genetic counselling should be offered to affected individuals and first-degree relatives to aid reproductive decisions, tumour surveillance and treatment.

Table 1. Biochemical changes post GEP-NET resection

	Gastrin – pmol/L (reference range 6-55)	Chromogranin A – ug/L (reference range 27-94)
At time of GEP-NET diagnosis	17,650	5,250
2 months after Lanreotide (just prior to Whipple Procedure)	1,030	183
6 months after surgery	13	N/A
12 months after surgery	35	616
18 months after surgery	16	206
24 months after surgery	28	444

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MEN2 be or not to be?

Rose Lin[1, 2], Suresh Varadarajan[1], Lachlan Angus[1, 2]

1. Department of Endocrinology, Northern Health, Epping, VIC, Australia

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

We present the case of a 26-year-old male with a 39mm right adrenal incidentaloma, with elevated plasma metanephrines and normetanephrines (Table 1) suggestive of phaeochromocytoma. He also had multiple TIRADS 3-4 thyroid nodules, with biopsy features highly suspicious for medullary thyroid carcinoma (MTC). Calcitonin and CEA levels were also elevated (Table 1). Concerningly, an adrenal MRI showed multiple sub-centimetre intrahepatic lesions, highly suspicious for metastases with a differential diagnosis of haemangiomata. The main concern was for metastatic phaeochromocytoma which could affect haemodynamic stability during planned operative management. Following multidisciplinary discussion, the liver lesions were felt

to be more likely metastatic MTC than phaeochromocytoma given the elevated calcitonin and CEA, with known cervical nodal metastasis. As such, following medical optimisation with alpha- and beta-blockade, the patient underwent right adrenalectomy, followed by thyroidectomy one month later. Plasma metanephrines normalised following adrenalectomy, and histology confirmed the presence of a phaeochromocytoma. Somewhat unexpectedly, there was loss of succinate dehydrogenase B (SDHB) expression. Histology of the thyroid confirmed the diagnosis of MTC with involvement of 6/29 lymph nodes resected. Post-operative calcitonin and CEA reduced substantially but remained elevated, suggestive of persistent tumour burden. The findings of a phaeochromocytoma and MTC are suspicious for multiple endocrine neoplasia type 2. The loss of SDHB expression in the phaeochromocytoma also raises the possibility of SDHB mutation. There is no known family history of thyroid, parathyroid, or adrenal malignancies or paragangliomas. SDHB mutations can predispose to non-medullary thyroid cancers (1), but not typically MTC. To our knowledge, there is one case report of a 60-year-old woman with MTC who had a SDHB gene deletion, with no underlying *RET* mutation (2). There are no cases of *RET* and SDHB mutation co-expression in patients with phaeochromocytomas. We eagerly await results of genetic testing in our patient.

Investigation	**Pre-Adrenalectomy**	**Post-Adrenalectomy**	**Reference Range**
Plasma Metanephrines	4498	234	<500 pmol/L
Plasma Normetanephrines	1433	395	<900 pmol/L
Plasma 3-Methoxytyramine	<100	<100	0 – 110 pmol/L
Chromogranin A	98	-	27 – 94 µg/L
Investigation	**Pre-Thyroidectomy**	**Post-Thyroidectomy**	**Reference Range**
Calcitonin	1860	178	<2.9 pmol/L
CEA	419	68	<6 µg/L

Table 1: Summary of investigations before and after adrenalectomy and thyroidectomy

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Carotenaemia as a clinical manifestation of Cushing's syndrome

Sarah Lum[1, 2], Anojian Koneshamoorthy[1]

1. Endocrinology Melbourne, Heidelberg Heights, VIC, Australia

2. Austin Health, Heidelberg, VIC, Australia

Cushing's syndrome (CS) resulting from excess production of endogenous cortisol is rare, with an estimated incidence of 2-8 people per million annually.(1) Of endogenous CS, adrenal cortisol production independent of corticotrophin is the underlying aetiology in approximately 20-30%.(1)

Many features of CS are non-specific including weight gain, hypertension, hyperlipidaemia and insulin resistance or diabetes mellitus, whilst more specific features include facial plethora, easy bruising, and violaceous striae.(2)

A 36-year-old woman, 18-months post-partum, presented with secondary amenorrhoea 6-months post cessation of breast feeding, lethargy, sleep disturbance, easy bruising, facial plethora and abdominal striae. She additionally presented with carotenaemia, with yellowing of her hands and feet. Her diet was not suggestive of carotene excess.

Biochemical testing confirmed CS, in addition to hypercholesterolaemia with a LDL of 4.8nmol/L and an elevated serum carotene of 21.2umol/L (1.0-5.5umol/L). A CT adrenal study revealed a right-sided adrenal lesion measuring 30 x 23 x 41mm with +12.72 HU.

Histology following laparoscopic right adrenalectomy confirmed an adrenal cortical adenoma measuring 35mm. Hydrocortisone therapy was commenced and weaned to 10mg twice daily. Lipid profile is improving without lipid-lowering therapy, with a LDL of 3.2 nmol/L, and reducing carotene level of 16.5umol/L. Regular menses returned 2 months post-operatively. There has been a significant improvement in the clinical features and symptoms of CS, as well as improvement in skin yellowing.

Excessive ingestion of foods high in beta-carotene is the primary cause of carotenaemia, and less commonly due to an underlying disease such as diabetes mellitus, hypothyroidism and nephrotic syndrome.(3) A mechanism of carotenaemia common to such conditions is reported to be related to the elevation in serum lipids.(3)

At the time of writing, there is no other known case report of carotenaemia associated with Cushing's disease or syndrome. Underlying dyslipidaemia associated with CS is a plausible correlation.

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When hoofbeats are zebras: An unexpected case of medullary thyroid cancer

Daisuke Miura[1], Elizabeth Wootton[1, 2], Suresh Varadarajan[2]

1. Austin Health, Melbourne, VIC, Australia

2. Northern Health, Melbourne, VIC, Australia

Case:

A 73-year-old woman with parathyroid hormone (PTH)-dependent hypercalcaemia (serum adjusted calcium 3.21mmol/L, PTH 37.5pmol/L) with sonographic features suggestive of a parathyroid adenoma underwent a right inferior parathyroidectomy. At the time of surgery, she was fully independent. Past history included follicular lymphoma under observation, previously treated breast cancer and COPD. Histology was in keeping with a parathyroid adenoma however identified areas of atypical cells and necrosis with absent PTH staining. A calcitonin level was 377ng/L. Subsequent thyroid ultrasound and fine needle aspirate demonstrated epithelioid cells with mild nuclear enlargement and calcitonin positive cells. Two months later she underwent a total thyroidectomy with lymph node dissection identifying bilateral medullary thyroid carcinoma (MTC) with metastasis to the contralateral central neck nodes and ipsilateral parathyroid gland. Calcitonin level one month post-operatively was markedly elevated at 1388ng/L.

She was hospitalised two months later for functional decline with severe hypocalcaemia (calcium 1.90mmol/L and PTH 3pmol/L) requiring intravenous calcium gluconate plus oral calcium carbonate and calcitriol. A whole-body CT and DOTATATE PET identified new lesions within the axial skeleton, lungs, and adrenal glands. An MRI brain demonstrated multiple intracranial and calvarial lesions. A T12 vertebral body biopsy confirmed metastatic MTC. Although planned for oncology follow up to discuss treatment and genetic testing, she developed respiratory failure and was transitioned to supportive care, passing away 6 months from the initial presentation.

Discussion:

Traditionally MTC has been associated with high mortality rates(1), although this may change with the increasing availability of targeted therapy using multikinase inhibitors (2), highlighting the importance of early detection to facilitate genetic evaluation. Genetic testing for this patient's family members remains under consideration. Given the potential for rapid progression, diagnosing MTC requires a high degree of clinical suspicion and involvement of multidisciplinary teams when faced with atypical histological findings for presumed benign lesions.

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Not a typical case of primary hyperparathyroidism

Sharanya D Mohan[1, 2], Patrice Forner[1, 2], John A Eisman[1, 2, 3, 4]

1. Endocrinology, St Vincent's Hospital , Sydney, NSW, Australia

2. University of New South Walves, Sydney, NSW, Australia

3. Garvan Institute of Medical Research, Sydney, NSW, Australia

4. School of Medicine Sydney, University of Notre Dame Australia, Sydney, NSW, Australia

Primary hyperparathyroidism is the most common cause of hypercalcemia. It is most commonly due to a single adenoma in 80-85% of cases, an atypical adenoma in 1.2-1.3%, and parathyroid carcinomas in <1%. Although atypical parathyroid adenomas make up 1-2% of primary hyperparathyroidism, there is limited knowledge about the pathophysiology, clinical presentations and significance of such a diagnosis.

A 91-year-old male with a background history of stable primary hyperparathyroidism presented with refractory symptomatic hypercalcemia. Despite having a long history of stable disease with parathyroid hormone (PTH) levels consistently around 30, he developed a sudden rise in PTH levels to 78, associated with the persistent hypercalcemia without major change in renal function. Following suboptimal calcium management with medical therapies, surgical resection was performed and pathology demonstrated features of an atypical parathyroid adenoma.

Post-operatively this patient had rapid correction of PTH and calcium levels. He was able to be discharged 7 days post operation with stable calcium levels.

This case highlights distinguishing features that can be used in clinical practice, that may suggest an atypical parathyroid adenoma and offers insight into the pathophysiology of atypical adenomas and the risk of developing parathyroid carcinoma.

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Hypercalcemia following use of calcium sulphate beads in total knee replacement

Sharanya Mohan[1, 2], Patrice Forner[1, 2], Jennifer Snaith[3, 1, 2]

1. Department of Diabetes and Endocrinology, St Vincent's Hospital, Sydney, New South Wales, Australia

2. St Vincent's Healthcare Clinical Campus, University of New South Wales, Sydney, NSW, Australia

3. Garvan Institute of Medical Research, Sydney, NSW, Australia

Background: Calcium sulphate bio-absorbable antibiotic beads are increasingly used for the management and prevention of prosthetic joint infections. Their benefits include the concentrated delivery of local antibiotics, while avoiding systemic toxicity. The development of post-operative hypercalcemia following use of these beads is an under-recognised complication.

Case: An 85-year-old female, with a normal pre-operative calcium and history of osteoporosis managed with denosumab injections, presented with symptomatic hypercalcemia 1 week following revision of a right total knee replacement. Antibiotic-loaded calcium sulphate beads were inserted to the joint intra-operatively. Day 7 post-operatively, she became acutely delirious, and was found to be hypercalcemic (corrected calcium 3.3mmol/L, parathyroid hormone level <0.3). Her multiple myeloma screening, ACE level and morning cortisol level were unremarkable, with 25-hydroxy vitamin D 62, and 1,25-dihydroxy vitamin D 13. She was treated with intravenous fluids for 5 days. Her confusion and corrected calcium improved to 2.51 by day 11 post-operation, allowing her to resume rehabilitation.

Conclusion: The use of intra-operative calcium sulphate antibiotic beads can cause severe symptomatic hypercalcemia and prolong hospital admissions. Risk of hypercalcaemia can be compounded by patient factors including post-operative immobility and surgical factors including the dose of calcium-beads used and joint-vascularity. This case raises awareness of the potential for unintended systemic absorption of antibiotic-eluting calcium beads.

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Stereotactic Body Radiation Therapy for Conservative Management of Pancreatic Insulinoma in an 85-Year-Old Male with hypoglycaemia unawareness: A Case Study

Lauren Moll[1], Thomas Dover[1], Kate Haigh[1]

1. Ipswich Hospital, Ipswich, QLD, Australia

Pancreatic insulinomas are rare functional neuroendocrine tumours which cause excessive insulin release and recurrent hypoglycaemia. Diagnosis can be challenging, with the 3 day fast test a significant burden on patients and resources. Surgery is the mainstay of treatment, with medical therapy used for bridging or for non-surgical management. This is the case of an 85 year old male referred for a vague history of hypoglycaemia. His background included smoldering myeloma, non-small cell lung cancer, epilepsy, and chronic lower limb lymphoedema. After a difficult and delayed diagnosis of insulinoma, he poorly tolerated medical therapy. After MDT discussion, conservative treatment with Stereotactic Body Radiation Therapy (SBRT) was used.

The diagnosis of insulinoma was complicated by a recent misdiagnosis of epilepsy and hypoglycaemic unawareness with no strong evidence of Whipple's triad. Diagnosis was made after an unrelated admission in which a hypoglycaemic seizure occurred with biochemistry revealing low glucose (2.5 mmol/L), elevated S-Insulin (22 U/L), pro-insulin (>100 pmol/l) and C-Peptide (1.6 nmol/L) with low beta-hydroxbutyrate (0.04 mmol/L). Magnetic resonance imaging revealed 2.3 cm pancreatic head lesion, which was intensely avid on DOTATATE PET.

The patient was commenced on Diaxoxide at 200 mg twice daily however dosing proved difficult due to fluid overload and renal dysfunction and careful titration was required with the use of Continuous Glucose Monitoring (CGM). Given the patient's age, surgical risk and difficulty with diazoxide, a multidisciplinary team opted for conservative management with SBRT. The patient responded well to SBRT, experiencing reduced hypoglycemia. Gradually tapering off diazoxide over three months post-treatment, he continues under long-term observation.

In conclusion, this case underscores the promise of SBRT as a viable non-surgical alternative for managing insulinomas patients with substantial surgical risks. Additionally, it underscores the importance of considering hypoglycemia unawareness when initially attempting to establish Whipple's Triad in the investigation of non-diabetic hypoglycaemia.

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A case of carcinoma of unknown primary within the sellar

Mitchell Munnings[1], Hang DT Nguyen[1]

1. Endocrinology, Launceston General Hospital, Launceston, TAS, Australia

Background:

Pituitary metastases occur in 1-4% of patients with metastatic disease, with breast and lung cancer accounting for over half of cases. [1,2] Carcinoma of unknown primary (CUP) is a carcinoma or undifferentiated neoplasm that through standard diagnostic investigations has not identified a primary tumour. [3] Survival rates in patients with CUP is historically poor. Incidence of CUP are declining as diagnostic methods become increasingly sophisticated, with recent analysis estimating CUP prevalence of <2% of all invasive cancers. [4]

Clinical Case:

63-year-old female presented with worsening headache, nausea, and vomiting. Biochemistry revealed hyponatraemia (Na 122mmol/L), elevated serum prolactin (2163 mIU/L), central hypothyroidism (TSH 1.65 mIU/L, FT3 2.7 pmol/L, FT4 8.0pmol/L), and equivocal cortisol (247nmol/L). Physical examination was unremarkable with no visual field defects detected at the bedside, however, formal perimetry demonstrated bitemporal hemianopia. MRI pituitary confirmed a lobulated mass measuring 17x23x27mm with suprasellar extension causing compression of the optic chiasm, extension into sphenoid and cavernous sinuses and evidence of internal haemorrhage.

Patient underwent transsphenoidal resection of the pituitary lesion with no residual disease on post-operative imaging. Histological and immunohistochemical (IHC) findings were consistent with large cell carcinoma of unknown lineage though similar to that seen in renal cell carcinoma. Prolactin and other anterior pituitary hormone levels were normal post-operatively. Primary malignancy was not detected on investigations including CT CAP, FDG-PET, breast US, and endoscopies. Patient received adjuvant radiotherapy to the pituitary fossa with 50.4Gy in 28 fractions.

Discussion:

Despite IHC features similar to that of renal cell carcinoma no evidence to date has yielded evidence of a primary lesion. Favourable characteristics of CUP include single-site disease and renal-like CUP. [3]

Conclusion:

We report a case of a sellar CUP that has to date achieved both local symptomatic control and disease free recurrence.

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Very severe hypertriglyceridaemia with lipaemia retinalis, eruptive xanthoma and pseudohyponatraemia

Ben Nash[1, 2]

1. Austin Health, Melbourne, VICTORIA, Australia

2. University of Melbourne, Melbourne, Victoria, Australia

An asymptomatic 45-year-old man was referred to the emergency department by his general practitioner in the setting of severe hypertriglyceridaemia (HT) on routine pathology. Prior medical history was significant for three prior episodes of hypertriglyceridaemia-related acute pancreatitis, obstructive sleep apnoea and type 2 diabetes with minimal adherence to biphasic insulin, fenofibrate and atorvastatin (4-5 missed doses per week). The patient had no history of medications associated with HT or alcohol excess. On arrival, he was found to have non-fasting serum triglycerides 156mmol/L, HbA1c 12.5%, C-peptide 1.28µg/L, and a paired serum glucose 9.8mmol/L. Pseudohyponatraemia was present, evidenced by sodium 131mmol/L via indirect measurement and 138mmol/L on direct measurement. Lipase, liver function tests, morning cortisol and thyroid function tests were within normal limits.

Examination revealed multiple eruptive xanthomas on extensor surfaces of the extremities, buttocks and feet. Anterior segment examination was unremarkable. Posterior segment examination and colour fundus photography revealed grade three lipaemia retinalis with diffuse white retinal vessels and salmon coloured retina. Visual acuity was 6/6 (right) and 3/36 (left) on a background of amblyopia from childhood refractory error. There was no evidence of retinal ischaemia.

The patient was fasted and a variable rate insulin infusion commenced. His TG progressively declined to 70.1mmol/L on day 2 of admission and 25.5 mmol/L by day 6. The patient was re-established on atorvastatin, fenofibrate, high dose omega-3 in addition to regular biphasic insulin and oral antihyperglycaemic medications prior to discharge on day 7. Repeat serum TG on

day 10 were 8.6mmol/L. Screening of immediate family members was completed. Although there was no known family history, a clinical genetics review and lipid electrophoresis is pending.

Evidence based guidance for management of severe HT is lacking. Pseudohyponatraemia is common. Lipaemia retinalis often doesn't affect visual acuity unless associated with vascular occlusion or retinal ischaemia.

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Management of multiple vertebral fractures during lactation in a patient with osteogenesis imperfecta type I following twin delivery

Chrislyn Ng[1], Anne Trinh[3, 2, 1], Roger Zebaze[1], Cat Shore-Lorenti[1], Peter R Ebeling[2, 1], Frances Milat[3, 2, 1]

1. School of Clinical Sciences, Monash University, CLAYTON, VIC, Australia

2. Monash Health, Clayton, Victoria, Australia

3. Hudson Institute of Medical Research, Melbourne, Victoria, Australia

Osteogenesis imperfecta is an uncommon bone disorder caused by mutations in type I collagen involved in bone matrix leading to increased fracture risk. There are several sub-categories within OI, with OI type I being the most common and mildest form. Women with OI considering pregnancy need to be aware of bone loss and fracture risk, particularly with lactation.

We report a case of a 38-year-old female with OI type I who presented with vertebral fractures (T7, T8 and T11) four months following twin delivery and post-partum lactation. The patient had significant debility resulting from her fractures with pain and inability to lift her children. Following endocrine review, she weaned breast-feeding but represented within weeks with further pain - MRI demonstrated new T12 and L1 fractures.

Dual-energy X-ray absorptiometry scan (DXA) revealed reduced bone mineral density (BMD) of 0.712g/cm² at the lumbar spine (LS) (T-score of -3.0), and 0.662g/cm² at the left total hip (LTH) (T-score -2.3). She received intravenous zoledronic acid 5mg. However, 12 months after her infusion, she experienced further thoracic pain after lifting and an MRI showed a new T7 fracture. High resolution peripheral quantitative computed tomography showed severe generalized microarchitectural deficits with reduced total, cortical and trabecular volumetric BMD in both the radius and tibia. Cortices were markedly thin with low trabecular bone volume fraction, trabecular number, as well as increased separation. The patient commenced teriparatide 20mcg daily for 12 months. Repeat DXA following teriparatide demonstrated a significant BMD improvement of 28.0% at the LS (0.912g/cm², T-score -1.2), and 11.6% at her LTH (0.739g/cm², T-score -1.7).

Bone loss with lactation is an important consideration for women with OI considering pregnancy. Women with OI should be assessed by an endocrinologist prior to conception to optimise bone health and have an individualised plan to support women and mitigate risk.

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A case of IgG4-related thyroiditis diagnosed during active surveillance for papillary thyroid microcarcinoma

Ho-Cheol Kang[1], Ji Yong Park[2]

1. Chonnam National University Medical School, Gwangju, South Korea

2. Internal Medicine/Endocrinology and metabolism, Chonnam National University Bitgoeul Hospital, Gwangju, South Korea

Immunoglobulin G4-related thyroiditis (IgG4-RT) is associated with chronic autoimmune thyroid diseases such as Hashimoto's thyroiditis, Graves disease and Riedel's thyroiditis. Although thyroid papillary carcinoma developed in the background of IgG4-RT has been reported, there is no report on IgG4-RT established from preexisting papillary thyroid cancer. Herein, we report a case of IgG4-RT documented by surgery during the active surveillance (AS) for papillary thyroid microcarcinoma (PTMC).

A 60-year-old male underwent third annual thyroid ultrasonography (US) for follow-up of a 2x2x2 mm-sized PTMC in the lower portion of the left thyroid lobe. The PTMC was cytologically confirmed 3 years ago by fine-needle aspiration (FNA) and the patient opted for AS rather than lobectomy. On US, the tumor was significantly increased to 2x5x6 mm (figure 1) and abutted the trachea and posterior thyroid capsule. Lobectomy was performed, and histology confirmed IgG4-RT without any foci of PTMC. Abdominopelvic CT showed no other organ involvement. The patient has been well without levothyroxine replacement after the surgery.

As AS is increasingly accepted for a therapeutic option in PTMC, our case raises an important clinical question. Whether the additional FNA is needed before the decision for surgery when the tumor on AS has been grown. Clinicians should be aware of that IgG4-RT may be focal and can be developed from the preexisting PTMC possibly due to anti-tumor immunity.

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Factitious thyrotoxicosis as an important diagnosis to keep in mind

Erin Paul[1], Sarah Lum[1], Scott Baker[1], Mathis Grossmann[1]

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

BACKGROUND

Factitious thyrotoxicosis is not benign and can cause severe hypercalcaemia (1), thyroid storm (2,3), periodic paralysis (4) and myocardial ischaemia (5,6). Delays in diagnosis can lead to prolonged therapy with anti-thyroid medications.

METHODS

We report on a 37-year-old female social worker with longstanding hyperthyroidism, on a background of Hashimoto's thyroiditis with positive TPO antibodies diagnosed at age 29.

RESULTS

At age 34, our patient presented with thyrotoxic symptoms including palpitations, fatigue, anxiety and alopecia, associated with TSH 0.01mU/L, fT4 58.4pmol/L, fT3 22.2pmol/L, undetectable TSH receptor antibody. Her regular levothyroxine dose of 50 micrograms daily was ceased and she was commenced on propranolol for symptomatic relief of suspected postpartum thyroiditis. Due to persistent symptoms and biochemical evidence of thyrotoxicosis, carbimazole was prescribed for presumed antibody-negative Graves' disease. Thyroid scintigraphy was not undertaken due to breast-feeding, frequent non-attendance at outpatient clinics and her moving interstate. At age 36, she was changed to propylthiouracil 100mg TDS with ongoing beta blockade due to carbimazole-related nausea, rising fT4, from 30pmol/L to 58pmol/L in the context of consistent poor adherence. A pertechnetate thyroid scan at age 37 calculated thyroid uptake as 0.42% (normal 1-5%) with no appreciable tracer uptake. At this time, TSH was <0.01mU/L (reference 0.38-5.30), fT3 > 45pmol/L (reference 3.3-6.8), fT4 > 75pmol/L (reference 8-16.5), thyroglobulin concentration <0.10 ug/L (reference 1.6-50) and thyroglobulin antibody 48kunits/L (reference <4) associated with an elevated corrected calcium of 2.70 mmol/L (normal 2.10-2.60) and ongoing severe symptoms of hyperthyroidism preventing employment including depression. Whole body I-131 uptake scan revealed absent tracer in the thyroid bed without ectopic I-131 avid thyroid tissue, excluding struma ovarii and confirming the most likely diagnosis of factitious thyrotoxicosis. She denied taking levothyroxine or iodine-containing supplements and had no known iodinated contrast exposure.

CONCLUSION

Factitious thyrotoxicosis is a crucial differential in refractory hyperthyroidism.

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Hypocalcaemia and Wolff-Parkinson-White Syndrome as Rare Manifestations of Muscular Dystrophy

Luis Pena[1], David Song[1]

1. Ballarat Base Hospital, South Geelong, VIC, Australia

This case report presents a 27-year-old male born to consanguineous Burmese parents, who was admitted to hospital due to per-rectal bleeding. He was incidentally found to have hypocalcaemia, vitamin D deficiency, and elevated parathyroid hormone. An electrocardiogram displayed a Wolff-Parkinson-White (WPW) pattern, despite the patient's lack of cardiac symptoms or awareness of this condition. Further questioning revealed a family history of sudden cardiac death and mobility impairments among male relatives on the paternal side. He did not show any phenotypic characteristics of muscular dystrophy.

Chromosome microarray analysis showed an exon deletion within the Duchenne Muscular Dystrophy (DMD) gene on the X-chromosome. Becker muscular dystrophy arises from mutations in the DMD gene, leading to the absence of dystrophin in muscle tissue. (1) This deficiency disrupts calcium homeostasis, causing dysregulated calcium influx and impaired clearance within muscle cells. Consequently, intracellular calcium accumulates, affecting both skeletal and cardiac muscles, resulting in cardiomyopathy and potential damage to the cardiac conduction system. (2,3,4,5,6).

Notably, this case report highlights the infrequent presentation of isolated biochemical disturbance and ECG changes within the context of muscular dystrophy. Recognizing the atypical yet meaningful presentations of muscular dystrophy, such as hypocalcaemia and WPW syndrome, underscores the necessity of an interdisciplinary approach. This approach involves genetic testing, clinical assessment, and cardiovascular evaluation to comprehensively grasp the diverse clinical manifestations associated with muscular dystrophy.

Keywords: muscular dystrophy, hypocalcaemia, vitamin D deficiency, Wolff-Parkinson-White syndrome, Duchenne Muscular Dystrophy, calcium homeostasis, cardiomyopathy.

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Diabetes burnout identification and the role of Inhaled Insulin in the dynamic management of Type 1 Diabetes and well-being in a young adult

Gnanagurudasan Prakasam[1, 2], Carly Haaland[2], Kevin Kaiserman[3], Joanne Rinker[3]

1. SUTTER MEDICAL CENTER, SACRAMENTO, SACRAMENTO, CA, United States

2. Pediatric Endocrinology, Center of Excellence In Diabetes and Endocrinology, Sacramento, CA, USA

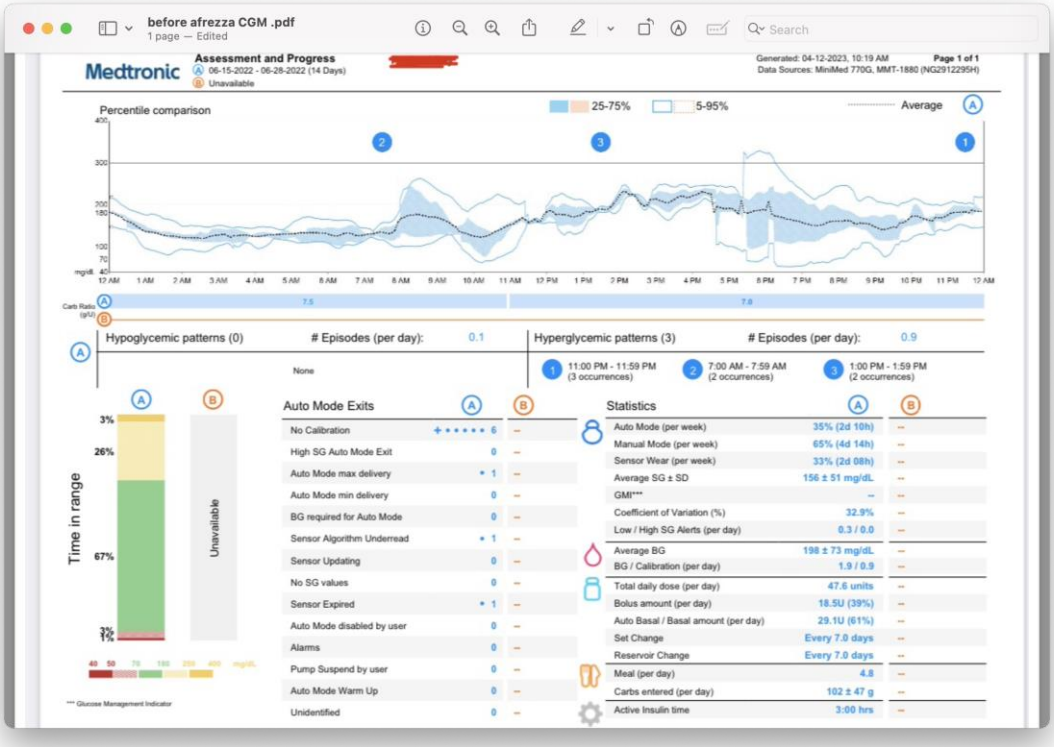
3. Medical Affairs and Safety, Endocrine Business Unit, Mannkind Corporation, Westlake Village, CA, USA

19 year-old female (diagnosed at 2years) with Type 1 diabetes, initially on injections (basal/bolus regimen), transitioned to insulin-pump at 5, recently on Medtronic 770G. Historical HbA1c was <8%. June 2022, A1c was 8.7%.

Person expressed significant diabetes burnout, especially concerned with transition to college. She disliked insulin pump for aesthetic reasons and expressed fear of hypoglycemia leading to skipping boluses. Pump data revealed 35% in auto-mode and 65% in manual-mode due to sensor failure. Time in range was 67% with 4% hypoglycemia. Wide glycemic excursions (3.3-19.4 mmol/l) noted. After discussing all treatment options, we decided on Afrezza, Technosphere® Insulin(TI) (insulin human inhalation powder)[1,2], powder form of bolus insulin taken by oral inhalation and basal insulin combination. With her fluctuating activity and eating habits, inhaled insulin would potentially give her better flexibility without the burden of the insulin pump[3] . She started on TI in October 2022 with bolus instructions 4-12 units depending on meal size (4,8 or 12 units for small, medium or large meals with no carb counting). Additional 4 units were prescribed 1 hour post-initial dose, for blood glucose(BG) greater than 8.3mmol/l. She also started on basal insulin, glargine 34 units everyday.

Three months after initiating TI her A1C improved to 8.2%. Her recent CGM report (February-2023) 'best day' shows average BG of 10mmol/l with 55% time 'in-range' and 0% hypoglycemia. In addition, we reviewed the well-being of the patient using the WHO -5 well-being index[4] and asked her to complete this before initiation of TI and 3 months after TI initiation. She reported improvements in both cheerful good spirits and felt calmer and more relaxed since initiation.

Diabetes management is a combination of glucose management and the patient's well-being. Diabetes management should be individualized and inhaled insulin[2] could be an integral part of this patient-centered approach.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Page 1 of 1
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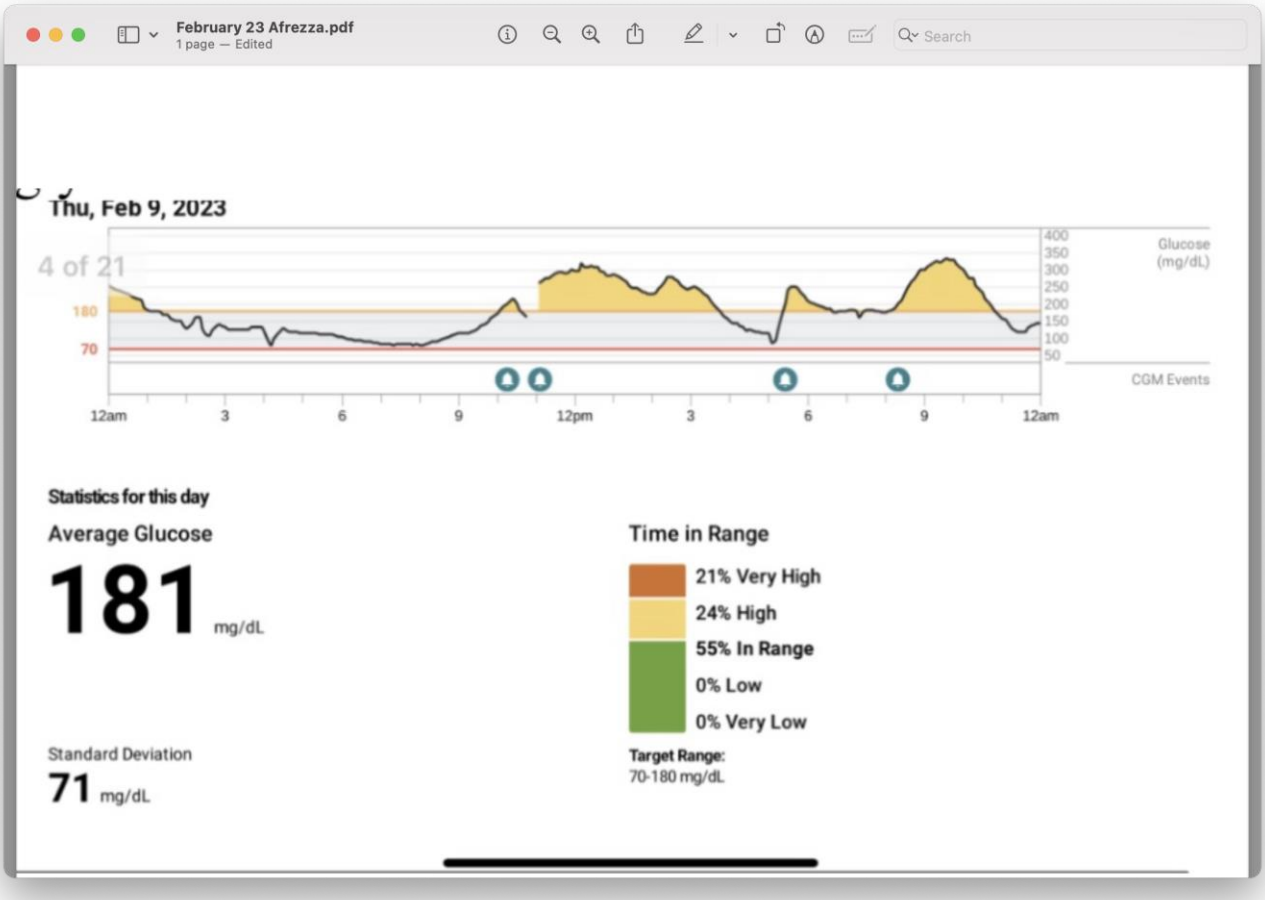
Percentile comparison
25-75%
5-95%
Average
Hypoglycemic patterns (0)
Episodes (per day): 0.1
None
Hyperglycemic patterns (3)
Episodes (per day): 0.9
1 11:00 PM - 11:59 PM (3 occurrences)
2 7:00 AM - 7:59 AM (2 occurrences)
3 1:00 PM - 1:59 PM (2 occurrences)
Time in range
3%
26%
67%
3%
1%
Unavailable
Auto Mode Exits
No Calibration 6
High SG Auto Mode Exit 0
Auto Mode max delivery 1
Auto Mode min delivery 0
BG required for Auto Mode 0
Sensor Algorithm Underread 1
Sensor Updating 0
No SG values 0
Sensor Expired 1
Auto Mode disabled by user 0
Alarms 0
Pump Suspend by user 0
Auto Mode Warm Up 0
Unidentified 0
Statistics
Auto Mode (per week) 35% (2d 10h)
Manual Mode (per week) 65% (4d 14h)
Sensor Wear (per week) 33% (2d 08h)
Average SG ± SD 156 ± 51 mg/dL
GMI*** --
Coefficient of Variation (%) 32.9%
Low / High SG Alerts (per day) 0.3 / 0.0
Average BG 198 ± 73 mg/dL
BG / Calibration (per day) 1.9 / 0.9
Total daily dose (per day) 47.6 units
Bolus amount (per day) 18.5U (39%)
Auto Basal / Basal amount (per day) 29.1U (61%)
Set Change Every 7.0 days
Reservoir Change Every 7.0 days
Meal (per day) 4.8
Carbs entered (per day) 102 ± 47 g
Active Insulin time 3:00 hrs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4 of 21
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70
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6
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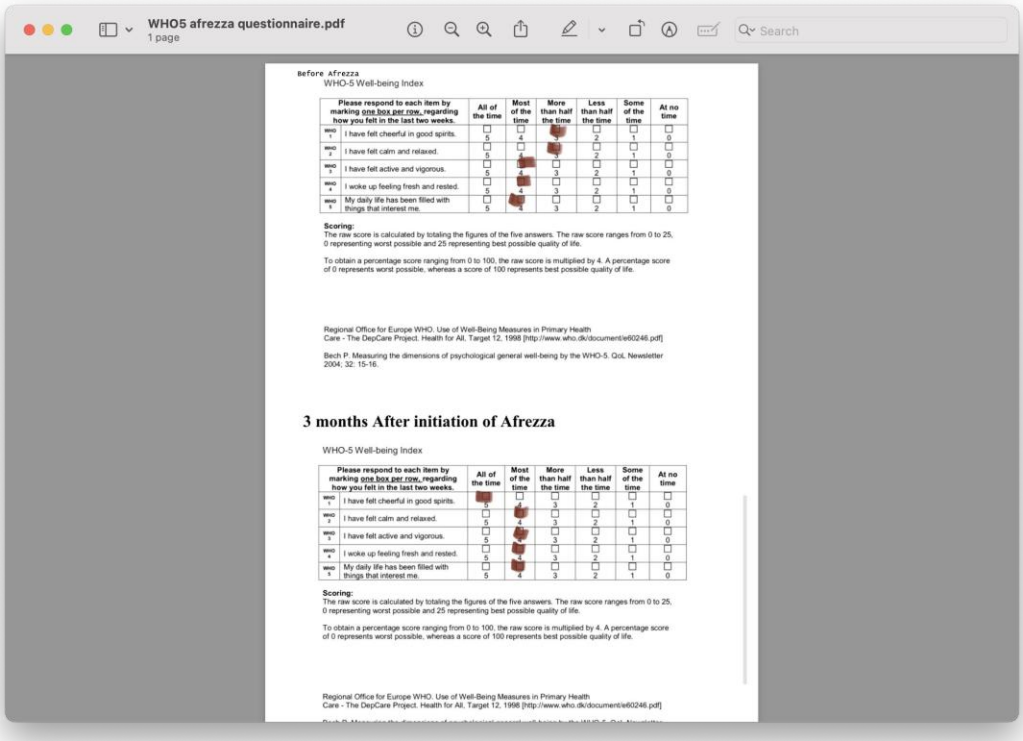
3
6
9
12am
Glucose (mg/dL)
CGM Events
Statistics for this day
Average Glucose
181 mg/dL
Standard Deviation
71 mg/dL
Time in Range
21% Very High
24% High
55% In Range
0% Low
0% Very Low
Target Range:
70-180 mg/dL



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Rare exclusively dopamine secreting paragangliomas

Subanki Rajanayagam[1], Josephine McCarthy[2, 3, 4, 5], Peter Fuller[3, 4], Debra Renouf[4, 6]

1. Endcrinology, Monash Health, Clayton

2. Department of Medicine, School of Clinical Sciences at Monash Health, Clayton

3. Centre of Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton

4. Endocrinology, Monash Health, Clayton

5. Endocrinology, Eastern Health, Box Hill

6. Endocrinology, Peninsula HEalth, Frankston

Exclusively dopamine secreting phaeochromocytomas and paragangliomas (PPGLs) are rare. (1,2)

Aim: To highlight important differences in the work-up and management of these tumours when compared to classical noradrenaline and adrenaline-secreting PPGLs.

Case 1: A 59 year old female was incidentally found to have a mediastinal mass. Her past medical history was significant for an abdominal paraganglioma resection over 30 years ago. Her son was diagnosed with a SDHB mutation. Lung malignancy was suspected, but this history prompted biochemical testing for PPGLs which showed a markedly elevated 3-methoxytyramine (3-MT) with normal levels of metanephrines and normetanephrines. Imaging revealed an intensely DOTATATE avid subcarinal mass. Initial management with metoprolol and prazosin resulted in hypotension warranting cessation of prazosin and dose reduction of metoprolol. She was reviewed by cardiothoracics; due to the location of the lesion and high surgical risk she proceeded with medical therapy.

Case 2: A 47 year old female presented with a 10 year history of a slowly growing right sided neck mass. She had no symptoms of catecholamine excess and was normotensive. Investigations revealed markedly elevated 3-MT levels with normal metanephrine and a slightly elevated normetanephrine level in the context of treatment with an anti-depressant. She was found to have a DOTATATE avid mass in the right carotid space in keeping with a carotid body paraganglioma with no evidence of metastatic disease. The patient is awaiting surgical resection of the lesion and genetic testing.

Conclusion: Paragangliomas which predominantly secrete dopamine typically present with a lack of hypertension and are often incidentally found on imaging. Unlike adrenaline and noradrenaline secreting phaeochromocytomas and paragangliomas, alpha blockade may not be indicated particularly in the absence of hypertension and needs to be considered on a case by case basis. We will discuss the investigations and treatment options for predominantly dopamine secreting paragangliomas.

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2. 2. Gangopadhyay , K., Baskar, V. and Toogood, A case of exclusive dopamine-secreting paraganglioma. Clinical Endocrinology 68: 494-495, 2008.

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Delayed gastric emptying with perioperative use of glucagon-like peptide-1 receptor agonists

Lisa M Raven[1, 2, 3], Robert B Feller[1, 2], Alina Soita[1], Campbell Brown[1], Jerry R Greenfield[1, 2, 3]

1. St Vincent's Hospital, Darlinghurst, NSW, Australia

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

3. Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) have increased in popularity in recent years due to their potent effect on glycaemia, adiposity and cardiovascular benefits in people living with type 2 diabetes (T2DM). There has also been an increase in off-label use for weight management in Australia. There have been recent updates from different American societies about the risk and optimisation of periprocedural gastroparesis with GLP-1 RA use.

We present three cases of retained gastric contents seen on gastroscopy. The first patient, a 62-year-old male (BMI 30 kg/m2), presented for routine follow up gastroscope for oesophagitis. He had commenced semaglutide 1mg the week prior to the procedure for weight management. Reported fasting time was 13 hours. Repeat gastroscopy 3 months later when semaglutide had been withheld for 3 weeks revealed an empty stomach after 12 hours of fasting. The second patient, a 61-year-old male (BMI 30 kg/m2), presented for elective gastroscopy for investigation of reflux. He had been using liraglutide daily injections for weight management. Reported fasting time was 10 hours. The final patient, a 55-year-old-female, presented for elective gastroscopy for investigation of iron deficiency. She had been taking semaglutide 0.5mg weekly for weight management. Reported fasting time was 14 hours.

These cases add to the growing literature of GLP-1 RA associated with increased retained gastric contents, with a case report of aspiration. GLP-1 RA delay gastric emptying, more prominently in short-acting formulation and with recent commencement. The American Society of Anesthesiologists recently recommended that GLP-1 RA be withheld for one dose prior to anaesthetic. However, it may be that a longer period of withholding, and/or prolonged fasting time are required, particularly if the GLP-1 RA was commenced recently. Further research, particularly in patients with T2DM incorporating glucose optimisation, is required to assess the perioperative risks of GLP-1 RA.

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Diagnostic accuracy of 4D-CT scan compared to Ultrasound and Sestamibi scan for localising parathyroid adenomas: A single centre experience

Ibrahim Shahid[1], Anojian Koneshamoorthy[1], Peter Santos[2], Jason Tan[3], Sara Vorgin[4], Vivian Grill[1, 5]

1. Department of Endocrinology and Diabetes, Western Health, Melbourne, Victoria, Australia

2. Department of Nuclear Medicine, Western Health, Melbourne, Victoria, Australia

3. Department of General and Endocrine Surgery, Western Health, Melbourne, Victoria, Australia

4. Department of Medicine – Western Campus, University of Melbourne, Melbourne, Victoria, Australia

5. Department of Medicine, The University of Melbourne, Melbourne, Victoria, Australia

Background: Four-dimensional computed tomography (4D-CT) has emerged as a new technique for pre-operative localisation in patients with primary hyperparathyroidism (PHPT). Accurate localisation of parathyroid adenomas allows for minimally invasive parathyroidectomy in lieu of traditional bilateral neck exploration with lower complication rates.

Aims:

To evaluate the diagnostic performance of 4D-CTs compared with technetium 99m-sestamibi (sestamibi) SPECT/CT and neck ultrasound (US) for preoperative localisation in patients with histologically proven parathyroid adenomas and hyperplasia.

Methods:

This is a single-centre retrospective study of patients with PHPT who underwent US, sestamibi and 4D-CT from April 2018 to October 2022, with subsequent parathyroidectomy. Reference standard for correct localisation was based on operative reports and histopathological confirmation. Each modality was then analysed individually for its diagnostic accuracy.

Results:

A total of 18 patients were identified who underwent parathyroidectomy following US, sestamibi and 4D-CT. The overall sensitivity of US, sestamibi and 4D-CT with respect to operative findings were 44% and 44% and 67% respectively. 4D-CT scan was superior in localising parathyroid adenomas in patients without prior neck surgery compared to sestamibi and US. 4D-CT imaging was particularly useful for localising smaller adenomas or ectopic adenomas which were not identified by sestamibi and US.

Conclusion:

At our centre, 4D-CT localised parathyroid adenomas with higher sensitivity in patients with PHPT without a prior history of neck surgery, compared with sestamibi or US.

Acromegaly presenting with sudden onset headache and acute Unilateral Oculomotor Nerve Palsy in a young woman

Abraham Shamshad[1], Scott McNeil[1, 2], Anna Davis[3], Jasprit Mann[4], Kathryn Hackman[1, 2]

1. Department of Endocrinology and Diabetes, Alfred Hospital, Melbourne, VIC, Australia

2. Department of Medicine, Monash University, Melbourne, VIC, Australia

3. Department of Radiology, Alfred Hospital, Melbourne, VIC, Australia

4. Department of Neurosurgery, Alfred Hospital, Melbourne, VIC, Australia

Objective

To report a case of acromegaly presenting with acute unilateral Oculomotor Nerve (CNIII) palsy.

Methods

We describe the clinical and investigation findings of a functional pituitary tumour causing CNIII palsy, highlighting the consequences of delayed presentation.

Results

A 22-year-old Korean tourist presented with acute onset headache, four days' left sided ptosis, reduced extra-ocular eye movements and deteriorating visual acuity. This was in the context of six months of oligomenorrhoea, acne, enlarging hands and progressive diastema. There was no weight gain or galactorrhoea. Notably she had a family history of a brain tumour of unknown aetiology in her brother during childhood, in South Korea, requiring resection.

Examination revealed left sided ptosis with an associated depressed and abducted eye and dilated, unreactive pupil. Visual acuity and colour vision were impaired bilaterally. She had bitemporal hemianopia, large hands and feet and mildly splayed teeth.

Pathology demonstrated elevated IGF-1 51.66 nmol/L (range 13.65-43.81) and Growth Hormone (GsH) 5.12 mcg/L (range 0.13-9.90). The remainder of the pituitary panel was unremarkable.

MRI revealed a 44×22×39mm sellar lesion, with cavernous sinus and suprasellar extension resulting in mass effect on the optic chiasm.

She underwent urgent endoscopic transsphenoidal resection. Histology showed focally infarcted pituitary neuroendocrine tumour with strong GH immunoreaction, confirming acromegaly. Prolactin, ACTH, LH, TSH and FSH staining were negative.

She developed polyuria post-operatively, passing up to 550mL/hr. Given the difficulty differentiating Arginine Vasopressin deficiency from GH associated free water clearance, she received desmopressin.

Post-operative visual acuity improved (Left 6/12 and Right 6/7.5). There was no improvement in extraocular movements and ptosis at day five, but modest improvement at three-week review.

Conclusions

This case highlights that large pituitary tumours can cause cranial nerve involvement and that prompt presentation with and management of CNIII palsy is essential to prevent long term neuropraxia.

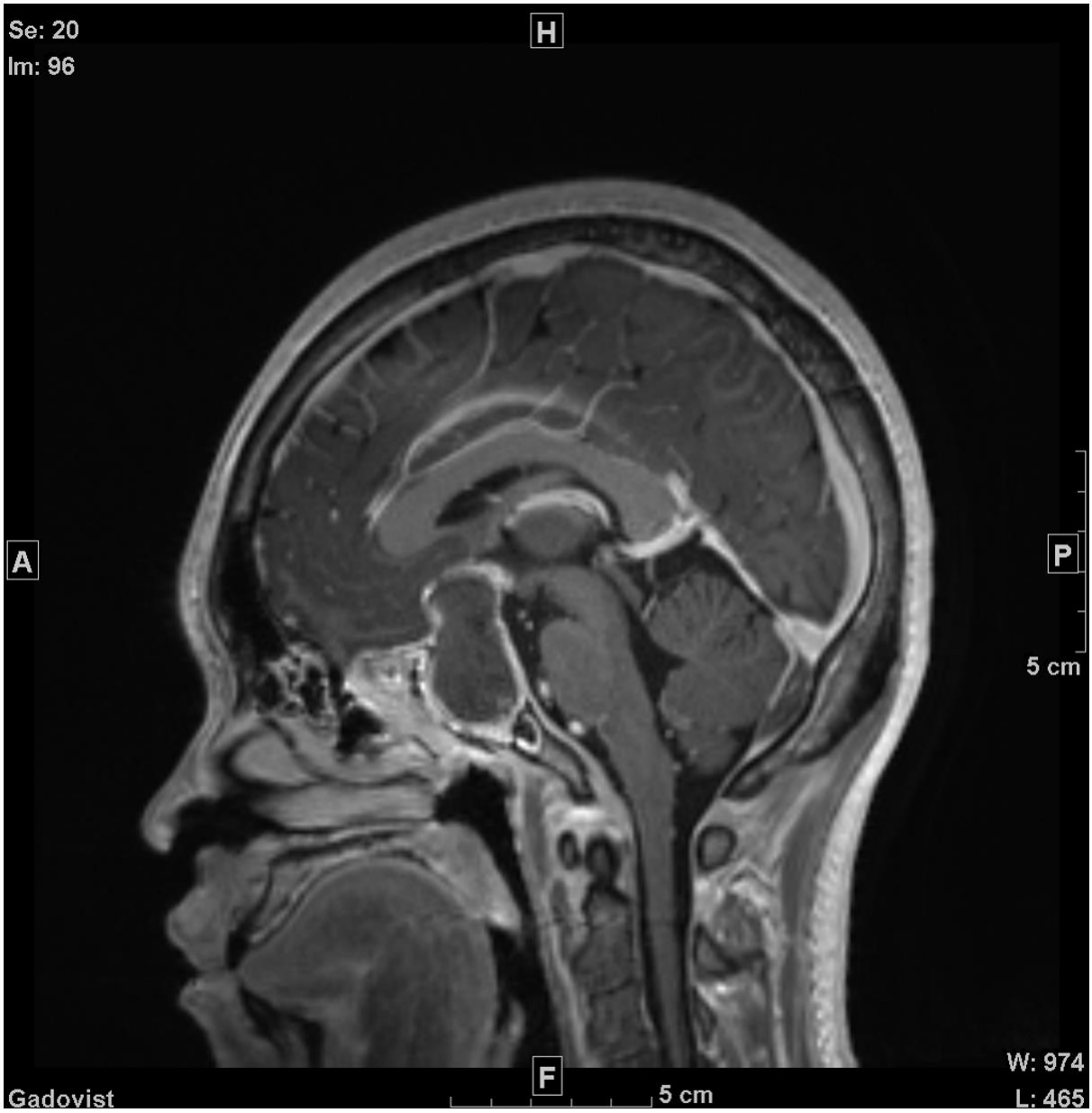
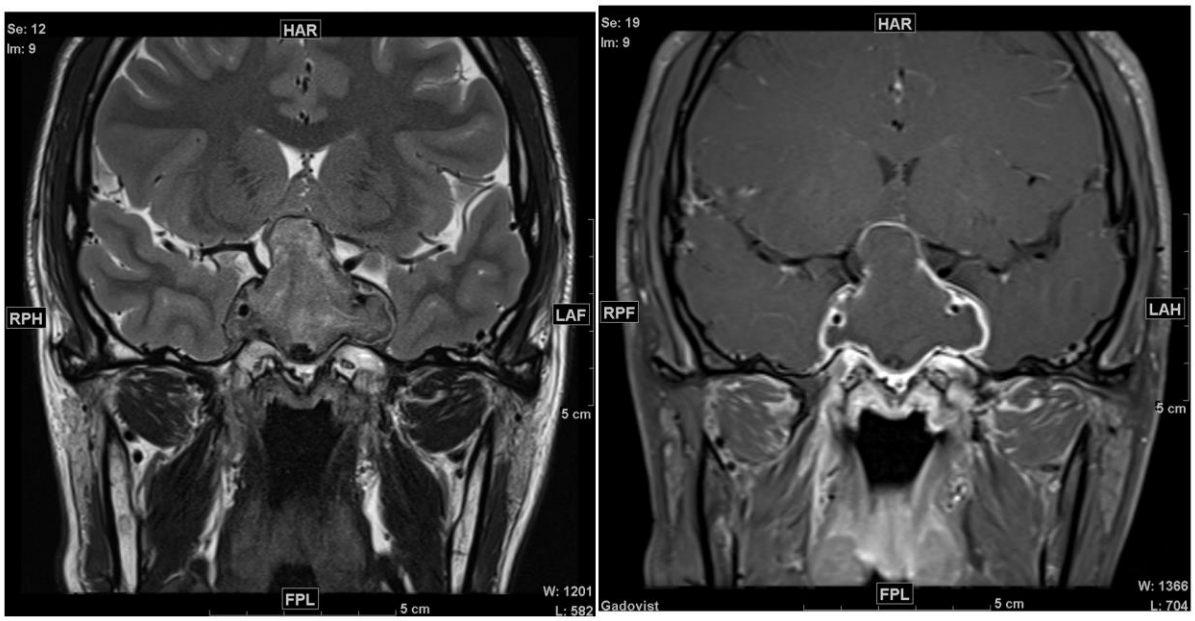


Image 1 (T1, Sagittal): A large mass, centred in the sellar, completely replaces the pituitary gland and extends into the suprasellar region. It measures approximately 20 x 37 mm (AP x SI) in this section.



Images 2 (T2, Sagittal) and 3 (T1, Sagittal): The mass extends into both cavernous sinuses with partial effacement of the cavernous Internal Carotid Arteries, as well as Left Oculomotor Nerve (CNIII), all of which are nearly completely encased. In this section the mass measures approximately 43mm (ML).

Images 4 - 6 Eye Movements (Day 5 post Transsphenoidal Resection of Pituitary Lesion)

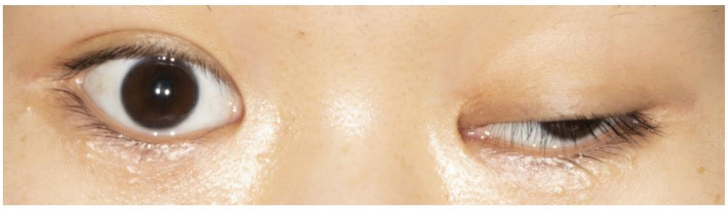


Image 4: Resting gaze: Left CNIII Palsy causing unopposed action of superior oblique (CNIV) and the lateral rectus (CNVI), pulling the left eye inferolaterally. Deinervation of Levator Palpebrae Superioris results in ptosis.

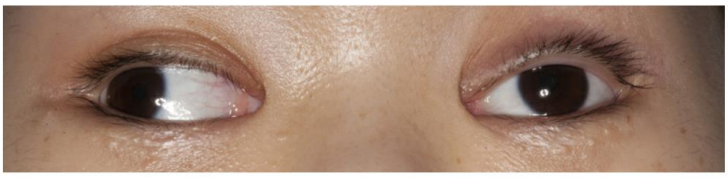


Image 5: Right lateral gaze (with manual assistance to raise left eyelid): Note inability to adduct the left eye, indicating inactivity of Left Medial Rectus.

Image 6: Left lateral gaze (with manual assistance to raise left eyelid): Note Left Lateral Rectus (CNVI) is unimpaired, as its innervation is independent of the affected Left CNIII.

Images 7 - 9 Eye Movements (Day 23 post Transsphenoidal Re Pituitary Lesion)

Image 7: Resting gaze: Some improvement of CNIII Palsy, with ptosis less sev previously, however the left eye is pulled inferolaterally.

Image 8: Right lateral gaze: Limited ability to adduct the left eye, though nov it to midline.

Image 9: Left lateral gaze : Left Lateral Rectus (CNVI) remains unimpaired.

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Resolution of pituitary 'macroadenoma' from treatment of underlying severe primary hypothyroidism

Arunan Sriravindrarajah[1, 3, 4, 2], Christina Lee[1], Kirby Wong[1], Anne Maree Kean[1]

1. Royal Prince Alfred Hospital, Sydney, NSW, Australia

2. St Vincent's Hospital, Sydney, NSW, Australia

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

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

The hypothalamic-pituitary-thyroid axis plays an important role in the regulation of normal thyroid function. Untreated primary hypothyroidism will lead to the loss of thyroxine inhibition on the hypothalamus, resulting in elevated thyrotophin-releasing hormone and possible hyperplasia of thyrotroph and / or lactotroph cells in the anterior pituitary[1].

A 52-year-old male presented with dizziness and nausea on a background of childhood hypothyroidism with total thyroidectomy at age 19. MRI pituitary demonstrated a 15x15x15mm sellar mass inseparable from the pituitary gland with suprasellar extension contacting and mildly displacing the optic chiasm. Biochemical investigations demonstrated severe primary hypothyroidism with TSH >99mIU/L (0.27-4.2), FT4 0.7pmol/L (12-25), FT3 1.1pmol/L (2.5-6.0) and secondary hypogonadism with FSH 6.6IU/L (1.5-12.4), LH 2.2IU/L (1.7-8.6) and testosterone 3.8nmol/L (10-30). The remainder of the pituitary panel was within normal range with cortisol (0835) 261nmol/L (170-500), ACTH 3.4pmol/L (<=10), prolactin 12.1ng/mL (2-16) and IGF-1 17.1nmol/L (7.2-25.5). He was commenced on levothyroxine 200mcg daily with repeat pathology after 6 months demonstrating biochemical euthyroidism with TSH 3.86mIU/L, FT4 16.8pmol/L, FT3 4.3pmol/L. His hypothalamic-gonadotrophic axis also normalised with FSH 5.9IU/L, LH 5.1IU/L and testosterone 19.3nmol/L. Repeat MRI pituitary showed an interval decrease in the size of the sellar mass to 15x15x8mm and no further contact or displacement of the optic chiasm. There were no clinical or radiological features at the time to suggest pituitary apoplexy. Multiple further surveillance MRI pituitary over the next 3 years showed a normal sized pituitary gland with the superior to inferior dimension of the gland gradually decreasing to 7mm. He remained biochemically euthyroid during this period.

This case demonstrates severe primary hypothyroidism may present with pituitary hyperplasia that may mimic a pituitary adenoma. Treatment with thyroid hormone replacement and subsequent normalisation of TSH levels would be expected to result in a decrease in the size of the pituitary enlargement.

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Finding the precipitant for an unexpected elevation in TSH – the importance of considering laboratory interferences

Arunan Sriravindrarajah[2, 1], Kiernan Hughes[1, 3], Sharanya Mohan[2, 1], Paul Bonnitcha[4, 1]

1. St Vincent's Hospital, Sydney, NSW, Australia

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

3. Garvan Institute of Medical Research, Sydney, Australia

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

Thyroid stimulating hormone (TSH) is a key investigation to assess thyroid function and adequacy of thyroid replacement. However, laboratory interferences can result in false elevations of this immunoassay and should be considered when biochemical testing is inconsistent with the clinical picture[1].

A 47-year-old male living on a remote cattle and sheep station in rural New South Wales presented for elective coronary artery bypass graft (CABG) on the background of triple vessel disease and hypothyroidism secondary to radioactive iodine (RAI) treatment of Graves' disease. Pre-operative examination demonstrated clinical euthyroidism, but investigations showed elevated TSH 41mIU/L (RR 0.4-4.8) and FT4 17.5pmol/L (RR 8-16) despite treatment with Thyroxine 200mcg daily (equivalent 2mcg/kg/day) and compliance with dosing recommendations.

He was diagnosed with Graves' disease 7 years prior via thyroid scintigraphy that showed uniformly increased uptake 24.7%. He was treated with RAI and subsequently received thyroxine replacement. However, TSH remained consistently elevated ranging from 17.7mIU/L to 19.3mIU/L, with FT4 and FT3 within normal ranges. MRI pituitary did not identify the presence of an adenoma. Previously, his thyroxine dose had been increased to 300mcg daily but he developed symptoms of thyrotoxicosis with TSH 43mIU/L, FT4 38pmol/L and FT3 10pmol/L (RR 3.5-6.0).

Given the discordance between TSH versus FT4, FT3 and clinical symptoms, his results were discussed with a chemical pathologist who investigated for laboratory interferences. Polyethylene glycol precipitation identified the presence of inactive macro-TSH (TSH-immunoglobulin complex) as the cause of the TSH elevation. He successfully received a CABG and was discharged on Thyroxine 175mcg daily, with advice to titrate Thyroxine dose based on FT4 rather than TSH.

This case demonstrates macro-TSH as a laboratory interference that can result in spurious elevation of TSH. Laboratory interferences should be considered when immunoassay results are inconsistent with the clinical scenario, and direct discussions with chemical pathologists can be invaluable.

1. Favrese, J. et. al. Interferences With Thyroid Function Immunoassays: Clinical Implications and Detection Algorithm. Endoc Rev. 2018;39(5):830-850)

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Hyponatraemia and cholestasis – remember the X factor

Arunan Sriravindrarajah[3, 2, 1], Sharanya Mohan[2, 1], Paul Bonnitcha[3, 1]

1. St Vincent's Hospital, Sydney, NSW, Australia

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

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

Hyponatraemia is an electrolyte disturbance estimated to occur in approximately 30-35% of hospital inpatients [1]. Pseudohyponatremia is an important differential that occurs when there are significant levels of lipids or protein in plasma which alter the standard 93:7 ratio of water to solute[2].

A 62-year-old female presented with a 12-month history of painless obstructive jaundice. PET-CT scan identified an FDG-avid 43x41x34mm mass in the duodenum resulting in proximal common bile duct dilatation. Pathology demonstrated a cholestatic pattern with GGT 1,800U/L (RR<35), ALP 825U/L (RR 30-100) and bilirubin 410umol/L (RR<18). Electrolytes showed near isotonic hyponatremia with serum sodium 121mmol/L (RR 135-145) and serum osmolality 296mosm/kg (RR 275-295). Further investigations demonstrated elevated total cholesterol 29.8mmol/L (RR<6.0), triglycerides 3.9mmol/L (RR<2.0) and blood glucose 17.2mmol/L (RR 3.0-7.8), with normal total protein 64g/L (RR 60-80) and serum urea 4.3mmol/L (3.5-8.0). On examination, she did not have clinical features of hyponatraemia.

Paired serum and venous blood gas (VBG) sodium levels were performed. They showed a significant discordance with serum sodium 122mmol/L compared to VBG sodium 134mmol/L, thus confirming the presence of pseudohyponatraemia. VBG measures sodium directly and is not affected by changes in the solute levels in plasma in contrast to serum sodium which measures sodium via indirect means. Lipid electrophoresis and apolipoprotein B100 were assessed, and the results were highly suggestive of the presence of lipoprotein X, a lipid often secondary to cholestasis and a known cause of pseudohyponatraemia. She subsequently received a Whipple's procedure, and there was an increase in serum sodium levels within 48 hours to 132mmol/L.

This case demonstrates lipoprotein X as the likely cause of pseudohyponatremia. VBG sodium should be measured when there is suspicion of pseudohyponatremia. The presence of lipoprotein X must be considered in all patients with cholestasis, and can be identified via lipid electrophoresis and measuring apolipoprotein B100.

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2. Theis, S.R. and Khandhar, P.B. Pseudohyponatremia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553207

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A tale of two sisters – delayed diagnosis of hypoglycaemia

Felicity Stringer[1], Christopher Preston[1, 2], Richard MacIsaac[1], Fiona Inchley[1], Livia Rivera-Woll[3], Stephen Farrell[1], Eric Yong[1], NIrupa Sachithanandan[1]

1. St Vincent's Hospital Melbourne, Melbourne, Victoria, Australia

2. Western Health, Melbourne, Victoria, Australia

3. Endocrinology Melbourne, Melbourne, Victoria, Australia

Case

A 47-year-old female presents with worsening neuroglycopenic symptoms of hypoglycaemia. The symptoms occur fasting and post-prandial, and improve with correction of hypoglycaemia.

Her past medical history was significant for neonatal hypoglycaemia, and she had an elder sister with an intellectual disability secondary to severe neonatal hypoglycaemia.

Initial screening pathology was unremarkable, however an inpatient 72-hour fast revealed hyperinsulinaemic hypoglycaemia, and she was commenced on diazoxide.

Due to her family history, genetic testing was undertaken, which was positive for ABCC8 compound heterozygote mutation. Unfortunately she did not tolerate diazoxide or acarbose medication. She continues to manage with dietary modifications aimed at avoiding hypoglycaemia, assisted by the use of a continuous glucose monitor.

Her sister has subsequently been further investigated and her 72-hour fast showed hyperinsulinaemic hypoglycaemia. Genetic testing revealed the same ABCC8 compound heterozygote mutation, and testing of their father (with their mother being deceased) is currently being undertaken.

Discussion

Monogenic hyperinsulinaemia effects 1/50,000 live births, and the most severe forms are caused by an inactivating mutation of the ABCC8 and KCNJ11 genes [1,2]. Together these genes are responsible for 36–70% of congenital hyperinsulinism cases [3-5].

ABCC8 and KCNJ11 encode the two subunits of the potassium-ATP channel [2]. This channel is critical in controlling glucose mediated release of insulin from pancreatic beta-cells, and dysfunction leads to dysregulated insulin secretion [2,4]. Patients typically present in early infancy with seizures, coma, and failure to thrive, with severe fasting hypoglycaemia [1]. Treatment for these patients includes somatostatin receptor analogues, and dietary modification [4,6]. Continuous glucose monitoring can be extremely beneficial at giving patients autonomy to manage their disease, and relieving anxiety associated with hypoglycaemia. Due to the mechanism of action of the medication, homozygous or compound heterozygous recessive mutations in the ABCC8 gene are unresponsive to diazoxide [1,2].

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Distinguishing subclinical Cushing's disease from a nonfunctioning pituitary tumour – unreliability of the intravenous dexamethasone suppression test

Tzu Mi Tseng[1], Anish Menon[1], Warrick Inder[1]

1. Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Background:

Subclinical Cushing's disease (SCD) is not a well-known entity with diagnostic challenges. This is the first report with a normal 4mg IVDST in SCD.

Clinical Case:

A 43-year-old woman was seen in the Endocrine clinic for evaluation of pituitary macroadenoma. She did not have symptoms and signs of Cushing's syndrome, acromegaly, panhypopituitarism.

Her baseline pituitary profile (table1) revealed a mildly elevated IGF-1. Subsequent oral glucose tolerance test excluded acromegaly with a nadir GH of 0.1 mg/L. MRI of pituitary fossa revealed a right-sided cystic pituitary lesion (Figure1) and the pituitary gland was expanded, encroaching on the suprasellar cistern but did not impinge on the optic chiasm. She was planned for trans-sphenoidal resection after a MDT meeting.

A preoperative hormonal profile was similar to baseline (table1). Given the high normal ACTH of 44ng/L, she underwent a 24-hour urine free cortisol and 1mg overnight DST (Table2). With the non-suppressed DST and mildly elevated urine free cortisol, a 4mg IVDST was done. It showed normal suppression of serum cortisol to <130 nmol/L on day2 (Table 3). This was interpreted as ruling out Cushing's disease according to the criteria established by Jung et al. (1).

Postoperatively, her morning cortisol continued to fall (+4h:1150nmol/L -> Day1:775nmol/L -> Day2:167nmol/L -> Day3:69nmol/L). She reported a worsening headache on day3, with no clinical signs of adrenal insufficiency. A diagnosis of glucocorticoid withdrawal syndrome was made (2). She was commenced on replacement hydrocortisone. Immunohistochemistry showed a corticotroph tumour with positivity for T-Pit and ACTH (Figure 3 and 4).

Conclusion:

- Suspect SCD with pituitary adenoma if there is high normal ACTH despite a normal cortisol level and absence of Cushingoid features.
- SCD diagnosis is confirmed if peripheral CRH stimulation and/or IPSS is in keeping with a pituitary source and subsequent tissue immunohistochemistry confirms T-Pit and ACTH staining.

Table 1

	Appointment 1 (baseline)	Appointment 2	5 month post op	9 month post op	Range/ Units
Cortisol	231	225	154	168	100 – 535 nmol/L
ACTH	51	44	17	26	9 – 51 ng/L
IGF1	39	39	34		10 – 32 nmol/L
Growth Hormone	0.9	6.2	1.3		ug/L
Prolactin	394	323	379		<500 mIU/L
FSH	3	3	5		IU/L
LH	3	2	4		IU/L
Estradiol	498	374	518		pmol/L
TSH	2.1	2.9	2.1		0.3 – 3.5 mIU/L
FT4	13.1	12.6	12.7		9 – 19 pmol/L
FT3	3.9	4.4	5		2.6 – 6 pmol/L
Comments	Breastfeeding at time				

Table 2

Investigation	Result	Range/units
24 hour urinary free cortisol		
U-cortisol	167	<110 nmol/d
U-Creatinine excretion	16.0	5.3 – 16.0 mmol/d
Urine Volume	0.96	0.5-2.1 L
1mg Dexamethasone suppression test		
Day 2 cortisol	111	<50 nmol/L

Table 3

4mg IV DST		
	Cortisol (nmol/L)	ACTH (ng/L)
-60min (BASELINE)	288	74
-5min (0935am)	342	54
+3h (1240pm)	182	59
+4h (1340pm)	162	49
+5h (1440pm)	130	49
+23h	112	55
+23.5h	120	56
Mean of day 2 samples	116	55.5

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TSH	2.1	2.9	2.1		0.3 – 3.5 mIU/L
FT4	13.1	12.6	12.7		9 – 19 pmol/L
FT3	3.9	4.4	5		2.6 – 6 pmol/L
Comments	Breastfeeding at time				

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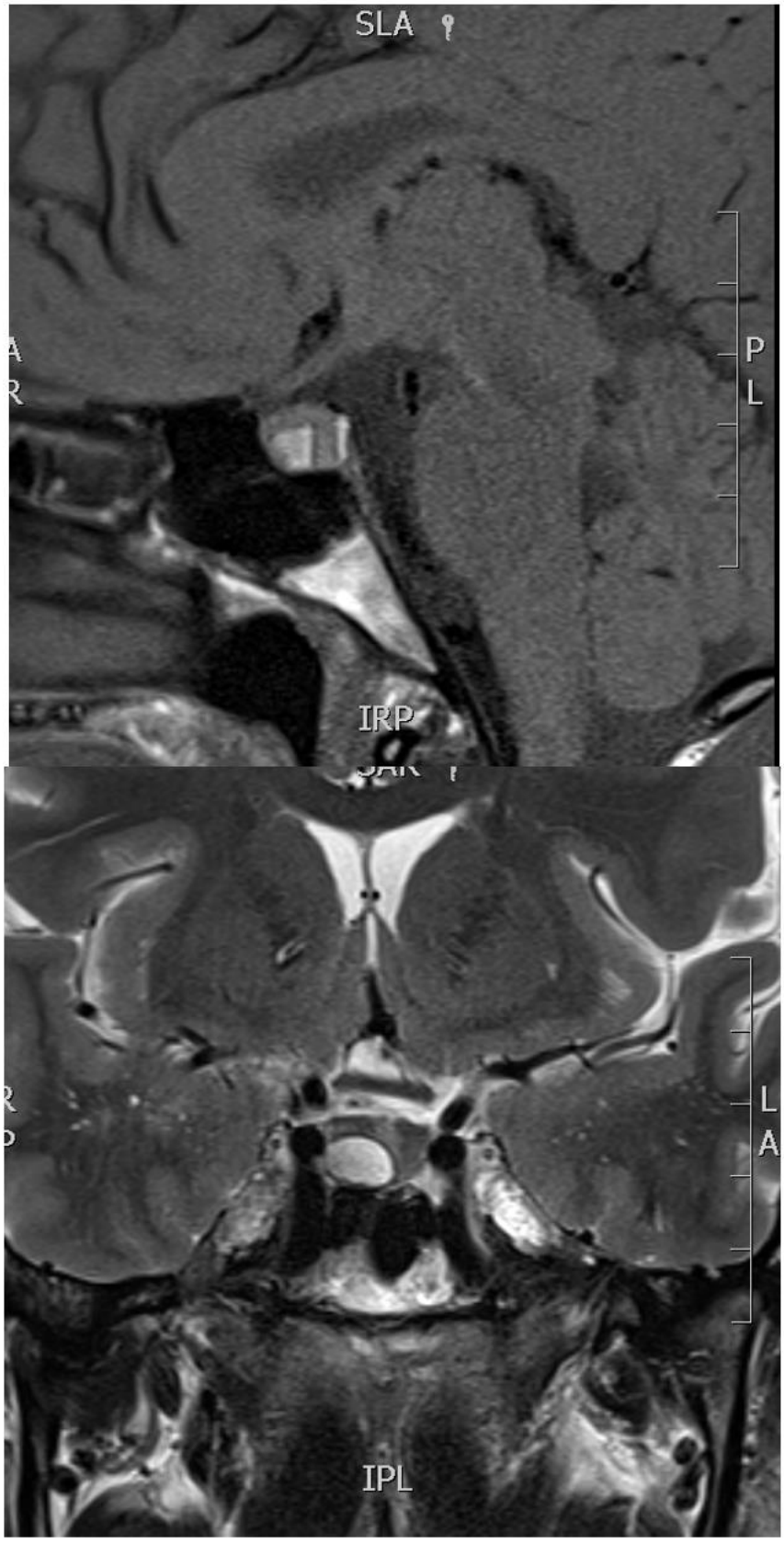
+5h (1440pm)	130	49
+23h	112	55
+23.5h	120	56
Mean of day 2 samples	116	55.5

Figure 1: MRI in sagittal T1 and coronal T2 view of pituitary fossa revealed a right sided cystic pituitary lesion, measuring 9 x 7 x 10 mm,



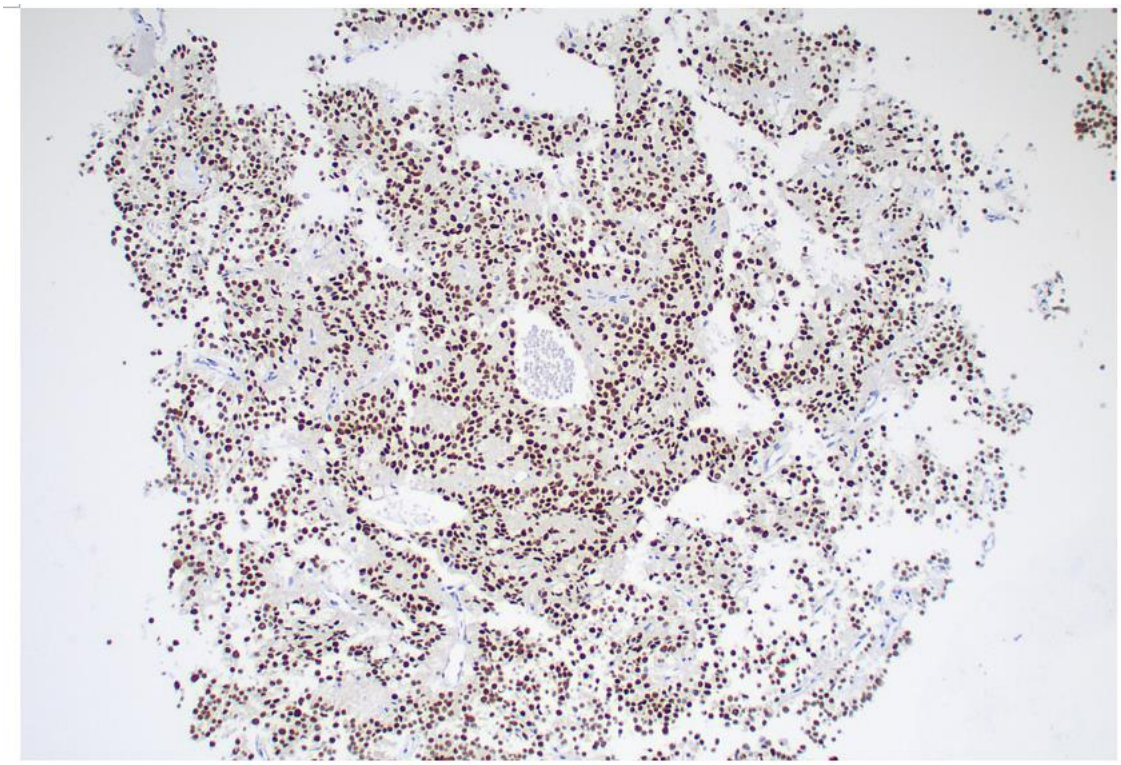


Figure 3: Positive immunohistochemistry stain for T-Pit x100

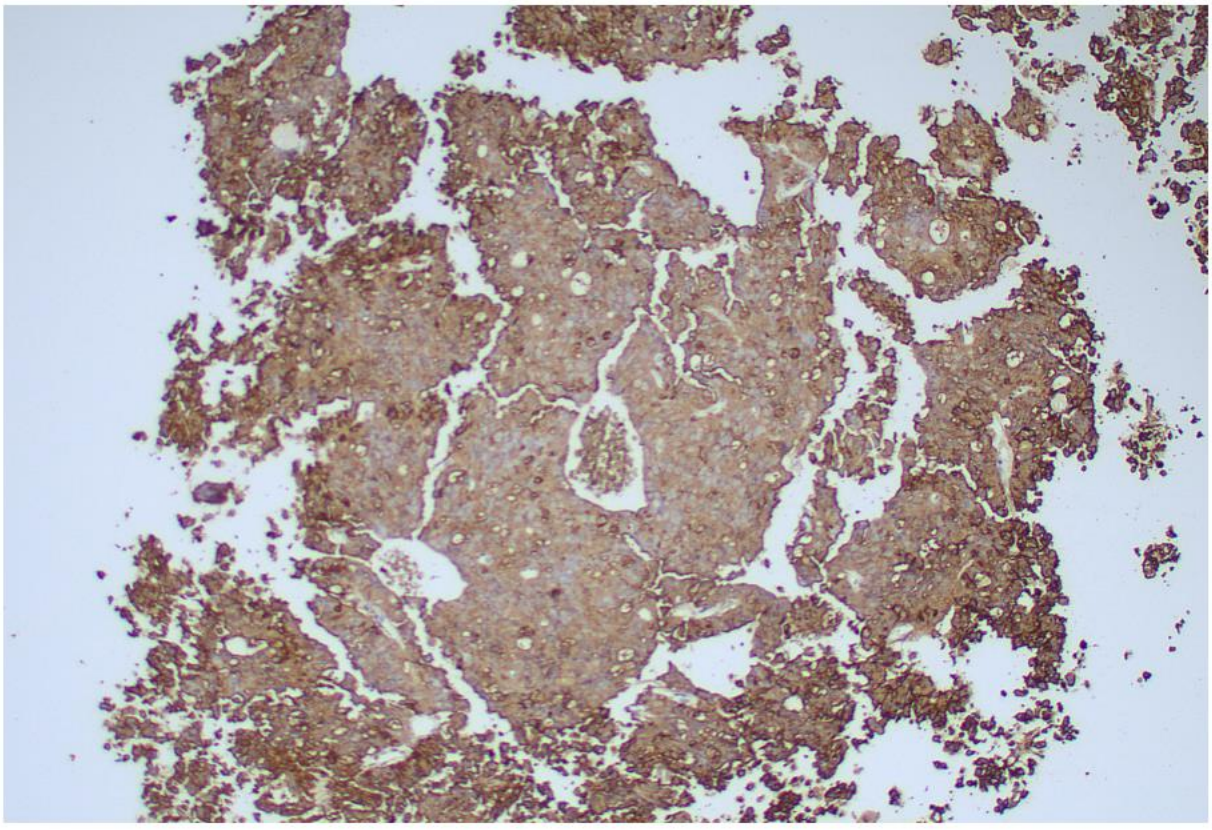


Figure 4: Immunohistochemistry stain for ACTH revealed strong cytoplasmic staining x100

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Spinal cord compression as the initial presentation of follicular thyroid cancer

Tegan van Gemert[1], Rachael Zuzek[1], Kathy Fu[1]

1. The Wollongong Hospital, Wollongong, NSW, Australia

Background: Differentiated thyroid cancer (DTC) carries very high overall 10-year survival rates, 93% for papillary carcinoma and 85% for follicular carcinoma.[1] The presentation of distant metastases drastically reduces these rates but distant metastases at diagnosis is uncommon.[2] Furthermore, DTC presenting initially with distant metastasis is even more uncommon, accounting for only 2% of thyroid cancer cases.[3]

Case Report: An independent 89-year-old female from home alone presented with lower limb weakness and left foot drop. She described progressive gait disturbance over 4 months and development of urinary urge incontinence. Examination revealed bilateral lower limb weakness and reduced ankle and knee jerk reflexes bilaterally. MRI spine showed a large mass involving the posterior elements of T10 resulting in compression of the thoracic cord and cord oedema. She was commenced on high dose steroids. She underwent excision of the large extradural tumour, partial T10 vertebrectomy and T9-11 laminectomy and fusion. The tumour histopathology was metastatic follicular thyroid carcinoma. Further investigation with thyroid ultrasound revealed a multinodular goitre including a left lower pole 22mm nodule, TIRADS 4. There was no neck lymphadenopathy. On FDG-PET this thyroid nodule was mildly FDG avid, SUV max 3.3. Nil other metastatic disease was identified. FNAB of the nodule confirmed thyroid carcinoma. Further treatment including total thyroidectomy followed by radioactive iodine was discussed. Due to the patient's age and functional decline she opted for no further treatment.

Discussion: The presence of distance metastases drastically reduces the 5 year survival rates of DTC to 77.6% for single organ metastasis, and 15.3% for patients with multi-organ metastases.[2] Our case demonstrates a rare presentation of spinal cord compression as the initial presentation of DTC. The best management approach of such patients is not clear given the rarity of these cases, but thyroidectomy to facilitate radioactive iodine therapy is usually recommended.[4]

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Recurrent pancreatitis in Familial Hypocalciuric Hypercalcaemia treated with cinacalcet

Tegan van Gemert[1], Rachael Zuzek[1], Matthew Luttrell[1]

1. The Wollongong Hospital, Wollongong, NSW, Australia

Background: Familial hypocalciuric hypercalcaemia (FHH) is a rare condition due to inactivating mutations in the calcium-sensing receptor gene (CASR). Usually, patients have mild hypercalcaemia with no or minimal complications.

Case: A 27-year-old male had a history of recurrent pancreatitis. Each admission was associated with significantly elevated corrected calcium up to 3.4 mmol/L with an inappropriately normal PTH. Calcium creatinine clearance of 0.00135 was suggestive of FHH. He continued to have recurrent pancreatitis with elevated calcium out of proportion to what is expected with FHH so consideration of dual pathology with primary hyperparathyroidism was considered. Sestamibi parathyroid imaging demonstrated a parathyroid adenoma posterior to the right inferior thyroid lobe with a correlating lesion also seen on 4D CT. He proceeded with surgical resection of the right inferior parathyroid, histopathology was consistent with hyperplasia. Despite this, he continued to have significantly elevated calcium and episodes of pancreatitis. Genetic testing was performed and identified a variant of uncertain significant affecting the CASR gene. Ongoing hypocalciuria and the genetic test result was thought to be supportive of a diagnosis of FHH. He was commenced on cinacalcet which led to a drastic reduction in hospital admissions, on occasions where he had run out of tablets, he represented to hospital within 1 week with abdominal pain and elevated corrected calcium to 3.3 mmol/L. With titration of cinacalcet to 60mg BD, calcium improved to 2.7 mmol/L and there had been no further hospital presentations.

Discussion: There are rare case reports of pancreatitis in FHH associated with variant mutations in the CASR.[1] Cinacalcet is a calcimimetic and has previously been used for patients with FHH causing complications.[2] Our patient responded very well to cinacalcet. Best long-term management of these patients is unclear, but some case series reveal sustained improvement over at least 3 years.[3]

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Synchronous metastatic thyroblastoma and metastatic lung adenocarcinoma

Karen Van[1], Shan Balachandran[1], Rosemary Wong[1], Michael Mond[1]

1. Eastern Health, Box Hill, VIC, Australia

CASE STUDY

A 44-year-old previously well man, an ex-smoker, with no pertinent family history, presented with a palpable right thyroid nodule and associated right cervical lymphadenopathy.

Thyroid ultrasonography identified a 47mm solid, hypoechoic right thyroid nodule, with anterior triangle and supraclavicular fossa lymphadenopathy. FNA confirmed a Bethesda 6 cytology suggestive of medullary carcinoma with a right lymph node positive for neoplasia. Serum calcitonin was <1.3pmol/L (RR <4.3). FDG-PET imaging demonstrated FDG-avidity of the right thyroid mass and right cervical lymphadenopathy. Small, non-PET-avid pulmonary nodules were identified bilaterally. Two-stage thyroidectomy with right modified radical neck dissection was performed due to patient preference. Histology indicated a high-grade malignancy with mixed epithelial and spindle cell components including a significant primitive component, and involving 17/38 lymph nodes. Limited somatic mutation analysis did not identify common thyroid cancer driver mutations.

Seven months later, he developed transient haematuria and dyspnoea. Whilst no cause for the haematuria was identified, a moderate left pleural effusion with multiple pulmonary nodules were suggestive of metastases. He underwent VATS and pleurodesis. Lung wedge resection showed invasive mucinous adenocarcinoma favouring a primary lung malignancy.

Following specialist opinion, the original thyroid carcinoma was determined consistent with metastatic thyroblastoma. He is currently undergoing chemotherapy for both malignancies and awaiting genetic screening for DICER1.

DISCUSSION

Thyroblastoma is a thyroid malignancy recognised as a discrete entity in the 5th Edition WHO Classification of Endocrine and Neuroendocrine Tumours.[1] Previously classified as malignant thyroid teratomas, molecular analysis has now allowed for definitive differentiation of benign/immature and malignant thyroid teratomas.[2]Thyroblastomas are histologically defined by the presence of primitive multilineage elements, including immature thyroid epithelium, cellular mesenchyme with frequent rhabdomyoblastic differentiation, and neuro- epithelial blastema.[2] All 11 reported cases are associated with DICER1 mutations.[3] We present a case with two distinct malignancies, with thyroblastoma being a unique, novel entity.

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A case report: recurrent pancreatitis and metabolic dysregulation in a patient with a novel PPAR-*γ* mutation

Kimberly Voon[1, 2], Yi Xian Chan[1], Wan Jia Loh[2, 3, 4, 5], Gerald Watts[2, 5]

1. Department of Endocrinology and Diabetes, Royal Perth Hospital, Perth

2. Cardiometabolic Service, Department of Cardiology and Internal Medicine, Royal Perth Hospital, Perth

3. Department of Endocrinology, Changi General Hospital, Singapore

4. Duke-NUS Medical School, Singapore

5. School of Medicine, University of Western Australia, Perth

Monogenic diabetes accounts for 1-5% of diabetes mellitus. Recognition helps to improve precision in treatment and allows for familial risk management. Adipose tissue is a dynamic endocrine organ, not only serving as a caloric reservoir for storing essential surplus nutrients but also playing a role in influencing metabolic health. Dysregulation of adipose tissue differentiation and deposition in peripheral tissues results in significant metabolic consequences. This can stem from dysfunction of the peroxisome proliferator-activated receptor (PPAR). In this case report, we describe a patient with hereditary pancreatitis who displayed emerging metabolic dysregulation, subsequently found to have a novel *PPARγ* mutation.

A 34-year-old Chinese woman with a background of recurrent pancreatitis thought to be secondary to a pancreatic divisum anomaly and hereditary pancreatitis (PRSS1 mutation carrier) was admitted to hospital for pancreatitis. On this occasion, she was identified to have new diabetes, with admission BGL 14.7mmol/L and HBA1C 9%, as well as severe hypertriglyceridaemia of 43.2mmol/L (<2mmol/L). Further assessment demonstrated features of partial lipodystrophy and she was confirmed to have a novel heterogenous p.Arg395His mutation affecting the ligand binding domain (LBD) of *PPARγ*. She was also identified to have hypertension and hepatic steatosis. She is awaiting further cardiovascular risk stratification. Cascade testing of her siblings is underway.

PPARγ mutations result in a wide spectrum of phenotypes including familial partial lipodystrophy, diabetes, hyperlipidaemia, hepatic steatosis, obesity and polycystic ovarian syndrome. Management hinges on identifying and ameliorating cardiometabolic complications. Synthetic *PPARγ* agonists such as thiazolidinediones may be useful adjuncts as it binds in

proximity to the LBD, leading to restoration of the molecule's transcriptional function. Thus, variants with mutations within the LBD such as our patient may gain the most therapeutic benefit. Further understanding of the genotype-phenotype correlation is required, as it may be used to guide therapeutic decisions for these patients.

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Insulin autoimmune syndrome and COVID-19 infection: a case report and literature review

Anindita P Wardhani[1], Jeremy Hoang[1]

1. Department of Endocrinology, Hornsby Ku-ring-gai Hospital, Northern Sydney Local Health District, Sydney, New South Wales, Australia

Background/Aim: Insulin autoimmune syndrome (IAS) is a rare disorder characterised by hypoglycaemia due to insulin autoantibodies. Prior exposure to certain viruses has been implicated in the pathogenesis. There is little literature on the relationship between IAS and SARS-CoV-2.

Method: We reviewed a case of symptomatic hyperinsulinaemic hypoglycaemia associated with markedly elevated insulin antibody and prior COVID-19 infection. We performed a literature review on IAS and potential mechanisms of SARS-CoV-2 in the pathogenesis.

Result: A 51-year-old non-diabetic patient was admitted with hypoglycaemia. Multiple stereotypical episodes of sweating and hot flushes had been occurring in the prior week. She had COVID-19 infection 4 weeks prior. 72-hour fasting test confirmed hyperinsulinaemic hypoglycaemia. At termination, she was symptomatic with glucose level 2.6mmol/L, insulin level 169mU/L, and c-peptide level 1.1nmol/L. Sulphonylurea screen was negative. MRI, contrast CT, Dotatate PET/CT and endoscopic US did not identify a source of hyperinsulinaemia. However, her insulin antibody was markedly elevated at 4243U/ml (Normal≤0.4U/ml). She was treated with high-dose Prednisolone. Her insulin antibody significantly decreased with no further hypoglycaemia.

A growing number of autoimmune diseases following COVID-19 infection have been observed[(1)]. We found two similar reports of hypoglycaemia, one of which was confirmed IAS[(2,3)]. It is proposed that post-COVID-19 autoimmunity is due to immune reconstitution following transient immune suppression[(4)]. This is a well-known phenomenon in other viral infections e.g. coxsackie B and hepatitis C, both of which have been implicated in IAS[(5-7)]. Additionally, the pancreatic islet is a target tissue of SARS-CoV-2 driven by ACE2 expression[(8)]. Recent studies have suggested viral infections as triggers of pancreatic beta-cell autoimmunity through the production of cross-reactive antibodies due to molecular mimicry[(9-12)].

Conclusion: We highlighted a possible novel role of COVID-19 in the pathogenesis of IAS. Clinicians should consider COVID-19 as a potential cause when evaluating similar cases of non-diabetic hypoglycaemia.

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Clinical Case of Eosinophilic Esophagitis (EoE) leading to likely diagnosis of congenital Connective Tissue Disease (CTD)

Dragon Ka Lung Wat[1], Ingrid Elisabeth Bonapart[2]

1. Resident, MAPU, Toowoomba Hospital, Toowoomba, QLD, Australia

2. Clinical Director MAPU / Endocrinologist, Toowoomba Hospital, Toowoomba, QLD, Australia

Aims

To raise awareness of the association of EoE with CTD. Risk of EoE is up to 8-fold increase in CTD population[(1)]. Recognizing undiagnosed CTD is of the utmost importance for more favorable outcomes. There is also a role for closer follow up of EoE disease in CTD patients, because of greater risks for more diffused eosinophilic extra-esophageal gastrointestinal disease and resistance to treatments[(2)].

Methods

Patient is a 19M and had 5 admissions for food bolus in 8 years. All of them required gastroscopy to resolve or assess the esophagus. Patient had clinical presentation related to Marfan Syndrome (MFS) and Ehlers Danlos Syndrome (EDS) like tall stature, high-arched palate, pectus incavatum and extensive striae over body. But without typical presentation like long extremities, heart murmur, ectopia lentis, vision issues and flat feet (pes planus) in MFS or hypermobility of joints and hyperflexibility of skin in EDS.

Results

Patient had been investigated for MFS at younger age but results were negative. Echocardiogram in 2020 was normal. All 5 gastroscopies were normal, food bolus passed or removed, biopsies had been taken in all gastroscopies and histology from all biopsies showed traditional EoE pictures. 4 out of 5 esophageal biopsies confirmed EoE with the highest count of 300 eosinophils/hpf. He also had positive family history of a sister with confirmed EDS.

Conclusion

Patient has been called back for further genetic testing for congenital CTD and screening for different features of CTD with pathology and imaging. We suspect patient to have congenital CTD with features from a few CTD variants. We need to confirm the genetic diagnosis and prevent unfavourable preventable outcomes. Early multi-disciplinary involvement is essential to control symptoms and minimise systemic involvement.

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Phaeochromocytoma resection unmasking Addison's disease in a case of Autoimmune Polyglandular Syndrome type 2

Alexander Yao[1], Michael Christie[2], Julie Miller[3], Christopher Yates[1]

1. Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

2. Anatomical Pathology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

3. Endocrine and Breast Surgery, Royal Melbourne Hospital, Melbourne, Victoria, Australia

Case Report

A 33-year old man with Hashimoto's disease was incidentally found to have a 4.1cm heterogeneously enhancing left adrenal mass on a CT performed to investigate right flank pain. There was no family history of endocrinopathies.

He reported 6 months of paroxysmal palpitations, headaches and agitation. Blood pressure and heart rate were 110/60mmHg and 70bpm, respectively. Adrenal hormone testing confirmed a phaeochromocytoma: secreting normetanephrine (10,524pmol/L) and 3-methoxytyramine (460pmol/L) (Table 1). Phenoxybenzamine and later propranolol were uptitrated before posterior laparoscopic retroperitoneal adrenalectomy. Histopathology confirmed typical phaeochromocytoma features with intact SDHA/B immunohistochemistry, however the adjacent adrenal cortex demonstrated lymphocytic infiltration and cortical cell destruction.

Post-operatively he developed symptomatic hyponatraemia. Short-synacthen testing confirmed primary adrenal insufficiency (Table 2), with elevated ACTH, low aldosterone and positive adrenal cortex antibodies (Table 3). Retrospective imaging review demonstrated right adrenal atrophy. Symptoms and hyponatraemia resolved with hydrocortisone and fludrocortisone commencement. Plasma metanephrines normalised post-operatively.

Genetic testing was declined due to anxiety, however evaluation for other endocrinopathies and MEN2-related diseases was unremarkable.

Discussion

Autoimmune polyglandular syndrome-type 2 (APS-2) has a prevalence of 1:1000-1:20,000 and is characterised by two of Addison's disease, autoimmune thyroid disease and type 1 diabetes with typical age of onset 20-40 years.1 APS-2 is a polygenic disease, with significant heterogeneity due to multiple genetic loci and environmental factors responsible. Major histocompatibility complex *(MHC)* genes located on chromosome 6 have been implicated. It appears that HLA-DR3 and HLA-DR4 haplotypes and the class 2 HLA alleles DQ2 and DQ8 increase predisposition.2

There have been 4 reported cases of phaeochromocytoma with APS-2.[3-6] This is the first where Addison's disease was unmasked by postoperative normalisation of catecholamines.

Table 1

Pre-op Investigations	Result	Reference Range
Normetanephrine	**10,524**	**<900 pmol/L**
Metanephrine	142	<500 pmol/L
3-Methoxytyramine	**460**	**<110 pmol/L**
Sodium	**133**	**135 – 145 mmol/L**
TSH	**7.69**	**0.50 – 4.00 mIU/L**
fT4	16.5	9.00 – 19.00 pmol/L
Thyroid peroxidase antibodies	**170**	**<34 kU/L**
Thyroglobulin antibodies	50	<115 kU/L
Corrected Ca	2.62	2.15 – 2.65 mmol/L
PTH	2.2	2.0 – 8.5 pmol/L
Vitamin D	**28**	**>50 nmol/L**
Calcitonin	1.4	<2.9 pmol/L

Table 2

Short Synacthen Test				
	Baseline	30mins	60mins	Reference Range
Cortisol	**12**	**<11**	**<11**	**>550 nmol/L**
ACTH	**659**			**7.2 – 63.3 ng/L**

Table 3

Post-op Investigations	Result	Reference Range
Sodium	**123**	**135 – 145 mmol/L**
Potassium	**5.9**	**3.5 – 5.2 mmol/L**
Bicarbonate	**16**	**22 – 32 mmol/L**
Serum osmolality	293	280 – 300 mmol/L
Urine osmolality	399	50 – 1200 mmol/kg
Urine sodium	57	mmol/L
Normetanephrine	544	<900 pmol/L
Metanephrine	<70	<500 pmol/L
Aldosterone	**47**	**100 – 950 pmol/L**
Renin	**205**	**10 – 50 mIU/L**
ARR	<1	<71
Adrenal cortex antibodies	**Positive**	
GAD/IA2/ZnT8/Insulin antibodies	Negative	
Coeliac serology	Negative	

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A Curious Case of Cushing's

Alexander Yao[1], Angeline Shen[1]

1. Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

A 44-year-old female migrant presented with clinical and biochemical evidence of active Cushing's syndrome (CS) on a background of Cushing's disease managed with a brief course of ketoconazole complicated by LFT derangement and followed by transsphenoidal surgery.

3 years later, she migrated to Australia and had manifestations of disease recurrence including new diabetes, anxiety, weight gain and hypertriglyceridaemia (Table 1). MRI did not identify any residual pituitary tumour. She refused repeat IPSS, pituitary surgery and bilateral adrenalectomy. She was managed with metyrapone and cabergoline which was complicated by adrenal crisis and subsequently commenced on a hydrocortisone and metyrapone 'block and replace' approach. She was lost to follow-up for a year and self-ceased her medications however appeared to have resolution of her metabolic complications, raising the possibility of cyclical-CS.

She re-presented during the COVID-19 pandemic with clinically active CS. However, investigations were delayed due to her fears of contracting COVID-19 (Table 2). A repeat MRI pituitary and CT CAP failed to identify a source. Ga68-DOTATATE PET/CT demonstrated a 14mm focally avid nodule in the right pulmonary region. Fine needle biopsy with endobronchial ultrasound confirmed a bronchial carcinoid tumour expressing ACTH. MDT consensus was to proceed with resection of the

lesion regardless of hormone secretion. Intra-operative pulmonary vein sampling is planned to be conducted at the time of the surgery.

Meanwhile she commenced on osilodrostat on compassionate grounds with dexamethasone and has had marked improvement of her metabolic profile and CS (Table 3).

Discussion

Cyclical-CS can be difficult to diagnose and manage and should be considered in patients with fluctuations in their clinical presentation.1 A block and replace approach may be helpful to prevent adrenal crisis.3

68Ga-DOTATATE scanning targets somatostatin receptor function and has an additional role compared to conventional imaging in the diagnosis of ectopic-CS or metastatic PitNETs.3

Table 1

Initial Biochemistry	Result	Units	Reference Range
Late Night Salivary Cortisol	**15**	nmol/L	(0.2 - 3.2)
24hr urine free cortisol	**1012**	nmol/d	(3 - 208)
8am cortisol	**1057**	nmol/L	(145 - 619)
ACTH	**30.6**	pmol/L	(< 20)
TSH	1.71	mIU/L	(0.50 – 4.00)
T3	3.9	pmol/L	(3.5 – 6.5)
T4	**9.1**	pmol/L	(10.0 - 19.0)
IGF1	18	nmol/L	(10 - 36)
GH	2	mIU/L	(0 - 21)
Prolactin	218	mIU/L	(59 – 619)
HbA1c	**7.0%**		
Triglycerides	**13**	mmol/L	(<2)
Total chol	3.9	mmol/L	(<4)

Table 2

2022 Biochemistry	Result	Units	Reference Range
Late Night Salivary Cortisol	**17**	nmol/L	(<8)
24hr Urine Free Cortisol	**304**	nmol/d	(<280)
1mg Dex suppression test	**366**	nmol/L	(<50)
ACTH	**64**	pmol/L	(7 – 63)
TSH	1.10	mIU/L	(0.50 – 4.00)
T3	3.9	pmol/L	(3.5 – 6.5)
T4	**7.2**	pmol/L	(10.0 - 19.0)
IGF1	18	nmol/L	(8.97 – 32.89)
GH	0.32	ug/L	(<8)
Prolactin	<13	mIU/L	(110 – 560)
LH	0.1	IU/L	postm (5.2 – 62.0)
FSH	0.9	IU/L	postm (27.0 – 133.0)
Oestradiol	129	pmol/L	postm (<505)
HbA1c	**13.8%**		
Triglycerides	**18**	mmol/L	(<2)
Total chol	3.6	mmol/L	(<4)

Table 3

Latest Biochemistry	Result	Units	Reference Range
Late Night Salivary Cortisol	**10**	nmol/L	(<8)
Morning Cortisol	127	nmol/L	(<540)
ACTH	19.3	pmol/L	(7 – 63)

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Pituitary metastasis

Sin Dee Yap[1], Brett Sillars[1]

1. Sunshine Coast University Hospital, Birtinya, QLD, Australia

MH is a 50-year-old smoker, who was referred to the emergency department with pan-hypopituitarism during work-up of increasing lethargy and recent diagnosis of a primary bronchial neoplasm on computed tomography of chest. He also reported erectile dysfunction, mild morning headache, blurry vision, nocturnal polyuria and polydipsia. Thyroxine 50 microg daily was started by his GP 2 weeks prior to his presentation. Planned bronchoscopy was postponed and endocrinology was consulted. His blood pressure was 102/65 mmHg without postural drop, otherwise clinical examination was unremarkable.

He received empirical intravenous hydrocortisone 100 mg intravenously awaiting cortisol levels. Diabetes insipidus was suspected clinically despite a normal serum sodium, with good clinical response to 200mcg of desmopressin. Desmopressin was subsequently withheld then reduced to 100mcg daily due to initial hyponatraemia. Thyroxine was continued.

Results of his anterior pituitary hormone panel are shown in Table 1 and table 2.

Table 1: Initial results of endocrine testing at external laboratory

Test (all done at 12.30pm)	Result	Reference range
ACTH	Not done	
Cortisol	49 nmol/L	140-640 nmol/L
$4S$TSH	1.1 mIU/L	0.4-4 mIU/L
FT4	< 5.4 pmol/L	8.5-27 pmol/L
FT3	2.4 pmol/L	2.8-6.8 pmol/L
LH	<0.1 IU/L	3-10 IU/L
FSH	0.3 IU/L	1.6-9.7 IU/L
Testosterone	<0.5 nmol/L	9-35 nmol/L
Prolactin	823 mIU/L	97-484 mIU/L
IGF-1	16 nmol/L	4-32 nmol/L
Sodium	134 mmol/L	135-145 nmol/L

Table 2: Bloods collected at 7.06am prior to hydrocortisone

Test	Result	Reference range
ACTH	16 ng/L	10-50 ng/L
Cortisol	123 nmol/L	140-640 nmol/L
Sodium (pre-desmopressin)	137 mmol/L	135-145 nmol/L
Serum osmolality	285 mmol/kg	275-295 mmol/kg
Urine osmolality	170 mmol/kg	

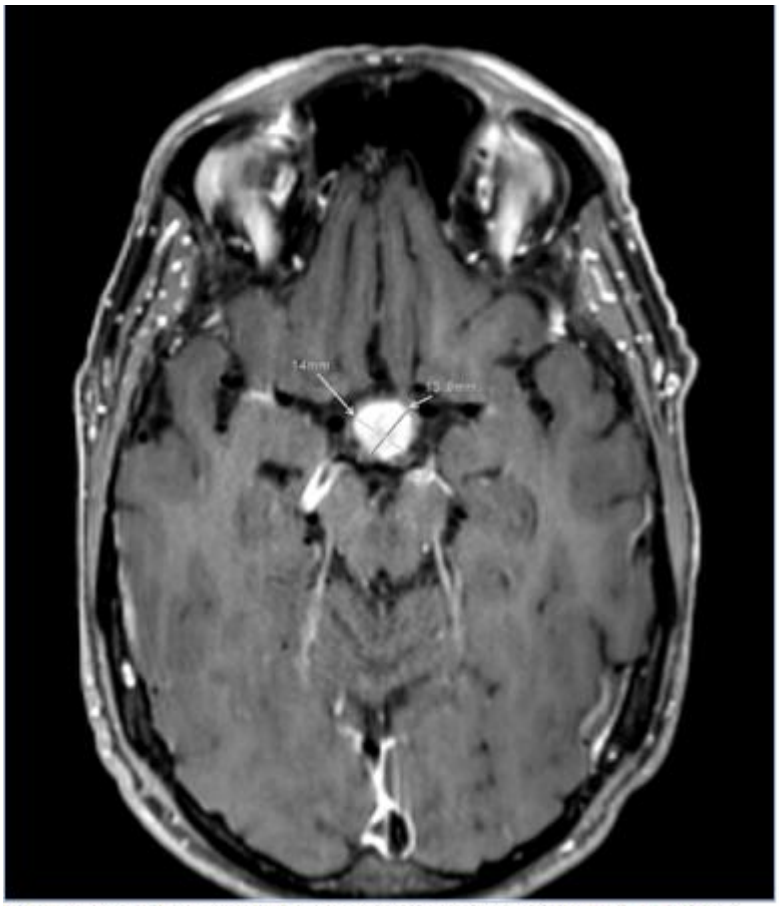

Figure 1: T1 axial view. 14 x 14 x 21mm enhancing lesion involving the hypothalamus, pituitary stalk and posterior pituitary gland.

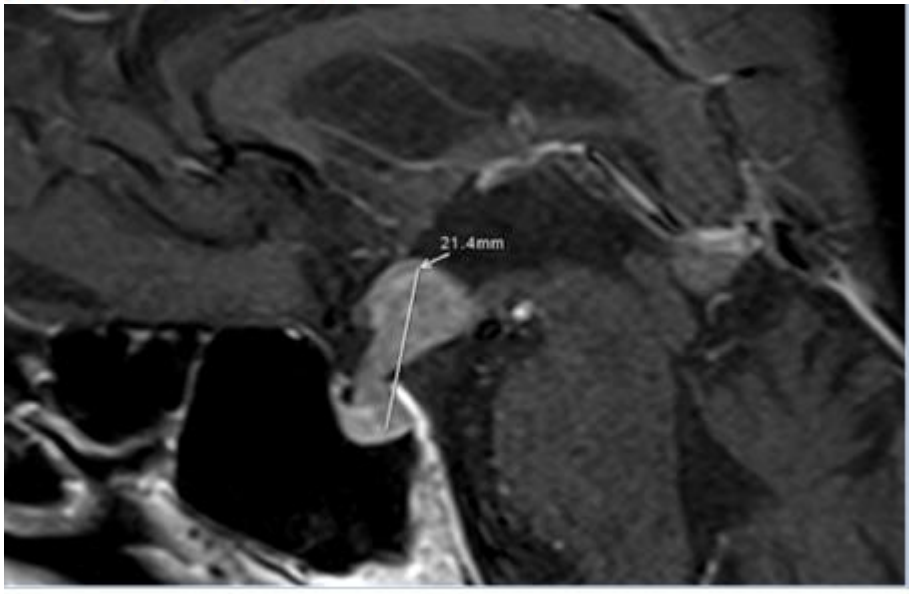

Figure 2: T1 sagittal view.

Pituitary metastases are rare. Diabetes insipidus and opthalmoplegia are associated with pituitary metastases[1]. Clinical presentation of pituitary metastases varies. Patients with pituitary metastases and hypophysitis can present with headaches and hypocortisolism. However, pituitary metastases are associated with diabetes insipidus and opthalmoplegia. Adrenal insufficiency is the most common in patients with pituitary metastases, followed by central hypothyroidism, hyperprolactinaemia and diabetes insipidus[1]. MH's clinical features were consistent with pituitary metastasis and a biopsy was not indicated. In this case, diabetes insipidus was suspected clinically despite a normal serum sodium, raising the possibility of secondary adrenal insufficiency masking the hypernatraemia associated with central diabetes insipidus. Treatment of pituitary metastases should be individualised and aim of treatment include management of the primary tumour and relieve of symptoms from mass effect with surgical resection and/or radiation therapy or comfort measures. Overall survival of patients with pituitary metastases is poor and depends on the disease burden of the primary malignancy.

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Macroprolactinoma in an adolescent female with primary amenorrhea

Ning Zhang[1], Eleanor White[1], Nicholas Little[2], Winny Varikatt[3], Mark Dexter[4], Sarah Glastras[1]

1. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Department of Neurosurgery, Royal North Shore Hospital, St Leonards, NSW, Australia

3. Tissue Pathology & Diagnostic Oncology, Westmead Hospital, Westmead, NSW, Australia

4. Department of Neurosurgery, Westmead Hospital, Westmead, NSW, Australia

Prolactinomas in childhood and adolescence are rare, accounting for less than 2% of all intracranial tumours (Hoffman et al. 2018). Clinical symptoms and signs of hyperprolactinaemia may manifest as delayed puberty, hypogonadism or galactorrhea, whereas tumour mass effects can present as headaches and vision impairment (Casanueva et al., 2006). In this clinical case study, we present the case of a 16-year-old female with primary amenorrhoea secondary to macroprolactinoma and explore the treatment options following a modest radiological and biochemical response to initial dopamine agonist (DA) therapy.

A 14-year-old female presented to her general practitioner with headaches, galactorrhea, hemianopia and primary amenorrhea. She had bitemporal hemianopia on visual field testing. The results of the initial laboratory tests in July 2021 included prolactin 26 286 mIU/L (Ref: 85-500) and magnetic resonance imaging (MRI) revealed a 28 x 18 x 15 mm pituitary mass with compression of the optic chiasm. Following tertiary endocrinology and neurosurgical reviews, medical management was recommended, and the patient was commenced on cabergoline 0.5mg weekly and gradually increased to a total of 3mg weekly. Despite a further 11 months of DA therapy, the patient remained amenorrheic and hyperprolactinaemic (4930 mIU/L, ref: 85-500) with new additional symptoms of low mood, lethargy, and weight gain. Visual field testing had improved with a 90% resolution of visual hemianopia. The patient underwent a transnasal transsphenoidal excision of the pituitary tumour. Post-operative MRI scans revealed a significant reduction in tumour mass however she remained hyperprolactinaemic and was recommenced on cabergoline. She remained amenorrhoeic and therefore was required to commence topical estrogen therapy.

We present the challenging case of managing a medically refractive macroprolactinoma during adolescence. Significant concerns included concomitant pubertal delay, reduced bone age, and delayed secondary sex characteristics. This case highlighted the need for additional management options including surgical and hormonal therapy.

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A rare case of hypercalcaemia secondary to advanced chronic liver disease

Rachael Zuzek[1], Tegan Van Gemert[1], Jane Zhang[1]

1. Wollongong Hospital, Figtree, NSW, Australia

Background: Hypercalcaemia in patients with chronic liver disease (CLD) without hepatocellular carcinoma (HCC) is rare and a diagnosis of exclusion.

Case: A 30-year-old previously well male initially presented with severe COVID pneumonitis, complicated by secondary sclerosing cholangitis resulting in CLD. He developed new hypercalcaemia which correlated with increasing liver enzymes and bilirubin (peak 278µmol/L). Peak corrected calcium (CorCa) was 3.47mmol/L with suppressed PTH 1.0pmol/L. There was no personal/family history of calcium disorders and no contributing medications. There was no bony abnormality or underlying malignancy on imaging; AFP was negative. IgG was mildly raised with no other features consistent with myeloma. He had normal renal and thyroid function. His urine calcium-to-creatinine ratio was normal. Vitamin D toxicity was excluded with a low vitamin D 27nmol/L. 1,25-dihyroxyvitamin-D was undetectable <12pmol/L and ACE was normal, ruling out granulomatous disease. There was no prolonged immobilisation. IV pamidronate and rehydration lead to initial improvement, however, his hypercalcaemia recurred (CorCa 2.7-3.12mmol/L). After successful liver transplantation his hypercalcaemia fully resolved and remained normal months later, leading to the diagnosis of hypercalcaemia of CLD.

Discussion: CLD is more often associated with hypocalcaemia due to hypoalbuminaemia. Hypercalcaemia in CLD associated with and without HCC was first described by Gerhadt in 1987 [1]. There are few reported cases and the cause is not clear. Elevated inflammatory markers (eg: TNF-1, IL-1) [2] and hyperbilirubinaemia may lead to upregulation of RANKL/OPG which increases bone resorption and downregulation of osteogenic factors such as RUNX2 [3]. Whether bilirubin is pathogenic or an indicator of disease severity is not certain [4]. CLD may also impair catabolism of metabolites involved in bone resorption [4]. The hypercalcaemia can be transient and responds to bisphosphonates and hydration [2,5], and in advanced cases, resolves with liver transplantation. Further research is needed to understand this rare cause of hypercalcaemia.

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Hereditary paraganglioma associated with SDHB mutation

Rachael Zuzek[1], Tegan Van Gemert[1], Alexia Pape[1]

1. Wollongong Hospital, Figtree, NSW, Australia

Background: Paragangliomas are rare neuroendocrine tumours derived from autonomic ganglia. Whilst most are sporadic, up to 40% of cases are hereditary [1], often associated with multiple lesions and younger onset.

Case: A 25-year-old female presented with a non-productive cough and palpitations. On examination she was hypertensive (162/110mmHg). Imaging revealed a large right paravertebral mass from T7-T9. Plasma metanephrines were elevated 7,180pmol/L, as was 24-hour urinary noradrenaline 4,211nmol/L and normetanephrine 17.6µmol/d. Ga-68 DOTATATE PET/CT confirmed intense activity at the lesion, with two further areas of activity in the neck and adjacent to the left adrenal gland. The patient underwent resection of all three lesions. Plasma metanephrines normalised after resection of the paravertebral tumour. Histopathology revealed a Zellballen pattern consistent with paraganglioma. Immunohistochemistry was positive for chromogranin with loss of SDH-B, suggestive of succinate dehydrogenase (SDH) deficiency. Genetic testing confirmed a SDHB mutation. There was no evidence of metastatic disease.

Discussion: Pathogenic genetic variants in SDH enzyme subunits are a common cause of hereditary paragangliomas [2]. SDHB mutations at locus 1p36.1-35 are the second most common and confer a 25% risk of developing a paraganglioma or pheochromocytoma by age 50 [3]. Patients tend to develop disease at a younger age compared with sporadic lesions [4]. There is no family history in the majority of patients, though the rate of de novo mutations is not known [5]. SDHB paragangliomas can be multiple, are often sympathetic and secrete noradrenaline, and more common in the abdomen/thorax [6]. SDHB mutations increase the risk of recurrent lesions and the likelihood of malignant transformation [2,4-6], requiring surveillance imaging. Furthermore, there is a 5% risk of renal cell carcinoma and 1% risk of gastric gastrointestinal stromal tumour [3]. In summary, there is increasing recognition of genetic factors in paraganglioma development, which has implications for prognosis and follow up.

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Antioxidants in sperm incubation media for IVF treatment improve subsequent mouse embryo development

Georgia Bennett[1], Thi Truong[1], Rebecca Kelley[1, 2], David K Gardner[1, 2]

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

2. Melbourne IVF, Melbourne, VIC, Australia

Aims: Truong and Gardner (2017) demonstrated that the addition of three antioxidants to sperm preparation media incubated for 1h had no effect on mouse embryo development. In IVF clinics, human sperm can be incubated for several hours before insemination. This study aimed to further previous mouse studies by utilising a clinically relevant sperm incubation time of 3h in the presence or absence of antioxidants, and evaluate the impact on subsequent embryo development.

Methods: Sperm were collected via backflushing the epididymis of F1-hybrid male mice. Sperm incubation occurred at 20% oxygen in the presence or absence of a combination of antioxidants (10µM Acetyl-L-Carnitine, 10µM N-Acetyl-L-Cysteine, 5µM α-Lipoic Acid). IVF was conducted under 5% oxygen and resulting embryos were cultured without antioxidants. After 4 days, the impact of antioxidants in sperm preparation on embryo development was analysed via time-lapse morhokinetics and by differential nuclear staining to determine cell counts.

Results: Antioxidant treatment during sperm incubation had no impact on fertilisation (87% vs 92%, control vs antioxidant) or blastocyst rate (90% vs 84%), but did result in a significant increase in trophectoderm (73.3 ± 2.8 vs 91.0 ± 2.5, $P<0.01$), inner cell mass (25.8 ±1 .1 vs 30.8 ± 0.9, $P<0.01$) and total blastocyst cell numbers (99.1 ± 3.5 vs 121.8 ± 3.2, $P<0.01$; $n>60$). Embryos derived from the antioxidant-treated sperm also developed more quickly at the 8-cell and morula stage (t8: 49.48 ± 0.49 vs 48.30 ± 0.32, $P<0.05$; tM: 56.86 ± 0.67 vs 54.79 ± 0.50 $P<0.05$; $n>69$).

Conclusion: The presence of antioxidants during mouse sperm incubation for 3h prior to IVF imparts a significant benefit on embryo quality and development. The data indicates that antioxidants could be beneficial for IVF clinics which currently incubate sperm at 20% oxygen for several hours before use.

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Effect of Zinc Supplementation on the Quality of Cooled Stored Equine Sperm

Patricio Palacios Benitez[1], Isabel I Ortiz[2], Jesus Dorado[2], Manuel Hidalgo[2], Juan Ramon Garcia-Diaz[2, 3], Andres Gambini[2, 1]

1. School of Agriculture and Food Sustainability, The University of Queensland, Gatton, Queensland, Australia

2. Veterinary Reproduction Group, Department of Medicine and Animal Surgery, Faculty of Veterinary Medicine, University of Cordoba, Cordoba , Andalucia, Spain

3. Facultad de Ciencias Agropecuaria, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Villa Clara, Cuba

Assisted reproductive technologies revolutionize animal breeding, notably improving genetics and production efficiency. Among these techniques, semen collection, cooling, and cryopreservation are key for accessing genetically superior stallions. However, preserving stallion sperm poses greater challenges compared to other species. This study aimed to explore the impact of zinc (Zn) sulphate supplementation on the in vitro quality of equine sperm during cold storage. Semen from three stallions was collected and diluted 1:1 (vivo) with the extender INRA96. Sperm morphology was assessed with Diff-Quick®, motility and kinetic parameters performing CASA, as well as membrane integrity and acrosomal reaction using a flow cytometer. Assessments were conducted on fresh samples, at 24- and 48-hour post-cooling, as well as following a heat-resistance test (240 min incubation at 37°C). In experiment 1, four Zn sulfate concentrations were tested: 0 mM, 1 mM, 2 mM, and 3 mM. In experiment 2, a broader range of concentrations of 0 mM, 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM, 1.6 mM, and 3.2 mM was assessed alongside the heat-resistance test. Data was analyzed with the statistical package SPSS v15.0. Our findings indicate that incorporating varying Zn concentrations to INRA96 extender doesn't significantly enhance sperm quality of cooled stallion semen assessed at 24 or 48 hours. Additionally, no benefits were observed for any concentration after the heat-resistance test. However, Zn concentrations surpassing 3 mM exhibited detrimental impacts on stallion's sperm quality parameters. These findings contribute to the understanding of Zn supplementation as a strategy for improving semen preservation in stallions and highlight the importance of maintaining a delicate balance in Zn concentrations to ensure optimal sperm function.

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Engineering functionalised surfaces that selectively capture suboptimal spermatozoa for applications in human IVF

Soraya Rasi Ghaemi[1], David Sharkey[1], Nicole McPherson[1], Krasimir Vasilev[2], Sarah Robertson[1]

1. Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia

2. Flinders University, South Australia, Adelaide, Australian Capital Territory, Australia

Selecting viable sperm for successful fertilisation and optimal developmental competence is an unmet challenge in reproductive medicine. There are limitations of methods based solely on motility and morphology criteria and this has prompted innovative approaches. Advances in understanding of the immunobiology of female reproductive tract sperm selection shows a role for immune cells in sperm selection. Knowledge of the relevant biomolecules provides novel avenues to develop surface chemistry-based approaches to emulate the molecular interactions between suboptimal sperm and immune cells. We have investigated plasma polymerisation approaches to recapitulate immune-mediated sequestration of suboptimal sperm on functionalised glass surfaces. We applied a plasma polymerised polyoxazoline (PPOx) film to glass using 2-methyl-2-oxazoline monomer. The PPOx film enabled covalent binding of antibodies reacting with suboptimal sperm in a concentration-dependent manner. In pilot experiments, samples of human donor sperm were introduced to the activated surface and the rate of sperm attachment was measured to define optimal surface coating parameters. We then applied similar surface functionalisation to antibody-coated glass channelled-slides and showed that unattached sperm recovered after introduction of neat semen exhibit superior functional characteristics compared with sperm prepared by standard swim-up or uncoated channelled slides. Recovered sperm were assessed by flow cytometry to measure proportions of viable sperm (propidium iodide) and apoptotic sperm (Annexin V expression), as well as reactive oxygen species (CellROX Green), and sperm DNA fragmentation (HALO-sperm assay)(n=3-5 per assay). Sperm recovered from functionalised channelled-slides had a significantly lower Annexin V+ sperm subpopulation (mean±SEM, 6.3±1.1%) compared with sperm prepared by standard swim-up (19.0±4.1%, $P<0.05$, ANOVA). The proportion of sperm exhibiting DNA fragmentation was also significantly decreased after functionalised channelled-slide use (5.7±1.2%) compared with swim-up (11.5±3.1%, $P<0.05$, ANOVA). In conclusion, our findings demonstrate the efficacy of surface functionalised antibody-coated channelled slides and their potential in preparing high-quality sperm for use in ART.

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Accuracy of sperm morphology assessment is dependent on microscope optics and morphological classification method

Katherine R Seymour[1], Taylor Pini[2], Kelsey Pool[3], Caitlin G Chambers[1], Jessica P Rickard[1], Simon P de Graaf[1]

1. School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia

2. School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia

3. UWA institute of Agriculture and UWA School of Agriculture and Environment, The University of Western Australia, Crawley, WA, Australia

Sperm morphology assessment is a critical determinant of fertility in livestock. However, anecdotal reports suggest the accuracy of sperm morphological classification varies considerably between assessors. This research aimed to (1) characterise the variation in sperm morphology classification between morphologists using both simple (two category) and complex (30 category) classification systems, and (2) characterise the variation between two types of microscope optics (phase contrast and differential interference contrast; DIC).

Images of individual ram sperm (n = 800/optic, 400x magnification) were labelled using up to 30 morphology categories by four experienced sperm morphologists, this labelling was then simplified to a normal/abnormal categorisation for comparison.

When using the normal/abnormal classification system with DIC, morphologists agreed on the label of 585 sperm out of 800 and when labelling with the 30-category system the morphologists agreed on 212 sperm out of 800. When using the normal/abnormal classification system with phase contrast the morphologists agreed on the label of 641 sperm out of 800 and when labelling with the 30-category system the morphologists agreed on 253 sperm out of 800.

In conclusion, sperm that were captured using phase contrast optics led to a higher degree of consensus amongst morphologists when compared to DIC. The variation decreased for both optics as the complexity of the labelling system increased. Further investigation into how standardisation may influence these results would be interesting to understand how this variation could be minimised.

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Serum derived complement proteins play an important role in sperm-neutrophil binding

Sophie Warr[1], Simon de Graaf[1], Taylor Pini[2], Jessica Rickard[1]

1. School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia

2. School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia

Semen deposition in the female tract elicits an immune-mediated response, releasing an influx of polymorphonuclear leukocytes (PMNs)[1]. In addition to protecting the reproductive tract from pathogens, this response also acts to select an elite population of sperm, capable of achieving fertilisation[2]. It has been hypothesised that the reduced ability of frozen-thawed ram spermatozoa to traverse the cervix may be related to their increased susceptibility to neutrophil binding and phagocytosis[3]. As such in the present study, we examined the binding of PMNs to fresh and frozen-thawed ram spermatozoa in the presence of ewe serum. Heat-treated serum was also included to assess the role of complement proteins. PMNs isolated from the blood of Merino ewes (*n*=2) were incubated (37°C; 120mins) with fresh spermatozoa diluted in Tris-citrate-fructose supplemented with 15% egg yolk (FrEY) and frozen-thawed spermatozoa diluted in an egg yolk-based diluent (FtEY) either in the absence of serum, 7.5% (v/v) serum, or 7.5% (v/v) heat-treated serum. Serum significantly increased sperm-neutrophil binding in FrEY and FtEY (84.85±1.6% and 80.23±2.1%, respectively) compared to that recorded in no serum (58.44±3.4% and 66.41±3.2%, respectively; p=0.025). The presence of heat-treated serum significantly reduced binding in FrEY by 22.25% and FtEY by 23.56% compared to that recorded in serum. In the absence of serum, FtEY recorded higher binding rates (66.41±3.2%) compared to FrEY (58.43±3.4%; p=0.025). Results in the absence of serum suggest that cryopreservation may alter lectin-mediated PMN binding, potentially due to modified sperm-surface carbohydrates. The deactivation of complement in heat-treated serum reduced binding compared to untreated serum, suggesting that complement-mediated binding is a key sperm-neutrophil binding mechanism. Further investigation is now required to explore factors which influence complement-mediated binding, such as seminal plasma, which could be used to suppress the immune response within the female environment, aiding the survival of frozen-thawed sperm in the cervix.

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Role of viscous loading in regulating bull and mouse sperm flagellar waveform

Farin Yazdan Parast[1], Avinash S. Gaikwad[2, 3], Sushant Powar[1], Ranganathan Prabhakar[1], Moira K. O'Bryan[2], Reza Nosrati[1]

1. Department of Mechanical and Aerospace Engineering, Monash University, Clayton, Victoria, Australia

2. School of BioSciences and Bio21 Institute, University of Melbourne, Parkville, Victoria, Australia

3. Institute of Reproductive Genetics, University of Münster, Münster, Germany

Sperm navigate through the female reproductive tract, encountering varying viscosities, to reach the oocyte for fertilisation [1]. Understanding sperm motion and flagellar patterns in a physiologically relevant context is crucial for reproductive insights and identifying infertility causes. Viscosity notably affects sperm motility, and it is suggested to influence long-range *in vivo* guidance [2]. However, the biomechanics underlying sperm flagellar activity in relation to fluid viscosity remain understood. Lack of high-speed high-resolution imaging techniques with automated image processing capabilities has been the main barrier to fully describing the flagellar beating behaviour. Here, we used a custom built high-speed, high-resolution dark-field microscopy system to study bull and mouse sperm flagellar dynamics in a physiologically relevant range of media viscosity, from 1 mPa·s to 200 mPa·s. Automated image analysis was used to extract sperm flagellar waveform and characterise bull and mouse sperm flagellar dynamics.

Differences emerged in the beating pattern for sperm in low versus high-viscosity conditions. Bull sperm exhibited a lower flagellar beating amplitude along the distal end of the tail when swimming in a high-viscosity compared to a low-viscosity buffer. However, mouse sperm in a high-viscosity buffer had a lower flagellar beating amplitude across the principal piece and a higher beating amplitude across the distal end of the tail compared to the low-viscosity buffer. Notably, bull sperm demonstrated a transition mode at 5 mPa·s and a regular circular pattern at 1 mPa·s and above 5 mPa·s. Conversely, mouse sperm maintained periodic flagellar beating in high-viscosity media but displayed distorted loops in low-viscosity media.

In conclusion, we resolved the dynamics of free-swimming bull and mouse sperm in viscoelastic media (1-200 mPa·s). Our findings indicated a highly reproducible flagellar waveform for both species in high-viscosity media.

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Will the combination of external (endocrine disruptor chemical) and intrinsic (growth factor) exposures influence the fate of testicular germ cell tumours?

Junlan (Iris) Ma[1, 2], Sarah C Moody[2], Kate L Loveland[1, 2]

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

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

Testicular germ cell tumours (TGCTs) are the most frequent solid tumour in young men (19 to 39yo). They arise from fetal germ cells that fail to differentiate and transform into tumours after puberty. TGCTs are classified into either seminomas, selectively marked by *SOX17* expression, or non-seminomas, most of which express *SOX2* but lack *SOX17*. Seminomas can be reprogrammed to the more clinically challenging non-seminomas *in vitro* and *in vivo*. Understanding the regulation of SOX2 and SOX17 may provide insight into germ cell tumour fate and cancer prognosis. This study, using TCam-2 cells, the established cell line representing seminoma, addresses the potential for the combined impact of an environmental chemical, mono-2-ethylhexyl phthalate (MEHP) and inappropriately elevated exposure to growth factor activin A to influence TGCT aetiology. The reprogramming response of TCam-2 cells was interrogated by exposure to these factors. Cells were cultured with activin A (5 ng/mL), MEHP (100µM), both combined, MEHP plus the potent activin/TGFβ/NODAL signalling inhibitor (SB431542) or vehicle controls for 48 hours (n=3) in serum-reduced conditions (2.5% FBS). Transcripts were measured by qRT-PCR. MEHP, activin A, and their combination each significantly upregulated *SOX2* (1.37-, 1.41-, 1.42-fold, respectively). However, SB alone and MEHP plus SB decreased *SOX2* (0.61-, 0.58- fold, respectively), suggesting MEHP and activin A work through the same pathway. *SOX17*, was unchanged by MEHP, but was upregulated (1.4-fold) by activin A, different from the outcome with *SOX2*. *ETV5*, encoding a transcription factor essential for maintaining spermatogonia stem cell self-renewal, was decreased by MEHP (0.54-fold), activin A (0.30-fold), and their combination (0.22-fold), indicating an additive effect. Cell migration assays and a non-seminoma model, NT2/D1 cells, will also be used to examine activin A and MEHP's effects on cancer progression. These outcomes demonstrate how concurrent environmental and intrinsic factor exposure may worsen TGCT outcomes for young men.

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Leveraging a mouse model to identify candidate secreted biomarkers of human early ovarian cancer

Sarah Sczelecki[1], Claire Henry[2], Janet Pitman[1]

1. Te Herenga Waka Victoria University of Wellington, Kelburn, WELLINGTON, New Zealand

2. Department of Surgery and Anaesthesia, University of Otago Wellington, Wellington, New Zealand

Ovarian cancer (OC) has the highest mortality rate of all gynaecological malignancies. This is partly due to the limited understanding of the initiating neoplastic transformations culminating in lack of reliable diagnostics for early detection. *In vivo* animal models have been used to enhance fundamental biological processes of OC development; however, those that can recapitulate initiating pathogenic events are limited. Therefore, there is an urgent need for novel models of early OC to improve the development of early-stage OC detection diagnostics.

We have recently genetically characterised a precursor lesion of OC in the Fanconi anaemia complementation group D2 knock-out (*Fancd2*$^{-/-}$) animal model [1-3]. However, its relevance as a model to study early human OC was previously unknown. Therefore, this study firstly compared differential gene expression (*c.f.* control tissue) of the precursor and late-stage OC phenotype (tubulostromal adenoma) from the *Fancd2*$^{-/-}$model to human high-grade serous and serous borderline ovarian tumours by total RNA sequencing of laser capture micro-dissected (LCM) tissue. Subsequently, the *Fancd2*$^{-/-}$ model was employed to provide proof-of-concept evidence that secreted extracellular vesicle (EV) encapsulated nucleic acid biomarkers of early-staged OC can be detected.

RNA-sequencing analyses of LCM tissue resulted in similar upregulation of key epithelial OC markers, such as *Cdh1, Muc16, Keratins, Epcam*, *Pax8* and *Wfdc2,* between the mouse precursor lesion and tumour and human OC specimens studied. Then, a comparison of the mouse and human secreted EV sequencing results also revealed shared upregulated EV-derived microRNAs between the mouse OC precursor and adenoma and low- and high-grade human disease specimens. Importantly, these candidate microRNA biomarkers of early OC displayed improved diagnostic value (by receiver-operating characteristic analysis) over the clinical gold-standard CA-125, effectively discriminating between OC and controls. Thus, this research characterised a clinically relevant OC model and identified novel candidate secreted EV biomarkers for earlier detection of human OC.

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Targeting of a novel cancer/testis epigenetic regulator for the treatment of cancer.

Tanya Soboleva[1], Xianzhao Jiang[1], David Tremethick[1], Gene Hart-Smith[2], Alex Sobolev[3]

1. JCSMR, ANU, Canberra, ACT, Australia

2. Macquarie Analytical & Fabrication Facility (MAFF), Macquarie University, Sydney, NSW, Australia

3. Institue of Gene Biology, Moscow, Russia

Epigenetic machinery plays an essential role in cell differentiation and maintenance of cell function. Not surprisingly, the corruption of epigenetic mechanisms results in disastrous consequences including oncogenesis.

It has been known for more than two decades that certain cancers upregulate testis-specific factors, known as cancer-testis antigens (CTA). The CTAs were largely considered not to be important for carcinogenesis but mainly viewed as convenient markers for the identification of certain types of cancer.

Our recent work has demonstrated that a testis-specific histone variant, H2A.B, is the first epigenetic CTA factor that is important for the maintenance of Hodgkin lymphoma (HL) carcinogenesis. Moreover, we have mapped and characterised post-translational modifications (PTM) of H2A.B N-terminus and identified their readers and writers. Finally, we have also shown that H2AB PTMs play a key role in H2A.B functional dynamics and epigenetic control of carcinogenic processes in HL. This presentation will discuss these novel findings and their implications.

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Circumventing L1 Reporter Silencing to Observe L1 Mediated Genetic Mosaicism

Alexander L. Carleton[1], Dorothy Chan[1], Juan Manuel Botto[1, 2], Geoffrey J. Faulkner[1, 2], Sandra R. Richardson[1]

1. Mater Research, BRISBANE, QLD, Australia

2. Queensland Brain Institute, Brisbane, QLD, Australia

Transposable elements (TE) are mobile genes able to move around the genome generating new endogenous mutations. Retrotransposons are a subset of TE's that move via a copy and paste mechanism called retrotransposition whereby the total number of these elements increases. The only autonomously mobile TE in mammals is the retrotransposon Long interspersed nuclear elements (LINEs/L1).

Since retrotransposition results in new insertional mutations, it stands to reason this random process can result in cellular dysfunction when insertions occur within crucial genes or regulatory regions having over 100 cases of human genetic disease caused by L1 mediated retrotransposition. New insertions are also a driving force of evolution generating genetic diversity within a population, and even generating genetic mosaicism within an individual. At early developmental stages, retrotransposition events can generate somatic mosaicism in organisms by causing mutations that are inherited by every cell within that developmental lineage.

So, to study L1 retrotransposition in cell culture and transgenic animal models, fluorescent reporters have been used which mark cells containing new insertions with fluorescent proteins. This system has been demonstrated in cultured PA-1 embryonal carcinoma cells to undergo reporter silencing, leading to underestimations of L1 activity in vivo.

To combat epigenetic silencing the L1-Cre reporter construct was developed. In this system, Cre recombinase is used as the reporter, where as few as four molecules of Cre recombinase can activate a secondary reporter placed in a safe harbour locus, marking the cell with a fluorescent protein.

Ultimately the L1-Cre system will be used in combination with transgenic mice containing multicolour conditional reporter genes to circumvent potential L1 reporter silencing, while allowing for multispectral visualisation and analysis of genetic mosaicism driven by L1's arising during mouse embryonic development.

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Mobile DNA activity in mammalian primordial germ cell specification

Renee Chu[1], Alexander Carleton[1], Stephanie Workman[1], Sandra Richardson[1]

1. MRI-UQ, Brisbane, QLD, Australia

Long Interspersed Element 1 (LINE-1 or L1) is an autonomously mobile DNA element found throughout mammalian genomes. L1s have the capability to retrotranspose, or "jump" into other regions of our genome, which can potentially result in cellular dysfunction and genetic disease. Importantly, as "selfish" elements, L1s create heritable insertions— new copies in cells that can be passed down to the next generation. Retrotransposition events in the germline have been detected in transgenic L1 reporter animals and in the early embryo, and exacerbated expression of mobile DNA in the germline has shown to cause sterility in mice. In humans, only two cases of heritable L1 retrotransposition events have been fully explicated; one potentially occurred during female germ cell development, and the other during pluripotent embryonic cell development. However, the exact developmental timing of L1 retrotransposition during this developmental period and the defence mechanisms involved to impede retrotransposition remains unelucidated.

Previously, Richardson et al. 2017 uncovered evidence that L1 may retrotranspose in early mouse primordial germ cells (PGCs). PGCs are specified early during mammalian embryonic development, ultimately giving rise to all germ cells of an adult organism. Little is known about the dynamics of L1 activity and regulation during PGC specification, and the founding population of ~40 mouse PGCs *in vivo* is technically challenging to study. Hence, we are using an *in vitro* model to investigate regulation of L1 expression and retrotransposition during cell fate transitions from mESCs to epiblast-like cells (EpiLCs) to primordial germ cell-like cells (PGCLCs). Our work will shed light on the unexplored conflict between L1 activity and genomic defences in this critical developmental niche, as well as further understand L1 mutagenesis as a potential contributor to reproductive dysfunction.

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Exposure to diethylstilbestrol causes transgenerational effects on female fertility and reproductive development through altering DNA methylation

Rachael Rogers[1], Deidre M Mattiske[1], Andrew J Pask[1]

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

Endocrine disrupting chemicals (EDCs) are pervasive toxins in our environment that can block and/or interfere with normal hormonal signalling and action within the body. We are continuously exposed to EDCs in our everyday lives as they are present in plastics, pharmaceuticals, herbicides, and pesticides. Numerous studies have demonstrated the detrimental effects of EDCs on fertility and reproductive development. However, the most alarming impact of EDCs is their ability to cause effects that persist for multiple generations. Previously, we showed that prenatal exposure to the potent estrogenic EDC, diethylstilbestrol (DES), causes transgenerational reproductive effects in mice. Specifically, we observed that female descendants went through puberty significantly earlier, had a significantly smaller anogenital distance and experienced reduced fertility up until the third, unexposed generation. To determine the mechanisms by which DES is able to cause these transgenerational effects, we conducted whole genome methylation analysis on oocytes collected from control and F1 – F3 DES exposed mice. The methylation percentage was compared between these groups, and we found that global methylation was significantly higher in DES F2 and F3 generations compared the F1 generation. This indicates that DES is able to alter the epigenome by disrupting normal DNA methylation, which may then result in the observed transgenerational phenotype. These data provide insights into the mechanisms through which EDCs are able to elicit their long-lasting, transgenerational effects.

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A rare case of ambiguous genitalia and gender dysphoria – a role for molecular genetic sequencing?

Ellen Wall[1], Veena Jayadev[2]

1. Department of Endocrinology, Concord Repatriation General Hospital, Sydney, NSW

2. Department of Andrology, Concord Repatriation General Hospital, Sydney, NSW

Aim: we report a case of a patient with pseudovaginal perineoscrotal hypospadias presenting with gender dysphoria in adulthood after being raised female, with the diagnosis of 5α-reductase deficiency confirmed following molecular genetic sequencing. This highlights the potential benefits of genetic testing over routine hormonal screening for this disorder which are often inaccurate.

Methods: case report of a man presenting with pseudovaginal perineoscrotal hypospadias who underwent hormonal, karyotyping and molecular genetic investigations. His background was complicated by a previous orchidectomy in infancy. We subsequently performed a literature review focusing upon the epidemiology and previous clinical presentations of 5α-reductase deficiency, it's known molecular variant associations (ie. mutations in *SRD5A2* gene)[1] and rates of comorbid gender dysphoria[2].

Results: karyotyping was performed which confirmed a 46 XY karyotype on cytogenetic analysis. There was presence of SRY mutation on quantitative fluorescent polymerase chain reaction. Biochemistry results are included below, pre- and post testosterone replacement after 44 weeks (Table 1).

Test	Result (baseline)	Result (post testosterone replacement)	Normal reference range (male)
Testosterone (nmol/L)	**1.2**	12.4	10-35
Dihydrotestosterone (nmol/L)	-	**0.3**	<0.7
T/DHT ratio	-	**41.3**	8-16
Oestradiol (pmol/L)	88	-	<156
FSH (IU/L)	**27.8**	1.7	2-12
LH (IU/L)	**21.1**	2.2	2-9
SHBG (nmol/L)	15.7	16.9	15-64
17-OH progesterone (nmol/L)	**1.0**	-	4.9-43.3

Whole exome gene sequencing was performed which demonstrated pathogenic compound heterozygous variants in the SRD5A2 gene (c.383_384delinsGA and c.607G>A).

Conclusion: 5α-reductase deficiency is an exceedingly rare cause of disorder of sex development in males which typically presents with ambiguous genitalia at birth[3]. It is phenotypically broad and may mimic more common conditions such as androgen insensitivity syndrome at birth[4]. Molecular genetic testing offers an alternative accurate diagnostic test to routine hormonal investigations and this earlier diagnosis may help limit the medical and psychosexual complications which are common in patients with disorders of sexual development.

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Endocrine disrupting chemicals found in personal care products perturb *in vitro* mouse blastocyst lineage cell counts and outgrowth areas

Bethany J Finger[1], Eve Morano[1], Alexandra J Harvey[1], Mark P Green[1]

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

Methylparaben (MeP), triclosan (TCS) and oxybenzone (BP3) are three common oestrogenic endocrine disrupting chemicals (EDCs) found in personal care products associated with negative reproductive health effects. Whether these chemicals can directly impact the pre-implantation embryo is unknown. The aim of this study was to determine how environmentallyelevant concentrations of MeP, TCS and BP3, both individually and in combination, affected mouse *in vitro* pre-implantation embryo development. Zygotes from 3–4-week-old CBAxC57BL6/J mice were cultured in G-media supplemented with or without EDCs over a range of concentrations found in human urine and serum. Cleavage and blastocyst development rates were recorded on day 5 prior to differential staining to determine inner cell mass (ICM), trophectoderm (TE) and total cell counts. An additional cohort of blastocysts were subjected to a further 96 hours of culture in the presence of individual and combined EDC conditions to quantify trophoblast outgrowth area. No changes in cleavage nor blastocyst rates were evident (P>0.1; n>4 cultures, n>140 zygotes per chemical per concentration). Relative to control embryos, TE and total cell numbers increased following culture with 250 nM MeP (P<0.001), 1500 nM MeP (P<0.05), and 2 nM BP3 (P<0.05). ICM and total cell number decreased in 2 nM TCS (P<0.05). Embryos cultured in both a low and high concentration of combined EDCs displayed decreased ICM:TE ratios (P<0.001). Blastocysts cultured in 250 nM MeP had an increased outgrowth area compared with control blastocysts (P<0.05;

n=3 cultures, n>38 blastocysts/group), consistent with the increase in TE cell number on day 5. Thus, environmentally relevant concentrations of common EDCs can directly alter *in vitro* preimplantation mouse embryo cell numbers and TE outgrowth. Further studies are required to elucidate the exact mechanism of action and the long-term consequences of EDC exposure of the preimplantation embryo.

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Sphingosine kinases are required for mouse preimplantation embryo development *in vitro*

Monique H Rayhanna[1], Michael B Morris[1], Margot L Day[1]

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

The addition of some growth factors improves the viability of preimplantation embryos *in vitro*. Sphingosine 1-phosphate (S1P) is a growth factor that, when added to culture media, improves oocyte maturation [1,2] and blastocyst formation [2], and decreases rates of apoptosis throughout development [3,4]. However, it is not known whether endogenous S1P signalling is also present in the embryo through activity of sphingosine kinase (SphK), an enzyme that catalyses the production of S1P from its precursor sphingosine.

The current study aimed to determine the expression and function of the two SphK isoenzymes, SphK1 and SphK2, in preimplantation embryos. SphK1 was expressed in the cytoplasm and nucleus at all stages of development, and staining for the phosphorylated or activated form of SphK1 was strongly cytoplasmic. Inhibition of SphK1 by PF543 reduced the percentage of embryos that cavitated and formed blastocysts. SphK2 was also expressed in the cytoplasm and nucleus at all stages of development, though staining at the 8-cell stage was weak. Phosphorylated SphK2 stained strongly in the membrane and nucleus at all stages, and inhibition of SphK2 by ABC294640 reduced embryo development at all stages.

Together, these results suggest that endogenous production of S1P by SphK is necessary for embryo development *in vitro*. Differences in localisation of the phosphorylated SphKs and timing of mortality upon inhibition point to SphK1 and SphK2 activating different signalling pathways to promote development, though these pathways remain to be elucidated. Identifying them would provide better insight into the molecular mechanisms underpinning preimplantation embryo development, and this knowledge could be used to improve *in vitro* embryo culture for clinical, research, and agricultural purposes.

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Differential miRNA profiles in bovine blastocysts produced via IVF and SCNT may explain the lower development rates of cloned embryos

Thiago Damasceno Teixeira[1], Bethany J Finger[2], Boris Novakovic[3], Elizabeth A Kleeman[4], Andrew French[1], Richard Fry[1], Frank Dunshea[1], Mark P Green[2]

1. School of Agriculture, Food and Ecosystem Sciences, University of Melbourne, Parkville 3010, Victoria, Australia

2. School of BioSciences, University of Melbourne, Parkville 3010, Victoria, Australia

3. Murdoch Children's Research Institute, Parkville 3052, Victoria, Australia

4. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville 3010, Victoria, Australia

Somatic cell nuclear transfer (SCNT) has been successfully undertaken in 17 mammalian species, however the efficiency of this technique remains low (less than 5%) for most species, except for cattle, with a live birth rate of up to 15%. The main factor associated with low efficiency has been perturbed nuclear reprogramming of cloned embryos. Since microRNAs (miRNAs) play an important role in cell reprogramming and embryo development, differences in their expression profile may underpin issues with nuclear reprogramming. The aim of this study was to investigate whether differences exist in the miRNA expression profiles of bovine blastocysts produced via IVF compared with SCNT. A total of 120 blastocysts were produced via IVF (n=60) using female sexed semen (n=60; n>3 cultures) or SCNT using a donor female fibroblast cell line (n=60; n>3 cultures) were collected on day 8 of development, total RNA extracted, and small RNA library prep and sequenced conducted on an Illumina NovaSeq™ X system (150bp, paired-ended). Illumina data files were processed, and differential expression analysed using DeSEQ2 on the online Galaxy Australia platform. A total of 268 expressed miRNA were identified. Of these, 20 differed significantly (FDR<0.05), including 9 up-regulated and 11 down-regulated in SCNT compared with IVF embryos. Those miRNAs were associated with different biological processes, including cell proliferation and apoptosis. These findings describe differences in the miRNA profile of IVF and SCNT-derived blastocysts in cattle. Further studies are required to confirm the exact function and effect of these 20 differentially expressed miRNA on embryo development, as these miRNAs may explain the different developmental potential of bovine blastocysts produced by SCNT.

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Somatic *KRAS* mutations are associated with endometriosis lesion subtypes, recurrence and patient age

Jacqueline F Donoghue[1], Meaghan Griffiths[1], Sylvia Beharis[1], Brodie Bilston[1], Leonie Cann[1], Georgina Prewer[1], Martin Healey[2], Peter AW Rogers[1]

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

2. Gynaecology Endometriosis and Pelvic Pain Unit, Royal Women's Hospital, Parkville, Victoria, Australia

INTRODUCTION: Endometriosis is a common gynaecological disease where endometrial tissue implants in locations ectopic to the uterus. Due to somatic mutations occurring in normal endometrial tissue, the question of what impact these mutations have on endometriosis lesion development remains to be answered. Further, *KRAS* mutations induce *PGR* hypermethylation and decreased epithelial cell expression of progesterone receptors leading to reduced progesterone/progestin therapeutic responsiveness. The aim of this study was to characterise the expression of two *KRAS* mutations in eutopic endometrium and endometriosis lesions over time to determine if there is an association between mutation expression, timing of diagnosis, patient age and disease status.

METHODS: DNA was isolated from FFPE tissue sections using the QIAamp DNA FFPE kit and screened using BioRad ddPCR Mutation Detection Assays for $KRAS^{G12C}$ and $KRAS^{G12D}$ and normalised to wildtype *KRAS*. Immunohistochemistry was used on FFPE sections to visualise progesterone receptor (PR) expression on cytokeratin positive glandular epithelium in lesions and curettes.

RESULTS: $KRAS^{G12D}$ was significantly increased compared to $KRAS^{G12C}$in patients with no endometriosis and first-time diagnosed in their 20's. $KRAS^{G12D}$ was also significantly increased over time compared to $KRAS^{G12C}$ in eutopic endometrium and was significantly associated with first time diagnosis in older women. Increased $KRAS^{G12D}$ was also significantly associated with recurrent endometrioma and first-time superficial lesions diagnosis whereas $KRAS^{G12C}$ was significantly associated with recurrent Pouch of Douglas lesions. PR expression in glandular epithelial cells was also significantly reduced in 77% of lesions while 23% of lesions exhibited weak or variable staining.

CONCLUSION: This is the first study of its kind to demonstrate a link between *KRAS* mutations, lesion subtypes, patient age, and timing of disease diagnosis and recurrence. This study also demonstrated a reduction/loss of lesion PR expression, supporting the need for further investigations into *KRAS* mutations as potential therapeutic targets to improve patient outcomes.

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Single-dose cisplatin does not impact endometrial decidualisation

Jordan Higgins[1], Lauren R Alesi[1], Fiona Cousins[2], Amy Winship[1], Karla J Hutt[1]

1. Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia

2. The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

With cancer survivorship at an all-time high, increased attention has been placed on uncovering the off-target side-effects of cancer treatments, like infertility. It is well-established that chemotherapies have profound impacts on ovarian function; however, impacts to the uterus are unclear. An adequately prepared endometrium – essential for pregnancy success – requires complex changes. Decidualisation describes the differentiation of uterine stromal cells into secretory cells; essential for trophoblast invasion, spiral-artery remodelling, and pregnancy establishment. Therefore, the aim of this study was to assess the impact of chemotherapy on decidualisation.

To study the impact of chemotherapy on the uterus independent of ovarian influence, adult female C57BL6/J mice were ovariectomised and treated with a single-dose of cisplatin (5mg/kg) or vehicle control (n=8/group), followed by artificial decidualisation 14 days later. Additionally, decidualisation was induced in a human endometrial stromal cells (tHESC) treated with 70μM cisplatin (IC_{50}) or vehicle control. Uterine-to-bodyweight ratios and qPCR analysis of key markers were used to assess decidualisation.

Decidualisation was induced *in vivo* (control: 75% vs. cisplatin: 88%). Uterine-to-bodyweight ratios were similar between control (0.010±0.003) and cisplatin-treated groups (0.008±0.002), suggesting single-dose cisplatin does not significantly impact decidualisation. No significant differences were observed in the expression of decidualisation markers *Bmp2, Hoxa10, Esr1,* or *Pgr* in uterine tissues. Significant increases in *BMP2* ($p<0.05$), *HOXA10* ($p<0.01$), and *PGR* (<0.001) expression in cultured tHESC demonstrated decidualisation was induced, but no significant differences were observed between untreated and cisplatin-treated cells. Increased γH2AX/TUNEL staining was observed in cisplatin-treated uterine tissues, suggesting elevated levels of DNA damage/apoptosis post-treatment.

Collectively, these data suggest single-dose cisplatin does not impact decidualisation. As women typically receive multi-dose regimens clinically, this should be the focus of future studies. Nevertheless, no available fertility preservation options exist to protect the uterus, thus characterising any impacts of chemotherapy on uterine function must be prioritised.

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Investigating the effects of Triclosan and Methylparaben exposure on endometrial function

Amy R. Shainfeld[1], Bethany J. Finger[1], Wei Zhou[2], Ellen M. Menkhorst[2], Evdokia Dimitriadis[2], Mark P. Green[1]

1. School of BioSciences, University of Melbourne, Parkville 3052 , VIC, Australia

2. Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville 3052, VIC, Australia

Impaired endometrial receptivity is a major bottleneck in pregnancy success and is associated with implantation failure. Exposure to endocrine disrupting chemicals (EDCs) has sparked much interest, due to their widespread use in consumer and industrial products. Triclosan (TSC) and Methylparaben (MeP) are commonly used antimicrobials and preservatives with known

oestrogenic and endocrine disruptor effects. These chemicals are linked with infertile pathologies in both human and domestic species, posing a significant risk factor for endometrial function. Hence, this study aimed to determine if *in vitro* exposure to TSC and MeP would impact endometrial epithelial cell adhesive, proliferation and oestrogen responsive gene expression. To evaluate the functional impacts of EDC exposure, Ishikawa cells were treated for 24 hours with varying environmentally relevant concentrations of TSC or MeP at 0, 10, 250 and 1500nM found in humans. Cells were then subjected to xCELLigence real-time monitoring for 72 hours to investigate changes in their adhesive and proliferative capacity. In separate cultures, qRT-PCR was performed to investigate changes in oestrogen responsive gene targets (*oestrogen receptor alpha, leukemia inhibitory factor receptor, homeobox A cluster and claudin 3*) involved in endometrial remodelling and receptivity. Repeated measures analysis and area under the curve were employed to determine statistical differences in cellular adhesion and proliferation. Non-parametric analyses were used to identify changes in gene expression. No differences in epithelial cell adhesion or proliferation were detected for either chemical (n=3 replicates per chemical per concentration; P>0.1). Equally, no changes in gene expression relative to control were evident (P>0.1) using the current *in vitro* exposure paradigms. Further studies are required to verify these findings by exploring variations in *in vitro* conditions and alternative function measures to better elucidate the effects of chronic MeP and TSC exposure on endometrial function.

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Changes in endometrial prorenin and the (pro)renin receptor across the menstrual cycle and in early pregnancy

Tess L Symington[1, 2], Joshua J Fisher[3, 2], Sarah J Delforce[1, 2], Wei Zhou[4, 5], Evdokia Dimitriadis[4, 5], Kirsty G Pringle[1, 2]

1. School of Biomedical Science and Pharmacy, University of Newcastle, Newcastle, NSW, Australia

2. Mothers and Babies Research Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

3. School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

4. Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria , Australia

5. Gynaecology Research Centre, Royal Women's Hospital, Parkville, Victoria, Australia

The endometrium is remodelled each menstrual cycle in response to hormonal fluctuations, and, to prepare for embryo implantation, transforms into the decidua. Both prorenin and its receptor, the (pro)renin receptor (PRR), initiators of renin-angiotensin system signalling, are abundant within the endometrium and decidua. Prorenin levels are increased upon decidualisation *in vitro*; therefore, we aimed to characterise prorenin and PRR expression across the menstrual cycle and in early gestation decidua, hypothesising they could be involved in both.

Endometrial biopsies were collected from fertile patients during the proliferative (n=5), mid (n=3), and late (n=5) secretory phases. Early gestation decidua (8-17 weeks) was collected from elective terminations of pregnancy (n=4). Prorenin and PRR proteins were localised using immunohistochemistry and staining intensity (H-score) quantified using HALO image analysis software. qPCR was used to determine mRNA expression of prorenin, PRR and markers of decidualisation in early gestation decidua (n=14).

The staining intensity of prorenin in decidual stroma was greater than in non-pregnant, cycling endometrium (P=0.0059), with no changes across the menstrual cycle. Glandular prorenin staining was more intense in mid and late secretory phases compared with the proliferative phase (P=0.042 and P=0.036). Stromal PRR staining intensity did not change across the menstrual cycle, however, was greater in decidua compared with non-pregnant endometrium (P=0.042). Glandular staining for PRR was greater in the mid-secretory phase compared with the proliferative phase (P=0.026). Decidual prorenin and PRR mRNA expression were positively correlated with gestation (8-17 weeks; P<0.001, r=0.65, and P=0.012, r=0.855, respectively), as well as with markers of decidualisation: prolactin (P=0.012, r=0.651 and P=0.0001, r=0.869) and insulin-like growth factor binding protein-1 (P=0.0035, r=0.723 and P<0.0001, r=0.923).

Conclusion:

These findings describe the cyclic nature of the renin-angiotensin system in the endometrium, with the increased prorenin and PRR abundance in the decidua suggesting a potential role in decidualisation.

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Extracellular Vesicles as Biomarkers of Endometriosis

Chloe James[1, 2], Johanna K Farley[1, 2], Natasha Borash[1, 2], Anna Tresso[1, 2, 3], Shima Bayat[2], Anup Shah[4], Joel Steele[4], Ralf Schittenhelm[4], Shanti Gurung[2], Beverley Vollenhoven[1, 2, 5], Thomas T Tapmeier[1, 2, 6]

1. Department of Obstetrics & Gynaecology, Monash University, Clayton, Victoria, Australia

2. The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia

3. University of Trento, Trento, Trentino, Italy

4. Monash Proteomics and Metabolomics Facility, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

5. Women's and Newborn Program, Monash Health, Clayton, Victoria, Australia

6. Endometriosis CaRe Centre, Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, Oxfordshire, United Kingdom

The endometriosis type characterised by intraperitoneal superficial lesions is virtually impossible to detect through ultrasound, and the need for invasive laparoscopy delays diagnosis by 8-10 years. Having shown that peritoneal fluid (PF) of women with

endometriosis contains endometriosis-specific sEV, we now isolated protein from sEV in PF and blood, and tested candidate biomarkers for endometriosis.

Women aged 18-50 years undergoing laparoscopic surgery for endometriosis or unrelated conditions were invited to participate in our study. After informed consent, sEV from matched PF and blood samples were isolated by differential ultracentrifugation, validated by Western Blotting, nanosight tracking analysis (NTA) and transmission electron microscopy (TEM), and analysed through label-based, quantitative proteomics. Data analysis was performed using Proteome Discoverer v2.4 in combination with statistical analysis by R (limma), considering a protein false discovery rate of 1% and a quantitative threshold of adjusted p-value <0.05.

We included 25 paired samples (n=11 controls, n=14 endometriosis) in the proteomics cohort. sEV identified by TEM showed a mode size of 121.8±18.0 nm (blood, n=5) and 155.9±37.2 (PF, n=6), and contained syntenin, ALIX, CD9, CD63 and CD81. Proteomics identified 7064 protein groups, with 3408 proteins consistently quantified across sample groups. Of these, 602 were found to differ significantly across all comparisons (P_{adj}<0.05). In PF, 533 proteins changed significantly in abundance, while in blood, four proteins changed in abundance, with proteinase 3 (PRTN3), the receptor of IL-32, significantly downregulated in blood-derived sEV from endometriosis patients. We are now validating the marker in a new cohort (n=21 controls, n=11 endometriosis to date).

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Rodent models of Autoimmune Thyroiditis: insights to impaired fertility and pregnancy success

Rebecca Brady[1], Nykola Kent[1], Anna Reid[1], James Cuffe[1]

1. University of Queensland, West End, QLD, Australia

Aims: Autoimmune thyroiditis (AIT) is a common cause of thyroid disease, characterised by auto-antibodies targeting proteins of the thyroid gland. AIT impact 1 in 5 women and is strongly associated with infertility and reproductive complications. However, there is limited research looking into how thyroid antibodies lead to reproductive dysfunction. Our laboratory has established two models of AIT to investigate how fertility is impacted by the two major thyroid antibody types, thyroglobulin-antibodies (TGAb) and thyroperoxidase antibodies (TPOAb)**.**

Methods: For our model of TGAb positivity, Lewis rats were provided sodium iodide in their drinking water (n=10 per group) and were injected with porcine thyroglobulin (2ng/ml) and Freund's adjuvant. For our TPOab model, Sprague Dawley rats were provided standard drinking water and injected with recombinant TPO protein (0.6ug/ul) and Freund's adjuvant. Control rats in each model received standard drinking water and were injected with vehicle controls. Estrous cycling was monitored using vaginal electrical impedance and cytology. Plasma was collected to assess thyroid antibody and hormone levels. Animals were mated and culled at embryonic day 20.

Results: Plasma TGAb was increased in AIT rats compared to controls and was associated with increased thyroxine without changes to TSH. TGAb impaired estrous cyclicity and fertility. Thyroglobulin-antibodies did not impact litter size but did cause reduced male fetus survival. Data collection from the TPO model is ongoing.

Conclusions: The findings from these models demonstrate that even a modest elevation in thyroid antibodies can lead to changes in fertility and reproductive health. These findings highlight the importance of using rodent models to investigate underlying pathophysiological causes impacting reproductive health.

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The inter-generational effects of genome-wide genomic instability on fertility

Jessica M Stringer[1], Stevan Novakovic[2], Elissah Granger[2], Caitlin Harris[2], Vanessa Tsui[2], Karla Hutt[1], Eva R Hoffmann[3], Davis J McCarthy[4], Wayne Crismani[2]

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

2. DNA Repair and Recombination Laboratory, St Vincent's Institute of Medical Research, Fitzroy, VIC, Australia

3. DNRF Center for Chromosome Stability, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

4. Bioinformatics and Cellular Genomics, St Vincent's Institute of Medical Research, Fitzroy, VIC, Australia

Fanconi anemia (FA) is a complex autosomal genetic disorder characterized by bone marrow failure, congenital defects and cancer predisposition resulting from the inability to repair DNA interstrand cross-links and increased genomic instability. Female FA patients have reduced fertility which typically manifests as premature menopause by their early 30s. Gametogenesis is heavily perturbed in *Fancm* loss-of-function mice (*Fancm*$^{-/-}$), consistent with the reproductive defects reported in humans with biallelic *FANCM* mutations. Despite the gametogenesis phenotypes in *Fancm*$^{-/-}$ mutants, both sexes are capable of producing offspring. In humans approximately 15% of female FA patients carry healthy babies to term. Importantly, we can mirror this stochastic and premature menopause fertility phenotype in our mouse models, by inbreeding the same *Fancm*$^{-/-}$ mutation onto two different mouse backgrounds (C57BL/6J and FVB). In the C57BL/6J mice a stochastic infertility phenotype was observed in the *Fancm*$^{-/-}$ female mice compared to wildtype controls (wildtype 10/10 vs *Fancm*$^{-/-}$ 2/5 females had multiple litters). However, the FVB *Fancm*$^{-/-}$ female mice have fewer litters (wildtype 4.57±1.9 vs *Fancm*$^{-/-}$ 2.44±1.0, P<0.01) and a significant reduction in age (days) at last litter (wildtype 174.7±63.3 vs *Fancm*$^{-/-}$ 116.3±25.6, P<0.02) compared to controls, indicative of premature menopause observed in female FA patients. When we cross the two inbred lines together we recover fertility to wildtype levels, however litter number, size and age at last litter diminished over subsequent generations (F3 and F4) of breeding in the *Fancm*$^{-/-}$ hybrid line compared to wildtype controls. As *Fancm*$^{-/-}$ results in a genome-wide increase in meiotic crossover frequency, responsible for shuffling the C57BL/6J and FVB genomes, we will use whole genome sequencing of

hybrid F4 parents and F5 offspring to map meiotic crossover sites and identify mutations associated with reduced fertility. This analysis will provide novel biomarkers for infertility and premature menopause relevant for FA patients and women in general.

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Exploration of responsible sequence for aberrant hypermethylation at maternal *H19*-ICR and BWS-like phenotypes in mice

Satoshi Hara[1], Fumikazu Matsuhisa[2], Shuji Kitajima[2], Hitomi Yatsuki[1], Musashi Ichimaru[1], Ken Higashimoto[1], Hidenobu Soejima[1]

1. Division of Molecular Genetics and Epigenetics, Department of Biomolecular Sciences, Saga University, Saga, SAGA, Japan

2. Division of Biological Resources and Development, Analytical Research Center for Experimental Sciences, Saga University, Saga, Japan

Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder with complex and diverse phenotypes, including overgrowth. BWS is caused by aberrant hypermethylation of imprinting control region within *IGF2-H19* locus (*H19*-ICR) on the maternal allele, which is normally unmethylated. Although mutations in the binding site of CTCF or OCT4 on the maternal *H19*-ICR cause aberrant hypermethylation and result in BWS, its molecular mechanism is not fully understood. In particular, the responsible sequence(s) for aberrant hypermethylation inducing BWS-like overgrowth, has not been identified in mice. To address this, we generated 10 strains of mutant mice harboring SOX2-OCT4 binding site (SOBS) and/or four CTCF binding sites (CTS1-4) in *H19*-ICR, either solely or simultaneously. We found that double mutations of SOBS/CTS3 or SOBS/CTS4 showed aberrant hypermethylation of the entire *H19*-ICR, biallelic expression of *Igf2*, decreased expression of *H19*, and overgrowth. On the other hand, CTS3/CTS4 double mutant mice showed limited hypermethylation to the regions encompassing CTS3, CTS4 and SOBS, and exhibited less frequent overgrowth compared to the SOBS/CTS3 mutants. Other mutant mice did not show significant changes in DNA methylation except at the mutated site(s), and their body weights were in normal range. Additionally, the accumulation of CTCF and RAD21 on CTS1-4 was significantly reduced in SOBS/CTS3 mutants, but did not change in SOBS/CTS4 or CTS3/CTS4 mice. These findings suggest that SOBS and CTS3 are essential for maintaining the unmethylated state of the maternal *H19*-ICR in mice.

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Placental gene function and maternal circulating microRNA levels related to gestational diabetes mellitus severity

Hasini Rathnayake[1], Mariama Kamara[1], Fabricio Da Silva Costa[2, 3], Avinash Kundar[1], Indu Singh[1], Olivia Holland[1]

1. School of Pharmacy and Medical Sciences, Griffith University, Gold Coast , Queensland, Australia

2. Maternal Fetal Medicine Unit, Gold Coast University Hospital, Gold Coast, Queensland, Australia

3. School of Medicine and Dentistry, Griffith University, Gold Coast , Queensland, Australia

Gestational diabetes mellitus (GDM) is a common pregnancy complication that affects maternal and perinatal health. Maternal blood glucose levels increase at ~24 gestational weeks as part of normal pregnancy but are increased pathologically in GDM. GDM is initially treated with diet, with medication indicated in more severe cases. GDM pathology and severity may be related to placental stress response, however, it is not known how maternal and placental systems interact in GDM pathophysiology. Gene expression can be downregulated by microRNAs, therefore we investigated whether placental stress response-related gene expression could be related to maternal microRNAs. Gene expression (84 genes of interest related to cell stress) and microRNAs (800 untargeted) were measured in placenta tissue (≥37 gestational weeks) and maternal plasma (24–28 gestational weeks), respectively, from uncomplicated, GDM-diet treated (GDMD), and GDM-medicated (GDMM) pregnancies (n=8/group). MicroRNA gene regulation was predicted via TargetScan. Eight genes (*CYP2C19, CYP2C9, HMOX1, ACADSB, FMO4, CAT, CRYAA, HSPD1*) were significantly downregulated in GDMM compared to GDMD pregnancies (fold regulation -1.54 to -2.99), and these genes were also upregulated in GDMD, and downregulated in GDMM, compared to uncomplicated pregnancies, respectively. Five microRNAs (miR-126-3P, miR-1322, miR-2117, miR-320C, miR-502-5p) were significantly upregulated in GDMM compared to GDMD pregnancies (fold change 0.59 to 0.73), and three of these microRNAs (miR-2117, miR-1322, miR 502-5p) are predicted to regulate four of the downregulated placental genes (*CYP2C19, ACADSB, FMO4, HSPD1*). Downregulated genes have roles in fatty acid metabolism (*CYP2C19*), blood glucose levels (*ACADSB*), oxidative and xenobiotic metabolism (*FMO4*), and mitochondrial function (*HSPD1*). Maternal circulating microRNAs dysregulated at 24–28 gestational weeks may influence placental gene expression and pathophysiology related to GDM severity. Measurement of these microRNAs at the time of GDM diagnosis at ~24 gestational weeks may be useful in risk prediction for medication necessity.

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Visualising LINE-1 retrotransposon expression dynamics in female and male germ cell development

Stephanie Workman[1], Sandra R Richardson[1]

1. Mater Research Institute, Brisbane, QLD, Australia

The autonomous retrotransposon Long Interspersed Element 1 (LINE-1 or L1) comprises approximately 17 and 18% of the human and mouse genomes respectively. Utilising the 'copy and paste' mechanism of retrotransposition, a subset of L1 elements have retained the ability to insert new copies of themselves throughout the genome. Doing so results in mutation at insertion sites that can be deleterious to genomic function and stability, sometimes contributing to isolated cases of disease in humans. To successfully pass their genetic information onto the next generation and beyond, retrotransposition competent L1s

must generate new insertions in pluripotent embryonic cells prior to germ line specification, or in cells of the germ lineage. DNA methylation of the L1 5'UTR internal promoter is a major determinant of L1 expression. In the mouse germ line, L1 DNA methylation dynamics differ dramatically between males and females. It therefore stands to reason that the developmental timing of heritable L1 retrotransposition events likewise differs between the sexes.

In this project, we are undertaking a systematic characterisation of sex-specific L1 expression dynamics during male and female germ cell development in the mouse. To characterise L1 mRNA expression with cell type specificity and subcellular resolution, we are utilising RNAscope single-molecule RNA fluorescence in situ hybridisation with a probe targeting the L1 TF 5'UTR (Bodea et al. 2022) alongside probes for germ cell specific genes. This method will produce a highly detailed picture of sex-specific LINE-1 expression with spatial and temporal resolution. Our results will provide insight into L1 activity in the germline and inform our understanding of sex-specific L1 mutagenesis.

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Defining the immune cell profile of the mouse seminal vesicle

Shanu Parameswaran[1, 2], Jemma Mayall[3], Fengyun Sun[4], Amanda L Anderson[1, 2], Brett Nixon[1, 2], Sarah A Robertson[5], Oliver J Rando[4], John E Schjenken[1, 2]

1. Infertility and Reproduction Research Program. Hunter Medical Research Institute, Hunter Medical Research Institute and School of Environmental and Life Sciences. University of Newcastle, Australia, New Lambton Heights, New South Wales, Australia

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

3. Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute and The University of Newcastle, New Lambton Heights, NSW, Australia

4. Department of Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, Worcester, MA, USA

5. Robinson Research Institute and School of Biomedicine, University of Adelaide, Adelaide, SA, Australia

Among the male accessory sex glands, the seminal vesicles and their secretions are the chief contributor to seminal plasma in all mammalian species. The bioactive factors secreted by the seminal vesicles play a pivotal role in supporting sperm survival and function, and modulate the female reproductive tract to facilitate implantation, thereby influencing developmental programming of offspring phenotype. However, there is little information on how seminal vesicles regulate synthesis and secretion of such factors. Given immune cells regulate secretory activity in other mucosal surfaces, this study aimed to assess immune cell profiles in the mouse seminal vesicles. To achieve this, we initially mined a recent high-throughput single-cell RNA sequencing dataset of adult mouse seminal vesicle tissue to identify immune cell populations that reside in this tissue. Immune cells identified from this analysis were then confirmed by flow cytometry and immunofluorescence using seminal vesicle tissue from adult Swiss mice. Analysis of single cell RNA-sequencing data identified that immune cells residing within mouse seminal vesicles primarily consist of macrophages, as well as dendritic cells, T cells, and natural killer (NK) T cells. Flow cytometry and immunofluorescence assessment confirmed these findings with the most prevalent immune cell population being F4/80+ macrophages (40% of CD45+ cells), followed by NKP46+ natural killer (NKP46+, likely NKT cells, 35% of CD45+ cells), CD4+ T cells (8% of CD45+ cells), and CD11c+F4/80- dendritic cells (6% of CD45+ cells). Interestingly, populations of F4/80+ macrophages were found in close proximity to epithelial cells, highlighting their potential to influence secretory function as occurs in other mucosal tissues. Our current studies will further characterise the macrophage subtypes present in seminal vesicles. Together, these data provide a resource for investigating immune cell activity in seminal vesicles and may identify novel immune cell subtypes that influence seminal vesicle secretory activity.

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Quantification of mononuclear phagocyte populations in the mouse epididymis: regulation by activin and effects of sexual maturation

Rama Ravinthiran[1], Rosemary Genovese[1], Rukmali Wijayarathna[1], Kate L Loveland[1], Andreas Meinhardt[2], Mark P Hedger[1]

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

2. Institute of Anatomy and Cell Biology, Justus-Liebig University Giessen, Giessen, Germany

The immunological environment of the epididymis constitutes a functional gradient from the caput through to the cauda, which correlates with regional differences in macrophage populations. The caput is dominated by resident macrophages with primarily immunoregulatory/anti-inflammatory properties that express the chemokine receptor CX3CR1 and the pan-macrophage antigen F4/80. In the cauda, macrophages lacking CX3CR1, with a pro-inflammatory phenotype, are more prominent. Since production of the immunoregulatory cytokine activin A is highest in the caput and lowest in the cauda, the relationship between activin and macrophage gradients was examined. Mice expressing a fluorescent transgene (*Gfp*) at the *Cx3cr1* locus were crossed with mice heterologous for activin A (*Inhba*$^{+/-}$) to produce transgenic littermates with either normal or low levels of activin A. Macrophage subsets were quantified by established stereological methods in adult and immature (56 and 25 day-old) mice by expression of CX3CR1-GFP and immunolocalisation of F4/80. Double-positive CX3CR1^{high}F4/80^{+} macrophages were the largest epididymal subset. In the adult, their volume density and number was highest within the duct epithelium and interstitium of the caput (30-40,000 cells/mm^3), and was significantly lower (15-20,000 cells/mm^3) in the corpus and cauda. This subset was not substantially altered in activin-deficient mice. CX3CR1^{null}F4/80^{+} macrophages were predominantly localised to the interstitium, with higher volume density in the corpus and cauda (10-15,000 cells/mm^3) than in the caput (4-5000 cells/mm^3). In

mice with reduced activin, the number of these cells increased approximately 2-fold within the interstitium in all regions. In the immature epididymis, however, the volume density of both macrophage subsets was evenly distributed across all regions. These data indicate that the differential macrophage gradient becomes established after sexual maturation and appearance of mature sperm. Activin suppresses the development or maintenance of the $CX3CR1^{null}$ pro-inflammatory population throughout the epididymis, but has no apparent effect on the number of $CX3CR1^{+}$ immunoregulatory/anti-inflammatory macrophages.

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Therian ancestry of INSL3 mediated testicular descent in mammals

Brandon R Menzies[1], Stephen R Frankenberg[1], Andrew J Pask[1]

1. The University of Melbourne, Vic, Australia

Descent of the testes from a position near the kidneys into the lower abdomen and/or scrotum during development is a fundamental process for most mammals that has been linked to improved testicular function. The INSL3 ligand and its cognate receptor (LGR8/RXFP2) are key to the gubernaculum-driven relocation of the testes from the kidneys to the lower abdomen with functional forms of their encoded genes located in conserved regions of the genomes of a range of eutherian mammals. However, the role of INSL3 in testicular descent in marsupials is not clear, and is important given the different mechanism of scrotum determination (X-linked determination) in marsupials compared to eutherians. We have compared functional forms of *INSL3* and *LGR8* from a broad range of marsupial orders, including Microbiotheridae, Dasyuromorphia, Notoryctemorphia and Diprotodontia, and show testicular expression and localisation of these genes during development in an Australian marsupial, the dunnart (*Sminthopsis crassicaudata*). Taken together, these data show that INSL3/LGR8-mediated testicular descent is a common feature of marsupials, all of which have either partial or completely descended testes. Thus, the genetic mechanism mediating this fundamental trait evolved in therian mammals and suggests that testicular descent, at least to the abdominal wall, was an ancestral feature of the group.

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The generation of a sperm-specific miR-30a/c2 overexpression mouse model for understanding paternal programming

Oluwabunmi I Olatunji[1, 2, 3], Hannah E Lyons[1, 3], Victoria Nikitaras[1, 3], Mark Nottle[1, 3], Nicole McPherson[1, 2, 3]

1. University of Adelaide, Adelaide, SA, Australia

2. Freemasons Centre for Male Health and Wellbeing, The University of Adelaide, Adelaide, South Australia, Australia

3. Robinson Research Institute, The University of Adelaide, Adelaide, South Australia, Australia

Sperm specific microRNAs (miRs) delivered at fertilisation are causative mediators in paternal programming. MiR-30 is one of the most highly abundant miRs in sperm and expression levels are modified in circumstances of obesity, stress, heat, and infertility. MiR-30 have been shown to be a causative mediator in the transmission of paternal stress. However, current studies determining sperm miR-30 post fertilisation have occurred following synthetic microinjections, which lack important post transcription modifications acquired during testicular and epididymal transit that provide protection from scheduled RNA degradation post fertilisation. To overcome these limitations, we aimed to create a genetically modified mouse model that overexpressed miR-30a/c2 in sperm, without overtly disrupting spermatogenesis or sperm function.

Testes-specific CCNA1-EGFP-miR-30a/c2 construct were microinjected into zygotes and transferred into pseudo-pregnant females. Pups were screened by PCR for integration of CCNA1-EGFP construct to confirm inheritance and breeding maintained through the female line. Male offspring body composition (N=8) (total body weight, liver, kidneys pancreases, seminal vesicles etc.) sperm function (sperm count and motility via CASA) and testicular histology were assessed. The abundance of sperm miR-30a/c2 were assessed through TaqMan qPCR (N=4) following Trizol total RNA extraction from frozen sperm pellet.

Male mice carrying the transgene were of normal weight and had similar body composition to those of age-matched wildtype males ($P>0.05$). No changes were seen in sperm motility, sperm concentration, or testicular morphology between transgenic and wildtype mice ($P>0.05$). We observed a 10-fold increase in sperm miR-30a and miR-30c2 abundance in transgenic mice compared with wildtype ($P<0.001$ and $P<0.01$ respectively).

Over expression of miR-30a/c2 in the testes, resulted in increased expression in sperm without modifying reproductive traits or body composition. Our mouse model, overexpressing miR-30a/c2 in sperm can be utilized as a research model for recreating normal physiological responses seen in aberrant paternal programming.

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Sub-chronic elevation of ambient temperature differentially alters male reproductive function depending on the duration of exposure

Shannon P Smyth[1, 2, 3], Natalie A Trigg[1, 2, 4], John E Schjenken[1, 2], Jacinta H Martin[1, 2], Amanda L Anderson[1, 2], Ilana R Bernstein[1, 2], David A Skerrett-Byrne[1, 2], Brett Nixon[1, 2], Elizabeth G Bromfield[1, 2, 3]

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

2. School of Environmental and Life Sciences, College of Engineering, Science and Environment, University of Newcastle, Callaghan, NSW, Australia

3. School of BioSciences, Faculty of Science, Bio21 Institute, University of Melbourne, Parkville, VIC, Australia

4. Departments of Genetics and Pediatrics, University of Pennsylvania Perelman School of Medicine and Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA

Extreme weather events associated with changes in climatic behaviour, including severe drought and heatwaves, are increasing in frequency, intensity, and duration. Despite compelling evidence that such weather events pose a potential risk to the reproductive capacity of ecologically and agriculturally significant animal species, our understanding of the impact of sub-chronic whole-body exposure to elevated temperatures remains limited. To address this, here we exposed male mice to heat stress conditions that mimic a prolonged heatwave (daily cycle of 8h at 35°C followed by 16h at 25°C) for a period of seven days. Neither the testes nor epididymides of heat-exposed mice exhibited gross histological changes or displayed significantly altered oxidative DNA damage, single-strand DNA lesions or apoptotic markers. Furthermore, spermatozoa of exposed males retained comparable functionality to sham controls and supported fertilisation and subsequent embryonic development. Intriguingly, however, the embryos sired by heat-exposed males experienced pronounced changes in gene expression linked to an acceleration of early embryonic development, aberrant blastocyst hatching and macrosomia. These data eloquently reveal the importance of stress signals encountered by sperm during transit through the epididymal environment and their role in modulating offspring outcomes. To expand on these findings, an extended fourteen-day exposure model was utilised to assess the impacts of sub-chronic heat stress when applied during both spermatogenesis and post-testicular sperm maturation. Preliminary data revealed a marked increase in TUNEL staining in the testes, and unexpectedly, an accumulation of immune cells within the epididymal lumen of heat-exposed males. In contrast to the seven-day model, spermatozoa from fourteen-day exposed males displayed significantly impaired motility and reduced viability compared to shams and age-matched controls. It is yet to be determined whether these changes impact offspring outcomes. Together, these in vivo models provide a new platform to extensively investigate the mechanisms through which increased ambient temperature alters male reproductive capacity.

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Exogenous oestrogen exposure causes cytoskeletal remodelling in a human testis cell line

Melanie K Stewart[1], Pascal Bernard[1], Ching-Seng Ang[1], Deidre M Mattiske[1], Andrew J Pask[1]

1. University of Melbourne, Parkville, VIC, Australia

The recent decline in male fertility has been linked to the increasing prevalence of endocrine disrupting chemicals that can interact with native oestrogen receptors to affect downstream signalling. There is a critical role for the cytoskeleton in facilitating spermatogenesis and Sertoli cell function, suggesting this could be a key target of oestrogenic EDCs to impact male fertility. We previously demonstrated that in human testis-derived NT2/D1 cells, exogenous oestrogen limits the bioavailability of the key testis factor SOX9 through the rapid stabilisation of microtubules, leading to a shift towards expression of ovarian developmental pathways. To further understand how oestrogen regulates the microtubule cytoskeleton, as well as actin and intermediate filaments, we performed proteomic and phosphoproteomic analyses. We show that oestrogen exposure leads to increased abundance of tubulin and hypophosphorylation of microtubule associated proteins, increased abundance of vimentin, and the rapid activation of the actin polymerising ARP2/3 complex. Together, these results demonstrate oestrogen treatment can target all three components of the cytoskeleton. Given how important the cytoskeleton is for spermatogenesis and Sertoli cell determination and function, these results provide a mechanism of how exogenous oestrogen could directly impact increasing rates of male infertility.

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Targeting female fertility in invasive vertebrate pests using gene drives

Stephen Frankenberg[1]

1. University of Melbourne, Parkville, VIC, Australia

Invasive vertebrates pests continue to have devastating impacts on biodiversity and agricultural productivity in both Australia and New Zealand. Gene drive technology has emerged as a potentially powerful tool for suppressing pest populations. By targeting genes for female fertility or development, suppression gene drives are more humane than conventional methods for pest control such as baiting, trapping and shooting. Our laboratory aims to demonstrate the feasibility of suppression gene drives through two parallel research endeavours. First, we are using the zebrafish as a vertebrate model to optimise the design of gene drives for their efficient propogation through a target population. Second, we are developing pipelines for producing animals of non-model vertebrate species (including fishes, amphibians and mammals) with targeted insertions of large DNA fragments using CRISPR.

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Development of an ESR1 yeast bioassay for the assessment of Phyto-oestrogen interactions in kākāpō

Amey Hughes[1], Daniel Harrington[2], Simon Hinkley[2], Janet Pitman[1]

1. Victoria University of Wellington, Wellington, New Zealand

2. Ferrier Research Institute, Victoria University of Wellington , Wellington

Kākāpō (Strigops habroptilus) are a critically endangered parrot that is endemic to Aotearoa, New Zealand (NZ). The Kākāpō Recovery Team, Department of Conservation and local iwi have brought this species back from the brink of extinction to ~250 individuals which reside in predator-free off-shore sanctuaries.

Kākāpō breed intermittently every 2-5 years, coinciding with the fruiting of *Dacrydium cupressinum (*NZ rimu) but the mechanistic link remains a mystery. One hypothesis is that phytoestrogens in rimu fruit bolster endogenous oestrogen levels, which act upon oestrogen receptors (ESR1) in the liver to stimulate egg yolk protein production leading to ovarian follicle maturation1. We have identified an amino acid indel in the ligand-binding domain of ESR1 of kākāpō that may increase their sensitivity to phytoestrogens2 and have obtained preliminary data of oestrogenic activity in crude rimu fruit extracts3.

The study aims were to 1) identify mutations within the ESR1 genes of individual Kākāpō and 2) assess the oestrogenic activity in extracts of rimu.

Mutations within the *ESR1* gene of 171 individual Kākāpō were examined using the Kākāpō125+ database. Despite single nucleotide polymorphisms (SNPs) being present within introns of most individuals, only one SNP (TCC<TCG) was present in the coding region in 33 individuals but did not result in an amino acid change (S<S).

The oestrogenic activity of rimu fruit, seeds and unfertilised ovules extracted in range of solvents (methanol, chloroform, acetone and water) were examined using a yeast bioassay transfected with human *ESR1*. All rimu specimens showed some oestrogenic activity, water-based extracts exhibiting the highest activity.

This study revealed no functional mutations in *ESR1* that may impair an individual's breeding success. Also the presence of rimu-derived phytoestrogens in aqueous extracts mirrors the birds eating habit of crushing the rimu fruit and discarding the chews supporting a physiological role of phytoestrogens in kākāpō.

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GM-CSF during *in vitro* oocyte maturation increases glucose uptake in oocyte

Anmol Saini[1, 2], Mark Nottle[1, 2], Nicole McPherson[1, 2, 3]

1. Discipline of Reproduction and Development, School of Biomedicine, University of Adelaide, Adelaide, South Australia, Australia

2. Robinson Research Institute, University of Adelaide, Adelaide, South Australia, Australia

3. Freemasons Centre for Male Health and Wellbeing, University of Adelaide, Adelaide, South Australia, Australia

We have previously shown that the addition of Granulocyte-macrophage colony-stimulating factor (GM-CSF) during *in vitro* oocyte maturation (IVM) can increase mitochondrial activity and reduce the level of reactive oxygen species (ROS) in cumulus-oocyte complexes (COCs). The present study was undertaken to determine if the addition of GM-CSF during IVM increases glucose uptake in COCs. C57Bl6 x CBA F1 female mice aged 21-23 days were injected IP with 0.1 ml of eCG and COCs aspirated from large antral follicles 46-48 h post-injection. COCs were placed in HEPES alpha-MEM media supplemented with 0 and 10 ng/ml of GM-CSF with 0.3mM of 6 NBDG. COCs were incubated for 0, 15, 30, 60 and 90 mins at 37 C in 6%CO_2, 5%O_2, and 89% N_2. Following incubations COCs were washed in G-MOPS (Vitrolife) and then loaded in groups of five onto glass slides for imaging. Glucose uptake in COCs was measured at excitation and emission wavelength of 488nm using The Cell Voyager CV1000 Confocal Scanner (Yokogawa) with Z-stack imaging and analysed using Fiji Image J software. The experiment was replicated three times with 25-28 oocytes per group. Data were normalised with logarithmic transformation and assessed using univariate general linear models with Bonferroni post hoc test using IBM SPSS. Adding GM-CSF during IVM increased glucose uptake at 15 minutes by 9.26 % ($P<0.001$) compared with control. While at 30 and 60 minutes there was an increase in uptake in the control group by 3.1% ($P < 0.05$) and 4.0% ($P<0.01$) respectively. No effect was observed at 90 minutes in both groups suggesting uptake had reached maximum levels. In conclusion, we have shown that adding GM-CSF during IVM increases the glucose uptake rate, which may contribute to the increase in mitochondrial activity and reduction in ROS levels reported previously.

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Study on domain-dependent regulation of follicle development by insulin-like growth factor binding proteins

Mitsuki Miyajima[1], Motoyuki Shimonaka[1]

1. Tokyo University of Science, Shinjuku-ku, Tokyo, Japan

An Insulin-like growth factor (IGF) is one of the regulators of development of ovarian follicles and its effects are thought to be regulated by several IGF-binding proteins (IGFBPs) at different stages of follicle development. IGFBPs contain an IGF-binding domain on the N-terminal side, and a thyroglobulin-type 1 (Tg-1) domain on the C-terminal side. Although the primary function of IGFBPs is to control the availability of IGF through their binding, the question has arisen whether Tg-1 domain, whose function is unknown, affects follicle development in any way. In this study, we investigated which domains are involved in follicle development at different stages by using recombinant proteins.

Five recombinant IGFBPs (1, 2, 4, 5, 6) and two chimeric proteins composed of N-terminal half of IGFBP 1 and C-terminal half of IGFBP 2 (IGFBP 1/2) and inversed order (IGFBP 2/1) were made by an E. coli expression system. To see the effects on follicle development, these recombinant proteins were added to cultures of granulosa cells or follicles in the presence and absence of FSH.

As a result, IGFBPs 1 and 5, but not 2, 4, and 6, additively promoted granulosa cell proliferation with FSH. To compare their effects on follicle development, we selected IGFBPs 1 and 2, which have different effects on granulosa cells, and found that

IGFBP 2 promoted follicle development more than IGFBP 1. To clarify the role of each domain, we compared the effects of IGFBP 1/2 and 2/1 on granulosa cell proliferation and follicle development. As a result, IGFBP 2/1 showed an additive effect with FSH on granulosa cell proliferation and also promoted follicle development. Taken together, it is suggested that IGFBPs regulate follicle development by controlling the availability of IGF via N-terminal domain, in concerted with by controlling granulosa cell proliferation via C-terminal Tg-1 domain.

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Clearing the slate: optimising and assessing the use of optical-clearing technologies in experimentally quantifying the ovarian follicle pool

Elyse O K Swindells[1], Jessica M Stringer[1], Karla J Hutt[1]

1. Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

Accurate assessment of follicle number in mouse ovaries is essential for evaluating the impact of endogenous and exogenous insults on female fertility. The current gold standard for counting follicles is stereology, which is an expensive, time consuming, 2D, section-based histological technique that provides only limited information about the spatial organisation of follicles in the 3D ovary. To overcome these issues, our long-term goal is to develop a fast and accurate automated method to evaluate follicle number and 3D location in whole mouse ovaries. The aim of this pilot study was to determine if follicles could be fluorescently-labelled, imaged and counted in whole ovaries with normal follicle numbers, and following follicle depletion. Female post-natal day 20 C57/Bl6 mice received 2 weekly doses of cyclophosphamide (150mg/kg, n=1) to deplete follicles, or saline (n=2) to retain normal follicle numbers. Ovaries were fixed (4% paraformaldehyde) and optically cleared using the aqueous-immersion-based CUBIC method. Oocytes were labelled by incubating ovaries with anti-cKit and anti-Vasa antibodies, followed by AlexaFluor™-647 and -568 conjugated secondary antibodies, respectively, with DAPI for nuclear staining. Ovaries were imaged via the 10x objective on a Leica Stellaris5 standard confocal and a 3i Mariannas Spinning Disk confocal microscope, then Imaris software was used for image analysis and follicle counting. Follicles at all stages of development could be clearly easily visualised, throughout the entire depth of the ovary (~1.5mm), with fewer follicles present in the ovary exposed to cyclophosphamide compared to the saline treated ovaries. After wholemount analysis, cleared ovaries were re-fixed in Bouin's, embedded in resin, and every 3rd 20µm section stained with PAS for stereology. Remarkably, tissue morphology was excellent and stereology is underway to determine if follicle numbers obtained from whole ovaries are comparable to the gold-standard. This study provides preliminary evidence indicating that follicle counting in whole ovaries is feasible.

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Ingenuity Pathway Analysis reveals novel pathways in adult mouse Leydig cells that are altered by *in vivo* LHCGR activation.

Liza O'Donnell[1], Anne-Louise Gannon[2], Ben Lawrence[2], Isabelle Stevant[3], Pauline Sararols[3], Serge Nef[3], Diane Rebourcet[2], Lee B Smith[1]

1. Griffith University, Southport, Queensland, Australia

2. The University of Newcastle, Callaghan, NSW, Australia

3. University of Geneva, Geneva, Switzerland

Steroidogenesis in adult Leydig cells (ALC) is stimulated by luteinising hormone receptor (LHCGR) activation. Many studies have investigated LHCGR activation in ALC but most use models that do not recapitulate the *in vivo* interstitial ALC niche which includes direct contacts with macrophages. To address this, we analysed ALC transcription during acute LHCGR activation *in vivo* and identified the transcriptional pathways that are regulated. Highly purified mouse ALC were isolated[1] after *in vivo* administration of vehicle or LHCGR agonist (human chorionic gonadotrophin, hCG) for 2 or 6 hours (h). RNASeq identified differentially expressed genes (DEG) altered by hCG and bioinformatic analyses including Ingenuity Pathway Analysis® (IPA) mapped the functional responses of ALC. Most known pathways associated with LHCGR activation[2] were altered by 2h. IPA revealed previously unrecognised regulators and pathways responsive to LHCGR activation. hCG acutely altered the production of, or response to, various cytokines. For example, IL6 was highly stimulated, and IL6-responsive DEGs were enriched in extracellular proteins that likely mediate interactions with interstitial macrophages. Novel LHCGR transcriptional targets were identified, such as activation of HIF1A-mediated transcription involving repression of its negative regulator, CITED2. Crosstalk between LHCGR and estrogen receptor signalling was also identified. hCG induced acute changes in genes encoding TGFβ family receptors, integrins and focal adhesion complexes, suggesting cell-cell interactions within the niche are integrally linked to ALC steroidogenic function. Acute stimulation of LHCGR downregulated the glucocorticoid receptor (GR) which mediates transcription of many genes important for ALC function, suggesting that steroidogenesis requires repression of a subset of GR-dependent transcription, possibly explaining why both excess and reduced GR signalling suppress steroidogenesis. This study reveals novel pathways in steroidogenically-active ALC that could be exploited for the design of therapeutic strategies to support ALC androgen production.

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The Role of Mitochondrial Complex I in Placental Development and Fetal Growth Restriction

Siddharth Acharya[1, 2], Veronica B Botha[1, 3], John E Schjenken[2, 4], Roger Smith[1, 5], Joshua J Fisher[1, 5]

1. Mothers and Babies Research Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

2. School of Environmental and Life Sciences, The University of Newcastle, Newcastle, NSW, Australia

3. School of Biomedical Sciences and Pharmacy, The University of Newcastle, Newcastle, NSW, Australia

4. Infertility and Reproduction Research Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

5. School of Medicine and Public Health, The University of Newcastle, Newcastle, NSW, Australia

Aims

Fetal growth restriction (FGR) is a pregnancy complication where the fetus fails to reach its pre-determined genetic potential, impacting the health trajectory for the lifespan of the fetus by programming lifelong disorders (1). One suggested mechanism behind FGR is placental insufficiency, underpinned by mitochondrial dysfunction within the placenta. Mitochondrial respiratory complex I (NADH dehydrogenase), is the first and largest protein complex of the electron transport chain, with a critical role in ATP synthesis. Dysregulation of complex I proteins alters the efficiency of the electron transport chain, resulting in bioenergetic insufficiencies (2). Such dysregulation may alter placental implantation, invasion, and vascularisation (3). We aimed to characterise the expression of complex I proteins between healthy and FGR placentas, to assess mitochondrial functionality in placental development.

Methods

Placentas from healthy full term (n=7) and FGR pregnancies (n=7) were sampled and prepared for PCR, western blotting, and immunohistochemistry, to characterise the expression of NADH ubiquinone oxidoreductase subunit S6 (NDUFS6), subunit S2 (NDUFS2), and subunit A6 (NDUFA6).

Results

Using PCR, we identified a significantly lower mRNA expression of NDUFA6 ($p < .001$) in FGR placentas compared with healthy placentas, a finding consistent with whole tissue western blotting data ($p = .0233$). We also identified a significantly lower expression of NDUFS6 ($p = .0499$) in FGR placentas. In healthy term placentas NDUFS6 expression was lower in mitochondria from syncytiotrophoblasts compared with cytotrophoblasts ($p < .05$), consistent with immunohistochemistry results ($p < .0001$). Western blotting demonstrated a significantly lower expression of NDUFS2 ($p = .0016$) in syncytiotrophoblast mitochondria compared with cytotrophoblast mitochondria.

Conclusion

Our findings elucidate a promising novel mechanism underpinning FGR pathology, whereby mitochondrial complex I dysfunction driven by subunit dysregulation may alter ATP synthesis, causing placental insufficiency. Further investigations will facilitate the development of diagnostics and therapeutics that can promote fetal health by supporting bioenergetics and metabolism.

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Women with pre-existing type 2 diabetes mellitus have altered gut microbiome composition in pregnancy

Sophie M Leech[1], Emily S Dorey[2], Helen L Barret[3, 4], Marloes Dekker Nitert[1]

1. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

2. Mater Research Institute , The University of Queensland, Brisbane, QLD, Australia

3. Obstetric Medicine, Royal Hospital for Women, Randwick, NSW, Australia

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

Introduction: Outside pregnancy the gut microbiome is thought to play a role in the pathogenesis of Type 2 diabetes mellitus (T2DM) with depletions in short-chain fatty acid (SCFA) producing bacteria and increases in intestinal permeability and inflammation. However, in pregnancy, only one study examining the relationship between pre-existing T2DM and the gut microbiome exists. As pregnancy is a time of significant hormonal and metabolic change, we aimed to examine the composition and functionality of the gut microbiome of pregnant women with pre-existing T2DM.

Methods: Stool from 9 women with pre-existing Type 2 diabetes mellitus and 26 normoglycaemic controls between 24- and 31-weeks' gestation was collected. The profile of the gut microbiome was assessed from shotgun metagenomic sequence data. Community composition was assessed using MetaPhlAn 4.0.6 and functional analysis was conducted with HUMAnN 3.6. Analysis was conducted with GraphPad Prism 9.0.2. and RStudio packages 'phyloseq', 'MixOmics', 'vegan', 'ANCOM-BC' and 'Maaslin2'.

Results: T2DM women had lower Shannon indices ($p = 0.0058$) with decreased evenness ($p = 0.0228$) and richness ($p = 0.0155$) of the gut microbiota. Beta diversity was also significantly different ($R2 = 0.043$, $p = 0.018$). No significant differences were detected using Maaslin2 at any taxonomy level after correction for multiple comparisons. However, ANCOM-BC identified multiple differentially abundant taxa such as depletions in Candidatus Avimonas narfia SGB14941 ($q = 0.045$) in T2DM women. Several functional pathways were differentially abundant including PWY-8190 which produces SCFA acetate and butanoate and was enriched in T2DM (Maaslin2: $q = 0.0017$, ANCOM-BC: $q = 0.0012$) and PWY-5747 (ANCOM-BC: $q = 0.036$), which describes the degradation of SCFA propionate.

Conclusion: The gut microbiome of women with pre-existing T2DM in pregnancy significantly differs from normoglycaemic women with changes in two SCFA pathways. As a next step, we will assess the SCFA concentration in serum and stool.

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Dysregulation of placental melanophilin during early pregnancy disrupts trophoblast differentiation.

Yimiao Yu[1], Guannan Yang[1], Leiani Santos[1], Wei Zhou[1], Shanti Gurung[2], Kim-Anh Lê Cao[1], Ellen Menkhorst[1], Evdokia Dimitriadis[1]

1. University of Melbourne, Carlton, VIC, Australia

2. Hudson Institute of Medical Research, clayton, Victoria, Australia

Preeclampsia is a severe pregnancy disease causing significant maternal and perinatal morbidity and mortality. Term preeclampsia (diagnosis>37 gestation weeks) accounts for 80% of the overall disease burden. However, due to our poor understanding of its underlying etiology, we still lack early prediction and prevention methods. Whether deficient early placentation contributes to term preeclampsia is hotly debated and has never been experimentally proven.

Our previous multi-omics on early pregnancy placental biopsies revealed melanophilin as associated with term preeclampsia. RNA sequencing data showed melanophilin is significantly downregulated in placental tissue from term preeclampsia compared to uncomplicated pregnancies. Melanophilin is well characterized as a key regulator of hair, skin, and eye colour, but more recently it is shown to regulate insulin granule release and enhance cancer progression. However, melanophilin has never been investigated in the placenta. We investigated the localization and expression of melanophilin in human decidua and placenta across gestation by qPCR and immunohistochemistry. siRNA knockdown of melanophilin in trophoblast cell lines (HTR8 and trophoblast stem cells [hTSC], n=4/group) was used to determine its role in gene expression (qPCR) and adhesion/proliferation (xCELLigence/MTT assay).

Melanophilin localized to extravillous trophoblast in decidua and syncytiotrophoblast, cytotrophoblast, stromal, endothelial and Hofbauer cells in placental villus. Melanophilin mRNA expression in placental villus was highest in the 1st trimester, reducing by 200% to be almost absent in the second trimester and at term ($p<0.05$). In vitro, melanophilin loss significantly promoted trophoblast adhesion (HTR8 1.3-fold; $p<0.05$) and proliferation (at 24h: HTR8 1.5-fold; hTSC 1.3-fold; $p<0.05$). Melanophilin knockdown in hTSC reduced mRNA expression of PlGF (0.4-fold) and regulated genes associated with trophoblast differentiation (HLAG, bhCG, GATA3, EOMES).

Here we show melanophilin was highly expressed in first trimester placenta and regulates trophoblast adhesion, proliferation and gene expression. Loss of melanophilin may contribute to the diminished trophoblast differentiation seen in preeclampsia.

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The retinoic acid receptors and binding proteins display distinct localization patterns in adult murine epididymal epithelial cells.

Anna-Lisa Pozzacchio[1], Sarah Hughes[1], Cathryn A Hogarth[1]

1. Department of Rural Clinical Sciences, La Trobe Rural Health School, La Trobe University, Wodonga, VIC, Australia

Retinoic acid (RA), the active metabolite of vitamin A, is essential for male fertility. While RA's role in testis germ cell development has been extensively studied, very little is known about whether RA is also important for epididymal function. Our laboratory previously discovered that blocking RA activity in the epididymis resulted in abnormal epithelial pathology and male infertility. This current study investigated the localization patterns of the RA receptors (RARα, RARβ, RARγ) and binding proteins (CRABP1 and CRABP2) in response to RA in epididymal epithelial cells using immunohistochemistry and immunocytochemistry. Our aim was to identify whether there are specific epididymal cell types and/or segments that actively signal using RA. Consistent with previous studies in the rat, RARα was present in the nucleus of all epithelial cells throughout the epididymis, with the strongest signal evident in the corpus. RARγ was present in the cytoplasm of all epithelial cells except the clear cells of the cauda, and RARβ was not detectable. CRABP1 was also not detected in the epididymis although CRABP2 displayed a distinct segment specific expression pattern, with positive principal cells only detected in the corpus and cauda regions. Analysis of staining patterns in the epididymal epithelial cell line, mECap18, revealed cytoplasmic signal for all RARs and CRABPs, although RARβ and RARγ were also detected in the nucleus. In the presence of RA, RARα and both CRABPs displayed a shift into the nucleus, in some cells as quickly as 20 mins. RARβ and RARγ showed no response to RA. This study has revealed that the RA signaling and binding proteins display distinct cellular localization patterns in the epididymis and show varying degrees of responsiveness to RA. Future studies will focus on identifying the key downstream targets of RA signalling in the epididymal epithelium.

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Investigating the conservation of CDC2-like kinases across eukaryotes and their role in male fertility

Rachel Ogle[1], Mark Baker[1], Jacob Netherton[1], Benjamin Robinson[1]

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

The widespread prevalence of CDC2-like kinases (CLKs) and their homologs across eukaryotes strongly suggests they are functionally important genes. The activation segment of these kinases exhibits unique temperature-dependent regulation of kinase activity, which increases as temperature declines. This is relevant in the context of spermatogenesis which is exceptionally temperature sensitive and must occur ~3°C below core body temperature. Testicular hyperthermia, even by an increase of 1-3°C, leads to reduced sperm output and quality. This temperature profile range is consistent with the activity of mammalian CLKs, which increase ~4-fold when the temperature is dropped from 38°C to 35°C. Furthermore, the knockout of

the CLK homologs in Drosophila melanogaster or Caenorhabditis elegans results in complete sterility, with testis-specific knockout of DOA further demonstrating its essential function in spermatogenesis.

To understand their diversification and determine whether these kinases serve essential functions in male fertility, we investigated their phylogenetic history, structural conservation, and impact of gene loss. Phylogenetic analysis reveals the expansion of CLK-related genes throughout eukaryotic diversification, with simple organisms including fly, worm and yeast possessing just one gene, whilst complex organisms such as mammals having a total of four homologs. Knockout studies conducted in various model organisms highlighted the essential nature of specific CLKs, and their critical roles in embryonic development, neural tissue formation, and reproductive function in diverse eukaryotes. These models also suggest that this indispensable function may have been partitioned through the mechanism of gene subfunctionalisation. By integrating findings of the phylogenetic analysis, structural comparisons, gene loss, and knockout studies, we can gain insight into essential functions of CLKs to further our understanding of their diverse roles, including male reproductive function.

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Understanding the molecular mechanism underpinning the adverse affects of testicular heat stress

Benjamin R Robinson[1], Mark Baker[1]

1. University of Newcastle, Callhagn , NSW, Australia

Male fertility is in significant decline across the western world. Consequently, the proportion of men requiring fertility treatment has risen from 12.4% in 2004 to 21.3% in 2017. The largest “diagnosed” cause of impaired male fertility is attributed to a combination of low sperm count, poor motility and abnormal morphology and is clinically known as Oligoasthenoteratozoospermia (OAT). The testis typically run at 3-4ºC lower than core body temperature. Remarkably, testicular hyperthermia is known to be a major cause of OAT, with a 1°C increase median scrotal temperature enough to cause a 40% reduction in sperm concentration in men. Testicular hyperthermia does not affect all cell but rather appears to be specific to pachytene spermatocytes and round spermatids.

To better understand the initial transcriptomic changes that result from testicular hypothermia we performed Next Generation RNA sequencing on round spermatids isolated from male CD1 mice (N=5) subject to testicular heat stress (42°C, 30min) versus controls (30°C, 30min). From this data we identified two key findings

Firstly, the significant upregulation of anti-apototic HSPA1A is attenuated in heat stress round spermatids compared to somatic cells which could countribute to their high sensitivity to heat stress. Secondly we identified two novel, testis specific long non-coding (LNC) RNAs that are unpregulated in response to testicular heating. Independent over expression of both LNC-RNAs leads to increased cell death in somaic cell lined in vitro. Work is continuing to better understand the molecular mechanisms behind both of these key findings

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Testicular histopathology and its association to semen quality and serum concentrations of reproductive hormones in 1400 men

Gülizar Saritas[3, 2, 1], Nina Moerup[2, 1], Trine H. Johanssen[2, 1], Anders Juul[2, 4, 1], Lise Aksglaede[2, 1], Sofia B. Winge[2, 1], Kristian Almstrup[3, 2, 1]

1. Department of Growth and Reproduction, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark

2. International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health, Copenhagen, Denmark

3. Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

4. Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Testicular biopsies may be performed as part of the diagnostic evaluation of the infertile man to describe spermatogenesis and to identify germ cell neoplasia *in situ*. Before biopsy sampling, assessment of semen quality and serum concentrations of reproductive hormones are performed, and the relationship between these parameters is well-described. However, the association between a given testicular histopathology and these parameters is not well-characterized, and we aimed to describe this association according to specific categories of testicular histopathology.

In total, 1400 men were grouped according to their testicular histopathology: complete spermatogenesis (n=977), reduced spermatogenesis (n=275), heterogeneous spermatogenic arrest (n=96), and homogeneous spermatogenic arrest (n=52). Immunohistochemistry (MAGE-A4, PIWIL1, TNP1) was used to identify spermatogonia, spermatocytes, and spermatids, respectively. Subsequent spermatogenic cell populations were quantified by image segmentation to describe the testicular histopathology. Data on semen quality and reproductive hormones were retrospectively evaluated as part of the clinical work-up.

A reduced number of spermatogonia was observed in the group with heterogeneous arrest compared to the complete spermatogenesis group. The spermatocyte and spermatid populations were also decreased; however, this was also observed in the groups with reduced and arrested spermatogenesis. Concentrations and total counts of sperm were reduced in the groups with reduced and arrested spermatogenesis compared to the complete spermatogenesis group, with the homogenous arrest group being the most severely affected. Compared to the complete spermatogenesis group, reproductive hormones in all groups showed significantly higher concentrations of FSH and LH and significantly lower concentrations of testosterone and inhibin B, with the heterogenous arrest group being the most severely affected.

Our data confirm the intimate relationship between testicular histopathology and semen quality and reproductive hormones showing the group with heterogeneous arrest have the most severe testicular dysfunction reflected by the lowest testosterone, highest LH and FSH, and decreased concentration and total counts of sperm.

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DNA fragmentation is correlated with abnormal morphology of frozen-thawed ram spermatozoa

Eloise Spanner[1], Maya Robertson[1], Simon de Graaf[1], Jessica Rickard[1]

1. Faculty of Science, University of Sydney, Camperdown, NSW, Australia

The morphological characteristics and DNA integrity of spermatozoa have been linked to fertility in a range of species (1, 2). Spermatozoa which record high morphological abnormalities are thought to be less fertile due to their inability to navigate the tract, fertilise the oocyte and often result in pregnancy loss (3). Compared to humans and other livestock species, where these two parameters have been shown to be correlated, little focus has been given to the potential interplay between the DNA integrity and morphological abnormalities of ram spermatozoa post-thaw (4). As such, this study directly compares the DNA integrity and morphology of ram spermatozoa following cryopreservation to understand the relationship between semen quality traits.

Semen was collected from Merino rams (N=250) and frozen using industry-standard techniques. Each sample was assessed for the percentage of abnormal sperm using phase-contrast microscopy (x400 magnification, 200 cells) and DNA fragmentation using flow cytometry (Acridine Orange; Thermo Fisher Scientific, NSW) immediately after thawing (3) and following 6hr of incubation at 37ºC to establish a DNA fragmentation index (DFI%).

Statistical modelling (R Studio) revealed DFI% at 0hr and 6hr post-thaw returned a positive correlation with morphological abnormalities (R^2 = 0.53, 0.33, respectively). There was also a significant increase in DFI± SEM% from 0hr (3.97±0.24%) to 6hr (6.91±0.51%, p<0.001) post-thaw.

These findings demonstrate a positive correlation between DFI% and morphological abnormalities of frozen-thawed ram spermatozoa and an effect of incubation time on DFI% in ram spermatozoa. The results imply that the processes that cause abnormal sperm morphology negatively affect the DNA integrity of sperm. Further research is required to explore the collective impact of such traits on the fertility of semen following artificial insemination and the acceptable levels for processed semen.

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Changes to the seminal environment induced by the metabolism of ram spermatozoa

Madeleine Van de Hoek[1], Jessica P Rickard[1], Simon P de Graaf[1]

1. School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW, Australia

Mass motility assesses the three-dimensional collective movement of a group of spermatozoa. While it is well established that motility (regulated by the energy generated by metabolism) rapidly declines post-collection, the metabolic indicators associated with this decline in ram semen have not been described. Therefore, the aim of this study was to describe how ram sperm metabolism alters the pH and sugar content of the seminal environment. We hypothesised that pH would decline as acidic metabolic by-products are produced and the concentration of simple sugars would be exhausted as a result of glycolytic metabolism.

Ejaculates (*n = 9*) were collected from Merino rams via artificial vagina and incubated at 30°C. Mass motility, assessed on a scale from 0 (no motion) to 5 (numerous rapid waves) and the pH of undiluted ejaculates was recorded every 2 minutes until motility ceased. The sugar concentration (fructose and glucose) of ejaculates was assessed (D-Fructose/D-Glucose Assay Kit; Megazyme, Ireland) immediately post collection and once motility stopped.

Immediately post ejaculation, semen exhibited a mass motility of 5.0±0.00, pH 6.9±0.08 and sugar content of 7.9±0.80 g/L. After 34±3.5 minutes, the mass motility of semen reached 0.0±0.00 and pH and sugar content decreased to 5.7±0.09 and 5.3±1.28 g/L, respectively. This suggest that the motility of undiluted ram semen does not cease because of a lack of available substrates for glycolytic metabolism. Although motility and pH were correlated (r= 0.63, p= < 0.001), previous studies have found that mammalian sperm can retain motility within a pH range of 5.0 to 8.5 [1]. It therefore remains unclear if pH decline is the cause of the reduction in mass motility or if these are two separate events occurring simultaneously. The next stage of this work is to explore this link further, as well as the influence of seminal plasma on sperm metabolism.

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Integrative transcriptomic analysis of miRNA-mRNA network in late-onset preeclampsia placentae

Luhao Han[1], Olivia Holland[1], Fabricio Da Silva Costa[2, 3], Anthony Perkins[4]

1. School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, Queensland, Australia

2. Maternal Fetal Medicine Unit, Gold Coast University Hospital, Gold Coast , Queensland, Australia

3. School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland, Australia

4. School of Health, University of the Sunshine Coast, Sunshine Coast, Queensland, Australia

Preeclampsia is a heterogeneous disorder of pregnancy with two main subtypes, early-onset and late-onset, suggesting potentially different underlying molecular mechanisms. Late-onset preeclampsia is more prevalent (approximately 70% of cases) and remains challenging to predict and manage. This study aimed to identify dysregulated genes, miRNAs and their interaction in term placentae from late-onset preeclampsia by utilizing *in silico* analysis.

Differential expressed genes (DEGs) and miRNAs (DEMs) were identified from subsets of public datasets GSE75010 (cases = 26, controls = 28) and GSE103542 (cases = 5, controls = 8) from Gene Expression Omnibus datasets. Differential expression and pathway analyses were performed using the R packages “limma” and “clusterProfiler”. A protein-protein interaction (PPI) network was generated via the STRING database. Target genes of DEMs were predicted with the R package “multiMiR”. DEGs that overlapped with the target genes of DEMs were defined as DEM-target DEGs for constructing a miRNA-mRNA network by Cytoscape software.

We identified 216 DEGs (96 upregulated, 120 downregulated) and 20 DEMs (10 upregulated, 10 downregulated) with |logFC| > 2 and $p < 0.05$. The PPI network of 216 DEGs was constructed to select hub genes according to the Maximal Clique Centrality (MCC) score using CytoHubba in Cytoscape. The top ten genes with the highest MCC score were *FN1, SEPRINE1, FLT1, TIMP3, ENG, EPAS1, PGF, LEP, SCARB1,* and *TIMP2*. miRNA-mRNA regulatory networks implicated miR-548c-3p/u, miR-3065-5p, miR-3921, miR-34c-5p, and miR-3163 interaction with multiple DEGs.

This study uncovered the top ten hub genes and ten DEG-related miRNAs associated with placental dysfunction in late-onset preeclampsia. In particular, miR-34-5p may play an important role in late-onset preeclampsia pathogenesis by regulating several hub genes such as *SERPINE1, FLT1, LDHA,* and *ADAM12*. These findings will be further validated in our local placental biobank (case = 26) to confirm their relevance in late-onset preeclampsia.

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Control of exocytosis in uterine epithelial cells during uterine receptivity

Emma Lucille Blayney[1], Alexander Cunningham[1], Sadaf Kalam[1], Samson Dowland[1], Laura Lindsay[1]

1. School of Medical Sciences, University of Sydney, Sydney, NSW, Australia

Implantation of the blastocyst into the uterine wall involves coordinated changes in uterine epithelial cells (UECs), trophoblastic cells and uterine luminal fluid volume and composition. One way the luminal fluid is altered is via UEC exocytosis. The SNARE protein complex including t-SNAREs (Syntaxin-2 and SNAP23) and v-SNAREs (VAMP) with the help of regulatory protein (Munc 18), regulate exocytosis in a variety of cell types. Currently regulation of exocytosis in UECs is unknown however the secretin hormone, which is released from decidual cells during early pregnancy, has been shown to regulate epithelial cell exocytosis in other tissues.

Immunofluorescence microscopy and western blotting identified changes in localisation and quantity of SNARE complex proteins SNAP23, syntaxin 2, VAMP and Munc 18-2 in uterine epithelial cells and luminal fluid at the time of uterine receptivity in rats. For the first time, secretin receptor was localised apically in rat UECs *in vivo* and found in receptive human endometrial epithelial cells *in vitro*.

An increase in apical vesicles within UECs is one of the many changes seen at the time of uterine receptivity. Apical secretin receptors in UECs could initiate an exocytosis event coordinating release of these vesicles into the uterine luminal fluid. Munc 18-2 localised to the perinuclear/Golgi area could be involved in movement of vesicles through the Golgi complex. SNARE proteins SNAP23, syntaxin-2 and VAMP, were found apically in UECs and regulate apical vesicle docking with the UEC apical plasma membrane. The presence of SNAP23 in uterine luminal fluid specifically at the time of receptivity may also play a novel role in uterine fluid. This study describes how exocytosis could contribute to uterine luminal fluid composition – an essential requirement for uterine receptivity and successful implantation.

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Nanosplit – Allele-specific read sorting programme for Oxford nanopore long read sequencing platform.

Teruhito Ishihara[1], Christian Belton[1], Gavin Kelsey[1]

1. Epigenetics Programme, Babraham Institute, Cambridge, United Kingdom

Genomic imprinting, a complex and important epigenetic phenomenon in mammals, causes a subset of genes to be expressed in a parent-of-origin-specific manner. Comprehensive searches for these imprinted genes in mice have been conducted primarily by allelic expression analysis of hybrid samples using Illumina-based sequencing platforms. However, this approach has limitations, including fragmentation of transcripts and PCR biases. These can lead to loss knowledge of the splice variant origin of each read and biased allelic specificity of sequencing libraries, respectively. To overcome these problems, direct cDNA/RNA sequencing on the Oxford nanopore long read platform, which avoids fragmentation of information and PCR bias, is a preferred alternative approach. However, while the programme SNPsplit can be used for Illumina-based platforms to separate sequencing reads by parental genome based on strain-specific single nucleotide polymorphisms (SNPs), the Oxford nanopore platform has no equivalent programme. In this study, we developed a new Python-based programme, Nanosplit, to achieve this in nanopore long read sequencing datasets. We performed nanopore sequencing on embryos and placentas of hybrid samples

obtained from crosses between the C57BL6 and CAST/Ei strains. Correct allelic sorting was confirmed by examining parental origin differences in known imprinted genes such as *Peg10* and *Igf2r*. The Nanosplit programme also allowed us to identify tissue-specific and/or isoform-dependent mono-allelically expressed genes that could not have been identified without long read sequencing. This study demonstrates the ability of Nanosplit to further advance the analysis of allelic expression and the identification of novel imprinted genes.

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A follow-up audit of the use of short synacthen test

Yi-An YP Pan[1], Peter PD Davoren[1]

1. Gold Coast University Hospital, Southport, QLD, Australia

The short synacthen test (SST) is a widely used dynamic test for assessing primary adrenal insufficiency (AI) and non-classical adrenal hyperplasia. A morning cortisol test can be performed as a preliminary test to rule out adrenal insufficiency and it is a reliable predictor of SST outcome.[1],[2],[3],[4] If cortisol levels are low, a paired ACTH test help differentiate between primary and secondary AI. This approach helps avoid unnecessary dynamic tests, reduces healthcare costs, and minimizes the risks associated with SST and ITT. An Oxford retrospective study demonstrated a cortisol level of 343 nmol/L could predict a 'pass' on the SST with 100% specificity, regardless of the type of AI. [1] However, a morning cortisol level of 415 nmol/L is necessary to predict a normal insulin tolerance test, highlighting that patients with normal SST results can still have abnormal ITT results.[2] The clinical context remains crucial, especially in cases with normal or low ACTH levels, as a normal SST does not necessarily indicate a normal HPA axis, central causes of hypoadrenalism might still be undiagnosed.

In 2019, a retrospective audit conducted at Gold Coast University Hospital (GCUH) demonstrated an over-reliance on the SST as the preliminary test and many could have been avoided. The aim of this subsequent 2023 audit is to evaluate the influence of the prior audit on the hospital's practices and identify how many SSTs could have been avoided with morning cortisol alone.

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