1

Multiple layers of complexity govern fertility, pregnancy, perinatal and lifelong health

Claire Roberts[1]

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For me it started with a 10g marsupial that I found had an invasive yolk sac placenta. Then David Barker showed epidemiological evidence that maternal undernutrition may restrict fetal growth and program the fetus for chronic disease in later life. But it is so much more complex than undernutrition. The mothers of the offspring Barker studied had a deprived existence in a multitude of ways. They and their *in utero* offspring would have been exposed to significant stress, to air pollution etc and their undernutrition was not simply calorie restriction but likely specific macro- and micro-nutrient restriction. The impact of their exposures would be strongly influenced by maternal and paternal genomes and pre-conception exposures. And, of course, the unique combination of maternal and paternal genetics that comprise the fetal/placental genome influence nutrient transport to the fetus and placental secretion of hormones and growth factors that orchestrate maternal adaptations to pregnancy. Parental genomes, diet, adiposity, cardiovascular and metabolic health conspire together with environmental factors in the first place to make conception possible or not. In turn they impact fetal growth and development, newborn and future maternal and offspring health. On top of this, the sex of the fetus has surprising impacts in many dimensions. The last decade has seen an explosion of cellular, molecular and clinical tools with which we are exploring impacts of genetic, epigenetic, nutrient, psycho-social and environmental factors on fertility, pregnancy health and beyond for mothers and children.

2

Improving care – theory to practice

David Maahs[1]

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A strong and collaborative multidisciplinary team to provide diabetes education and on-going support is the foundation for successful diabetes care. Historic and novel care delivery models will be discussed including tele-health, tele-education, and remote monitoring models accelerated during the Covid pandemic with attention to disparities in care and involvement of team members. Discussion will be encouraged to share best practices and solutions to barriers that impede providing best possible diabetes care.

3

Driving Positive Behaviour Change

Hayden Fricke[1]

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In order to consistently deliver quality patient outcomes most of us need to learn to work in highly effective multidisciplinary teams. However, some teams are better than others. Why? What gets in the way of truly becoming highly cohesive and high performing? Individual team members must change themselves and their own behaviours in some small but important ways in order to develop the qualities needed to work well as a multidisciplinary team. We know that behaviour change is hard. There are many barriers to changing yourself. For healthcare teams we need to change our normal operating approach in order to do things like build trust, adapt quickly to circumstances, continue to learn, evolve and grow rather than having a fixed mindset. Hayden will share a framework and practical tips around how to drive positive behaviour changes that stick for yourself and your team members, with the ultimate goal of creating highly effective multidisciplinary teams.

4

Impact of 6 months use of intermittently scanned continuous glucose monitoring on habitual sleep patterns and sleep quality in youth with type 1 diabetes and high-risk glycaemic control

Shelley Rose[1, 2, 3], **Barbara C Galland**[1], **Sara E Styles**[3], **Esko J Wiltshire**[2, 4], **James Stanley**[5], **Martin I de Bock**[6, 7], **Paul A Tomlinson**[8], **Jenny A Rayns**[9], **Karen E MacKenzie**[6, 7], **Benjamin J Wheeler**[1, 10]

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Aims: To date, few objective or subjectively measured data exist to describe the impact of continuous glucose monitoring (CGM) technology on sleep timing, duration, and quality in young people with type 1 diabetes (T1D). This study aims to evaluate the impact of first-generation intermittently scanned CGM (isCGM) on habitual sleep patterns, including sleep onset and offset timing, sleep duration, disturbances (frequency and duration of night-time awakenings), sleep efficiency and perceived sleep quality in youth with high-risk glycaemic control.

Methods: We recruited 64 youth aged 13-20 years (mean age 16.6 ± 2.1 years, 48% female, diabetes duration 7.5 ± 3.8 years, 41% Māori or Pacific ethnicity, HbA1c 96 ± 18 mmol/mol [10.9 ± 3.8%]). Thirty three participants were allocated to the 6-month intervention group (isCGM plus self-monitoring blood glucose [SMBG]), while the remaining 31 participants were allocated to the SMBG control group. At baseline and 6 months, participants completed seven days of actigraphy to objectively measure sleep timing and duration. They were also asked to complete the Pittsburgh Sleep Quality Index (PSQI) to assess perceptions of sleep timing and quality over the prior month.

Results: At six months, subjective measures for overall sleep quality, time taken to get to sleep, sleep duration, sleep efficiency, night-time disturbances, use of sleep medications, and daytime dysfunction were similar between the groups. Although participants using isCGM reported later average bedtimes over the prior month compared to controls, regression analyses of the actigraphy data found no strong/substantial evidence for differences in objectively measured sleep patterns between the groups after adjusting for age, school term time, and baseline sleep values.

Conclusions: Access to isCGM in addition to SMBG was not sufficient to impact on either objective or subjective measures of sleep outcomes in youth with T1D and high-risk glycaemic control and highly variable sleep patterns.

5

Intensive management from diagnosis improves HbA1c at 12 months post diagnosis results from a prospective cohort study in newly diagnosed children with type 1 diabetes

Caroline Griffin[1], Karen Mackenzie[1], Janet Ferguson[1], Neil Owens[1], Sharon Walsh[1], Olivia Sanders[1], Chloe Hudson[1], Martin de Bock[2]

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2. Otago University, Christchurch, Canterbury, New Zealand

Background: Intensive management is known to improve long term outcomes in type 1 diabetes, however there is a lack of data to document the impact of this when instituted from diagnosis.

Aim: To examine the impact of intensive management of type 1 diabetes from diagnosis on HbA1c 12 months from diagnosis

Methods: A prospective, continuous cohort of 70 sequentially newly diagnosed children (diagnosed July 2018- November 2021) were reviewed 12 months after diagnosis following implementation of an intensive management protocol from diagnosis and was compared to a retrospective cohort of the previous 70 children diagnosed pre-implementation (diagnosed December 2015- June 2018). Intensive management involved carbohydrate counting and flexible insulin dosing from first meal on sub-cutaneous insulin, targeted blood glucose levels from 4 – 8mmol/L irrespective of time of day, avoidance of twice daily insulin regimens, and promotion of continuous glucose monitoring (unfunded). Baseline demographics of both cohorts were documented, and 12 month data on HbA1c, diabetes technology use, and insulin regimen were compared.

Results: Table 1 documents the baseline and 12-month data of the two cohorts. There was an 11mmol/mol (0.7%) difference between the HbA1c at 12 months between the two groups 12 months.

Conclusion: Intensive management from diagnosis improves long term HbA1c in children with type 1 diabetes. Whilst there have been improvements over all ethnic groups, more is needed to be done in improving outcomes and access to CGM for minority ethnic groups.

Table 1:

	Cohort 1 n=70	Cohort 2 n=70
Gender	38 female 32 Male	32 female 38 male
Age at diagnosis	1year 7months- 14years 7 months median age at diagnosis 8 years 7 months	2years 5months- 15 years 3 months median age at diagnosis 10 years 4 months
Ethnicity	NZ Euro 81% (n=57) Maori 3% (n=2) Pacific 4% (n=3) Other 11% (n= 8) (African, , Other Euro, Asian)	NZ Euro 77% (n=54) Maori 13% (n=9) Pacific 4% (n=3) Other 6% (n=4) (African, Other Euro, Indian)
Length of Hospital Stay (days)	3.25 average 3 median	3.27 average 3 median
Number of clinics attended in first 12 months	5.7 average 6 median	5.5 average 5.5 median
DKA at diagnosis	33% 11 severe 10 moderate 2 mild	40% 14 severe 11 moderate 3 mild
Regimen at diagnosis	BD 89% (n=62) MDI 10% (n=7) Other 1% (n=1)	BD 26% (n=18) MDI 73% (n=51) Other 1% (n=1)
Regimen at 12 months post diagnosis	BD 66% (n=46) MDI 23% (n=16) CSII 11% (n=8)	BD 11% (n=8) MDI 83% (n=58) CSII 6% (n=4)
CGM use (real time and intermittently scanned) at 12 months post diagnosis (if used 3 months prior to 12-month HbA1c)	57% 40/70 using 97.5% Libre n=39 2.5% CGM n=1	75% 53/70 using 94% Libre n=50 6% CGM n=3
Median HbA1c 12 months post diagnosis	7.9% (63mmol/mol)	7.2% (54mmol/mol)
Median HbA1c 12 months post diagnosis by ethnicity	Nz Euro 7.8% Pacific Island 8.7% Maori 9.2% Other 8.0%	Nz Euro 7.0% Pacific Island 8.1% Maori 8.6% Other 7.0%
% meeting target HbA1c ≤7.0% 53mmol/mol at 12 months post diagnosis	13%	44%
% meeting target HbA1c ≤7.0% 53mmol/mol at 12 months post diagnosis by ethnicity	Nz Euro 14% (n=8/57) Pacific Island 0% (n=0/3) Maori 0% (n=0/2) Other 11% (n=1/8)	Nz Euro 54% (n= 29/54) Pacific Island 0% (n=0/3) Maori 22% (n=2/9) Other 50% (n=2/4)
CGM usage by ethnicity	Nz Euro 58% (n=33/57) Pacific Island 33% (n=1/3) Maori 50% (n=1/2) Other 62% (n=5/8)	Nz Euro 81% (n=44/54) Pacific Island 33% (n=1/3) Maori 55% (n=5/9) Other 75% (n=3/4)

6

Who's Right? A Rights based framework for the healthcare needs of those with a Variation in Sex characteristics.

Denise steers[1], Angela Ballantyne[1], Esko Wiltshire[1]

1. University of Otago, Wellington, WELLINGTON, New Zealand

In this poster we present a novel human rights framework for supporting ethical and clinically appropriate treatment for people born with Variations in Sex Characteristics (VSC). This framework supports and enables a spirit of collaboration in the treatment for persons with VSC that is specific to Aotearoa/NZ. It incorporates Ti Tiriti O Waitangi, Human Rights, Clinician Best Practices and a Future Focus as the four key elements to delivering well informed healthcare.

Internationally, there is a move to introduce legislation to mandate specific treatment pathways for VSC, for example in the ACT in Australia the Variations in Sex Characteristics (Restricted Medical Treatment) Bill 2022. Legal avenues have been sought by VSC and human rights activists due to ongoing lack of trust that health professionals are willing to provide health care for those with a VSC that supports bodily autonomy and self-determination.

In Aotearoa/New Zealand a different approach has been forged via continued dialogue between those with lived experience and those working in health. The Ministry of Health is currently working on developing a model of care that privileges the knowledge of those with lived experience alongside that of the health professionals. There is a spirit of collaboration to bring about change that champions a human rights informed model of care. This involves updating best practice guidelines and education of health professionals/parents/patients and the public and development of resources including sustainable VSC peer lead support.

We as health care professionals in Aotearoa/NZ have the chance to demonstrate trustworthiness. The capacity to grow our self-understanding and acknowledge the need for providing a model of care that allows for self -determination and bodily autonomy to be highlighted while supporting beneficence and non-maleficence. We offer a frame work and some educational tools to support such a change.

7

Addressing preventable infertility through understanding ovary development and improving fertility education

Jessie Sutherland[1]

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

Dr Jessie Sutherland leads an independent research program in reproductive health and fertility at the University of Newcastle, established in 2015. The goal of her research program is to eliminate preventable infertility on a global scale. To achieve this, her multidisciplinary team focusses on improving the reproductive health knowledge of young people and determining the underlying causes of infertility in women.

In recognition of her national research excellence, she is the dual recipient of an ARC Discovery Early Career Researcher Award and NHMRC Peter Doherty Australian Biomedical Fellowship. In demonstration that her research is relevant, needed, and useful, she had secured over $1.8M in competitive independent funding from Government, Industry, and Philanthropy and published 40 scientific papers with >900 citations. She is the first Newcastle-based researcher to establish a formalised partnership agreements with Family Planning NSW, Sexual Health Victoria, and Your Fertility and secure seed funding and a collaborative commercial agreement from industry partner Cooper Medical Fertility Solutions. Dr Sutherland is also an advocate for equity and diversity in the University sector and is an Academic representative the College of Health, Medicine and Wellbeing Equity Diversity and Inclusion Committee.

8

Spermatogonial stem cells and oncofertility: from chemotherapy target to potential treatment tool

Tessa Lord[1, 2]

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

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

Dr Lord is an ARC DECRA fellow at the Priority Research Centre for Reproductive Science, University of Newcastle, where she has been leading a research group since 2019. Her research aims to characterise molecular pathways that regulate testicular stem cell function and apply this knowledge to bring important spermatogonial stem cell (SSC) technologies to fruition. One potential application of such technologies is the reversal of infertility in survivors of childhood cancers.

Unfortunately, approximately 50% of male survivors of childhood cancers will be rendered permanently infertile because of chemotherapy- or radiotherapy-induced destruction of SSCs. Further, unlike adult men, pre-pubertal patients are not able to produce a semen sample for cryopreservation, so currently have no options to safeguard their future fertility. In these circumstances, cryopreservation of a testis biopsy, followed by transplantation of captured SSCs back into the patients' testes in adulthood, may be a potentially feasible alternative. However, advances in our fundamental knowledge of these cells are required to translate such experimental techniques into the clinic. During her time as a postdoctoral researcher at Washington State University, Dr Lord designed a novel, high-throughput pipeline to screen >1400 transcription factors for a role in regulating SSC function. Of putative candidates identified, three have been further characterised (thus far), revealing an important role in regeneration of spermatogenesis by SSCs after chemotherapy. Additionally, through the manipulation of molecular pathways identified in these experiments, Dr Lord has more recently made advances in maintaining SSCs in *in vitro* culture as a precursor to transplantation treatments (NHMRC Ideas grant funded project). Findings stemming from this research have the potential to significantly impact the wellbeing of paediatric cancer survivors.

9

New FKBPL and CD44-based diagnostics for preeclampsia: from discovery to a point of care test

Lana McClements[1]

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

Dr McClements is a qualified Clinical Pharmacist who is now a Senior Lecturer at the Faculty of Science and the Institute for Biomedical Materials and Devices at the University of Technology Sydney (UTS). She graduated with MPharm from King's College London (UK) and after five years of working at two hospitals in London as a Clinical Pharmacist, Dr McClements was awarded a PhD scholarship and completed her PhD at Queen's University Belfast (Northern Ireland, UK) in 2014. In 2018, Dr McClements moved to UTS to take up a lectureship position where she now leads a team of five PhD students, two-three Honours students and three Research Assistants. As a chief investigator over the last 5-7 years, she has led impactful research program that strives to improve women's health from pregnancy and beyond, and from bench to bedside. She discovered and patented two new blood-based biomarkers of impaired angiogenesis, FKBPL and CD44, in pregnancy, which are being commercialised for prediction and diagnosis of preeclampsia. Recently, she was awarded a 2022 Heart Foundation Future Leader Fellowship to develop new personalised treatments for preeclampsia that target FKBPL signalling. Furthermore, Dr McClements' group designed innovative 3D multicellular models of early placenta and women's heart disease for high-throughput screening of biomarkers and therapeutics. Her funded work by the Cardiac and Vascular Health SPHERE (Maridulu Budyari Gumal) Clinical Academic Network that includes multidisciplinary team from UTS, University of New South Wales, and South-Eastern Sydney LHD (SESLHD) will facilitate the development of a new 3D platform for personalised medicine management of women at high risk of developing heart disease post-hypertensive disorders in pregnancy. She is also evaluating an emerging treatment for preeclampsia based on mesenchymal stem cell-derived extracellular vehicles, funded by the NSW Cardiovascular Research Network (CVRN) through the National Heart Foundation of Australia. Dr McClements contributes to a culture of excellence beyond the borders of her own research including her leadership of the Academic Women in Science (AWiS) network (~100 women) and as the Chair of the Science

Equity and Diversity Committee at UTS. She was awarded high commendation as an Emerging Health Researchers by BUPA Foundation in 2020 and was a finalist in the UTS Vice Chancellor's Award for Research Excellence (Early Career Researcher 2020 and Research Leadership and Development 2022) and 2020 Johnson & Johnson Maternal Health Quickfire Challenge in the USA.

10

Men do more than you think? The contribution of fathers to early embryogenesis.

Nicole McPherson[1]

1. University of Adelaide, Adelaide, SA, Australia

Dr McPherson (BHCS, PhD) is an emerging research leader in male reproductive biology who is currently supported by a DECRA (2022-2024) at the University of Adelaide. Dr McPherson leads the Male Reproductive Life Course Research Group within the Freemasons Centre for Male Health and Wellbeing and the Robinson Research Institute and also holds a Research Scientist position for the Monash IVF Group. Her research vision is for the development of a male-centred approach to pre-conception health and pregnancy care that includes programs focusing on the delivery of tailored pre-conception lifestyle advice to men, male centred pre-conception testing/diagnostics for healthy conception, and advancements in male infertility treatments.

11

Exposure to agricultural azoles disrupts retinoid signalling in fetal rodent testes

Cassy M Spiller[1], **Monica Kam Draskau**[2], **Chun-Wei Allen Feng**[1], **Terje Svingen**[2], **Josephine Bowles**[1]

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2. Division of Diet, Disease Prevention and Toxicology, National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark

Background and Aims: Disorders of human male reproductive health include cryptorchidism, hypospadias, infertility/subfertility, testicular germ cell cancer and primary hypogonadism. The 'testis dysgenesis syndrome' hypothesis proposes that ALL of these problems have a shared origin during fetal life: if testis development is perturbed during a critical window of time whilst in the womb, reproductive health and function is affected. These disorders are escalating at such high rates that it is presumed that environmental causes are to blame, and the key suspect is our increasing exposure to 'endocrine disrupting chemicals'.

The balance of retinoic acid (RA) signalling is particularly crucial for correct fetal testis development: Normally, a P450 enzyme, CYP26B1, degrades RA during testis development, but if CYP26B1 does not function, ectopic RA leads to partial testicular feminisation and perturbation to secondary sexual structures.

Methods: By combining classic developmental biology and mouse transgenic expertise with reproductive toxicology, we have developed a novel ex-vivo testis culture system as a read-out for RA-Cyp26b1 signalling perturbation. Transgenic fetal testes at 12.5 dpc were cultured in hanging drops for 48 hours in the presence or absence of a panel of agricultural chemicals before harvesting for qRT-PCR analysis, histological examination, staining or imaging.

Results: We have used this transgenic system to evaluate the molecular effects of a common agricultural azole, Flusilazole, and related compounds. Flusilazole is a fungicide that works by inhibiting the fungal P450 enzyme CYP51, though it is likely that it can also inhibit mammalian CYP enzymes. We found that ectopic RA signalling could be detected in testes in response to azole exposure at a range of concentrations.

Conclusions and Significance/Impact: Our work indicates possible consequences for reproductive development and function following common azole exposure. Our ongoing work will have future translational importance, in particular, for the refinement of current chemical screening methodologies.

12

Characterising the impact of paternal environmental drivers on seminal plasma composition

David A Skerrett-Byrne[1, 2], **Lachlan M Moldenhauer**[3], **David J Sharkey**[3], **Hon Y Chan**[3], **Peck Y Chin**[3], **Nicole O McPherson**[3], **Tod Fullston**[3], **Cottrell T Tamessar**[1, 2], **Jacinta H Martin**[1, 2], **Natalie A Trigg**[1, 2], **Amanda L Anderson**[1, 2], **Ilana R Bernstein**[1, 2], **Simone J Stanger**[1, 2], **Lily A MacDougall**[1, 2], **Shannon P Smyth**[1, 2], **Elizabeth G Bromfield**[1, 2], **Shaun D Roman**[1, 2], **Brett Nixon**[1, 2], **Sarah A Robertson**[3], **John E Schjenken**[1, 2, 3]

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

Parental lifetime experience influences the health and well-being across generations. Maternal exposures are influential, but paternal exposures are emerging as playing an equally important role. While our understanding of the impact of paternal exposures on offspring health is increasing, the mechanisms underlying these changes remain to be fully defined. To date, studies have largely focused on genetic and epigenetic alterations to sperm cargo as the primary mechanism for the transmission of paternal experience. However, our recent findings demonstrate that non-germ cell factors are sensitive to paternal environmental stressors in a manner that may also influences fetal development and offspring health. Here, we discuss our recent research in mice that explores the influence of a variety of different paternal environmental exposure models, including obesity, heat and reproductive toxicants, on seminal fluid composition and function. Our studies demonstrate that the seminal vesicles, the primary contributor to seminal plasma in mice and most mammalian species, are sensitive to environmental insults. Across all exposure models, we provide evidence that the seminal vesicles respond through altering the composition of their secretions, including cytokines mediating male-to-female signalling at coitus. Using a paternal obesity model, we further demonstrate that these changes ultimately interfere with the establishment of an optimal female reproductive tract immune environment that is required to facilitate embryo implantation and reproductive success. These findings demonstrate that paternal exposures alter the composition of male seminal fluid, including, and raise the prospect that male seminal fluid signalling factors form a novel pathway that contributes to paternal programming.

13

Androgens and the epigenetic clock

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2. Department of Physiology, University of Otago, Dunedin, New Zealand

Epigenetic clocks are powerful biological biomarkers capable of precisely estimating chronological age and identifying novel factors influencing disease pathology using only DNA methylation data. We developed the first epigenetic clock for domesticated sheep (*Ovis aries*), and discovered that castrated male sheep have a decelerated aging rate compared to intact males (Sugrue et al., 2021). We identified several CpG dinucleotides that become progressively hypomethylated with age in intact males, but remain stable in castrated males and females. Using this data as a starting point, we have created a new methylation clock that can predict male-specific aging with surprising accuracy. Functional experiments using hormonal supplementation suggest the 'ticking-rate' of this clock is dependent upon androgen exposure; a finding that could have implications for reproductive disease diagnosis.

1. Sugrue, Victoria J., et al. "Castration delays epigenetic aging and feminizes DNA methylation at androgen-regulated loci." Elife 10 (2021): e64932.

14

New insights into ovarian development and function

Anthony D Bird[1], Diana Vidovic[1], Julia Kaburaki[1], Pascal Bernard[1], Elly Jarred[2], Annie Novak[3], Jeremy Le[1], Joan Jorgensen[3], Patrick Western[2], Andrew J Pask[1], Dagmar Wilhelm[1]

1. University of Melbourne, Melbourne

2. Hudson Institute of Medical Research, Melbourne

3. University of Wisconsin, Madison, USA

Normal development of the ovaries during embryogenesis is critical for their function and fertility of the adult female. Arguably, the most important signalling pathway for normal ovarian development is the canonical WNT signalling pathway activated by WNT4 and RSPO1. Recently, it has been shown that ATP6AP2 functions as a bridge between the WNT receptor LRP6 and the vacuolar H^+-ATPase (V-ATPase). This interaction is crucial for canonical WNT signalling after binding of the ligand. These data, together with our observation that *Atp6ap2* is expressed in the developing gonads, led to our hypothesis that the multi-functional protein ATP6AP2 is important for ovarian development. To test this hypothesis, we generated mice with conditional deletion of *Atp6ap2* in the somatic cells of foetal gonads using the *Nr5a1*-Cre line. Characterisation of this mouse line revealed that ATP6AP2 is important for the formation of primordial follicles, granulosa cell differentiation as well as granulosa cell and ultimately oocyte survival. In conclusion, our data demonstrate that these mice provide a novel experimental system to investigate the development of primordial follicle and the molecular pathways driving this process, which will be crucial for the understanding of physiological as well as pathological development and function of the ovary.

15

How psychological safety can help drive high performing teams

Hayden Fricke[1]

1. Steople, Brighton East, VIC, Australia

Google conducted a global study of all their teams to find out what the most important factors were that drive team performance and productivity. They found that psychological safety accounted for 36% of team productivity. This research brought to light the years of research conducted by Amy Edmondson from Harvard Business School that demonstrated similar outcomes across a range of industries, particularly healthcare settings. Psychological safety is where team members are not fearful of speaking up. Clearly it is incredibly important to team performance but how do you create it? Hayden will share stories of teams that produced incredible outcomes through having high psychological safety. He will provide practical tips and tools to help build psychological safety within multidisciplinary teams.

16

Automated Insulin Delivery

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2. Children's Hospital Westmead, Westmead, NSW, Australia

What you need to know about the Medtronic 780G algorithm, and case study presentations

17

Optogenics to stimulate the steroidogenic pathway

Diane Rebourcet[1]

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Steroid hormones regulate many crucial physiological processes and any alteration in their production or activity can have major pathophysiological implications that can significantly affect quality of life. Whilst hormone replacement therapy can be helpful, its long-term effects remain unclear and require continuous delivery for months/years thus presenting a significant financial burden for healthcare providers. There is a recognized need to develop safer and more effective therapies to support lifelong health, using new technologies. Current developments in gene/cell-based therapies lack regulation and switch on/off accuracy, which remains a critical issue for clinical applications.

Initially developed in neuroscience to control neural activity, optogenetics has provided a new tool-set permitting an unmatched and precise spatiotemporal manipulation of signalling and cellular processes by light. This discovery thus enables technologies where light at specific wavelengths controls gene transcription. This rapidly developing field offers new prospects for the development of precise and regulated biomedical technologies that could be applied to the development of therapies that support healthy, endogenous androgen production.

18

High FSH doses during ovarian stimulation in small ovarian reserve heifers cause follicular hyperstimulation dysgenesis

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2. Victoria University of Wellington, Wellington, WELLINGTON, New Zealand

High gonadotropin doses used during assisted reproductive technology (ART) cycles cause ovulatory follicle dysfunction, oocyte and embryo wastage, and decrease live birth rate. We used the small ovarian reserve heifer model to investigate the hypothesis that excessive FSH doses during ovarian stimulation induce premature luteinization and ovulatory follicle dysfunction.

Compared to heifers treated with an industry standard FSH (70 IU Folltropin-V) dose, we observed heterogeneity in follicular fluid (FF) estradiol:progesterone ratios and cumulus cell-oocyte complex (COC) morphology in follicles from excessive (210 IU) dose treated heifers. Overall, >70% of follicles from 210 IU treated heifers contained expanded COC with the majority also exhibiting increased FF concentrations of key endocrine markers of luteinization (progesterone and/or oxytocin; $P<0.05$). Subsequent RNA-seq analysis identified increasing transcriptome alterations in the oocyte, granulosa and cumulus cells as the severity of follicle phenotypic heterogeneity increased. Ingenuity pathway analysis indicated processes associated with ovulation and luteinization occurred concurrently and that oocyte quality is likely reduced in these follicles.

Thus, excessive FSH doses during ovarian stimulation induced follicular hyperstimulation dysgenesis, characterised by ovulatory follicle dysfunction. These changes result in predicted deficiencies in the oocyte, potentially explaining the negative relationship between excessive FSH doses and ART outcomes.

This project was supported by the NIH-USDA Dual Purpose Program by Agriculture and Food Research Initiative Competitive Grant no 2017-67015-26084 from the USDA National Institute of Food and Agriculture awarded to JJI and KL, and in part by the NIH, Eunice Kennedy Shriver National Institute of Child Health and Human Development (T32HD087166).

Utilising a micro-device for simplifying and improving ART

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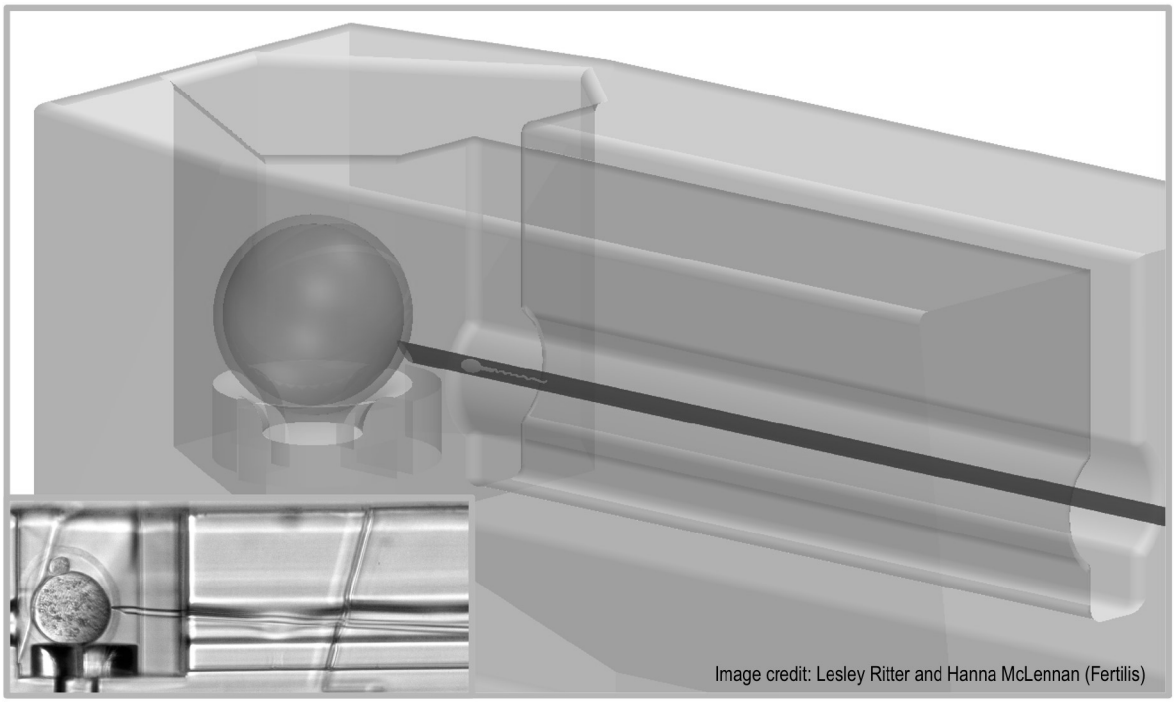

Image legend: Design and fabrication of a device on the micron scale for oocyte microinjection and cryopreservation.

Abstract: Intracytoplasmic sperm injection (ICSI) and oocyte cryopreservation are important assisted reproductive technologies routinely performed in clinical IVF. However, both procedures are technically challenging, requiring manual handling by highly skilled embryologists and adherence to stringent time frames. The stress induced by these processes may lead to poor clinical outcomes. Therefore, we hypothesised that minimisation of oocyte handling will simplify both procedures and in turn, improve IVF outcomes. To address this, we designed and fabricated a micrometre-scale device that houses multiple oocytes in a linear array. The device was fabricated by two-photon polymerisation and consisted of two components: the *Pod* and *Garage*. An individual oocyte is housed within a Pod, with multiple Pods docked into a Garage. To demonstrate the utility of this device for ICSI, presumptive zygotes were microinjected with fluorescent microspheres within the device and cultured to the blastocyst stage. Compared to standard culture, the Pod and Garage had no impact on embryo development and DNA damage levels in resultant blastocysts. Importantly, the device allowed for ICSI to be performed without the need of a holding pipette, thus simplifying the process. To evaluate the suitability of this device for cryopreservation, we demonstrated that the device could withstand repeated freeze-warm cycles with no observable structural impact. Vitrification and warming of oocytes within the device had comparable survival, developmental competency, and metabolic profile to those vitrified using standard practice. Importantly, the device allowed for reduced manual handling of oocytes during cryopreservation. Additionally, vitrification within the device occurred within 3 nL – an approximate 1000-fold reduction in the volume of cytotoxic cryoprotectant solution compared to standard practice. Overall, this work demonstrated the capability of this device to simplify the technically challenging procedures of ICSI and oocyte cryopreservation and may lead to improved outcomes for patients.

Conflict of interests: J. G. Thompson is a Director and Chief Scientific Officer of Fertilis Pty Ltd. All the other authors declare no competing interests. A PCT patent (PCT/AU2020/051318) has been granted.

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New placenta-on-a-chip and bioprinted models of early placenta to elucidate pathogenesis of preeclampsia

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Preeclampsia is a cardiovascular disorder of the second half of pregnancy that is characterised by the new onset of hypertension in conjunction with end-organ damage including placental dysfunction. There are three main phenotypes of preeclampsia: i) early-onset and ii) late-onset preeclampsia diagnosed before 34 weeks or from 34 weeks of gestation, respectively, and iii) post-partum preeclampsia. Despite preeclampsia being the leading cause of morbidity and mortality in pregnancy, still there is no cure. The pathogenesis of preeclampsia is associated with abnormal placentation occurring in the early stages of pregnancy where spiral uterine artery (SUA) remodelling is impaired, which often occurs due to inappropriate function of trophoblast cells (differentiation, migration, invasion) or underlying endothelial dysfunction. Limited knowledge of the molecular and cellular regulation of these aberrant processes has impeded the development of effective treatments for preeclampsia. This is further complicated by the fact that there are differences in the pathogenesis and features between three phenotypes of preeclampsia. Moreover, there is the lack of reliable and specific model systems of human pregnancy. Collecting first trimester placentae or primary trophoblasts is challenging, and it cannot be done routinely. To address this gap, we have developed a low-cost, relevant and reproducible 3D bioprinted model of trophoblast organoids that recapitulates three major trophoblast lineages of human placenta (E-cadherin+ villous cytotrophoblasts, β-hCG+ syncytiotrophoblasts and HLA-G+ extravillous trophoblasts (EVTs)). This 3D model of early placenta can be used as a tool to study early placental development and function. Live cell imaging revealed spontaneous organoid formation from single cells within a few days. Trophoblast organoids also demonstrated invasive capabilities of the matrix. In addition, we established a multicellular placenta-on-a-chip model representative of first trimester trophoblast cell migration and invasion of the endothelial-cell vascular networks as well as the heightened inflammatory environment of preeclampsia, within a microfluidics chip. We have utilised these models to elucidate the role of new angiogenesis- and inflammation-related signalling mechanisms on placental development and growth, in the context of preeclampsia. Similarly, the 3D placental platforms can be used for high-throughput screening of clinically available and emerging treatments for preeclampsia by investigating the effects/mechanisms on trophoblast differentiation, migration, invasion and remodelling of the vasculature or vascular dysfunction.

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Inequities in endocrine diseases among indigenous populations

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Reproduction, and the process of replenishing the next generation of whānau and family is a highly regarded, celebrated and culturally significant process for many Māori and Pacific people. However, difficulties conceiving and maintaining a pregnancy can pierce through these sociocultural ideals, shaping discordant realities that render material, social and psychological challenges. While studies of Māori perspectives on assisted reproductive technologies (Glover et al., 2008), Māori fertility and infertility (Reynolds & Smith, 2012), have been undertaken, technological innovation and social change has been rapid, with new logics and metrics to understand and interpret through cultural ways of making meaning. While important insights have been gleaned for Pacific women's experiences of infertility (Foaese, 2018), and the implications of the Body Mass Index for Māori and Pacific access to fertility care (Parker & Le Grice, 2022; Shaw & Fehoko, 2022) there remains further areas to glean insight into Māori & Pacific experiences with fertility services. Here, I present on a qualitative focus group study supported by Fertility New Zealand & The University of Auckland. Six focus groups were held with 19 Māori & Pacific participants between 2020 and 2021, and accounts from these participants were analysed and made meaning of through thematic analysis. In this talk, I explore some of the interconnected themes that foreground the challenges raised by infertility across participants' social and whānau lives, intimate relationships, the impacts of grief, loss, and trauma, and struggles with racialised exclusion. There is an urgent need for holistic and integrative pathways to healing from the material, social and psychological challenges of infertility, drawing from the deep puna of mātauranga and Pacific knowledges across Te Moana Nui a Kiwa.

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Partnerships, Reciprocity, and Co-design: How to work effectively with First Nations people and Research

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This presentation will explore 2 case studies that show the efficacy of working collectively and collaborative with First Nations communities. The objective is to provide strategies that if employed may help to support the development of effective partnerships between Aboriginal Nations peoples and their communities? Moving beyond codesign and embracing co-decision making principles.

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Pacific youth, sexuality and reproduction: A Pacific approach towards tapu (forbidden) research topics

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Research involving Pacific peoples must be culturally responsive to participants' needs, especially if the research topic is tapu (forbidden, sacred) like sexuality and reproduction. This can be achieved by employing Pacific research epistemologies, methods and methodologies. I present our study, Te Tīpani Project, to illustrate the use of multiple Pacific epistemologies when investigating Pacific youth knowledges of sexuality and reproduction. We revitalised a popular Pacific research paradigm (Fonofale) and used the Kakala research methodology to guide our steps. First, we consulted with community experts to understand the needs of our cohort. Second, we constructed a preliminary online survey, completed by eighty-one Pacific tertiary students, and interviewed eight respondents using the Talanoa method. Third, we committed to disseminating our findings back to the community through various initiatives. This study highlighted strengths previously unidentified: Participants had complex understandings of sexuality and reproduction; Formal and non-formal learning environments both played vital roles in participants' knowledge acquisition; Participants demonstrated help-seeking behaviours when faced with sexual and reproductive challenges. Using Pacific research epistemologies enabled us to approach our cohort with cultural consideration and responsiveness. This study initiated a relationship with our local Pacific community and supports ongoing research improving teaching pedagogies within anatomical science education.

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The Gomeroi Gaaynggal Study: Improving Outcomes for Aboriginal Women and their Babies

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The Gomeroi Gaaynggal (babies from the Gomeroi lands) cohort is the largest longitudinal cohort of Indigenous women and their children that begins in pregnancy and continues through early childhood worldwide. It is based in the regional town of Tamworth, NSW, within the Aboriginal land of the Kamilaroi/Gomeroi people. The Gomeroi Gaaynggal study was developed in partnership with the local Aboriginal community to address the disparity in health outcomes in their community. It was run alongside an Aboriginal Arts Health Program. Between 2009 and 2019, 403 pregnant women participated in the pregnancy study and 185 women and their children participated in the follow up study. During pregnancy we collected data on maternal nutrition and body composition, mental and physical health, fetal growth, and pregnancy outcomes. In the follow up study we collected data on maternal health, breastfeeding, introduction of first foods, and growth of the children. We are continuing to work with the community to develop community-based programs to provide a healthy start to life for all Aboriginal Australian children.

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High saturated-fat diet is lipotoxic for human islets, compared to preservation of beta-cell function with high monounsaturated fat diet

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Chronic consumption of high saturated-fat diet (HSFD) in animals causes beta-cell dysfunction due to lipotoxicity. Lipotoxicity in vivo in human islets is currently unproven.

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

METHODS: Immunodeficient RAG1-null mice (C57Bl/6 background) were studied. Recipients were made diabetic by Streptozotocin. 40 female mice received 2000IEQ human islets from normal glucose tolerant donors. Islets were isolated from research-consented organ donors at Tom Mandel islet transplant program, Melbourne. Mice with functioning grafts (random-fed BGL (rBGL) <10mmol/L, n=33) were then fed chow, high-saturated (HSFD, 45% calories from lipids) or high monounsaturated (MUFD, 45% of calories from lipids). Glucose tolerance tests (GTT) were performed before and during assigned diets. Energy expenditure was measured in metabolic cages.

RESULTS: Mice fed HSFD gained >10% of body-mass by 16 weeks of diet. In contrast, MUFD mice had significantly lower weight-gain which was not different from chow mice. Food intake was not significantly different between MUFD and HSFD

mice, nor was voluntary exercise. By mixed model analysis with Tukey's correction for multiple comparisons, GTT was significantly worse in HSFD mice versus chow ($p<0.0001$), but not MUFD vs chow.

At cull, HSFD mice had greater adipose tissue mass: inguinal $p<0.005$ versus MUFD, $p<0.005$ versus chow, epididymal fat $p<0.005$ versus MUFD and $p<0.0005$ vs chow. Mice fed HSFD also had reduced graft final beta-cell volume which was 46% lower than chow and 23% lower than MUFD.

CONCLUSION: HSFD caused weight-gain and detrimental effects on human islets even though 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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Detecting primary aldosteronism in Australian primary care: a prospective study

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Objective: To identify primary aldosteronism (PA) in newly diagnosed, treatment-naïve, hypertensive patients in primary care.

Design: Prospective study conducted in 2017-2020.

Setting: General Practitioners (GPs) from multiple practices across Melbourne (Victoria) were invited to screen their patients for PA at the time of the diagnosis of hypertension. Screening for PA was performed by measuring the aldosterone-to-renin ratio (ARR). Those with ARR ≥70 pmol/mU underwent the saline suppression test in a specialist referral centre to confirm the diagnosis of PA.

Participants: 247 primary care adults with blood pressure >140/90 mmHg on two or more occasions and not taking antihypertensive medications.

Main outcome measures: The diagnostic rate of PA, calculated as the percentage of patients with confirmed PA divided by the number of hypertensive patients screened for PA.

Results: Among the 247 participants, 62 (25%) had a positive screening test result and 35 (14% of all the participants; 95% confidence interval 10% to 19%) were confirmed to have PA. None of the patient characteristics (age, sex, blood pressure or serum potassium) distinguished the PA from the non-PA group.

Conclusion: PA was diagnosed in 14% of patients with newly diagnosed hypertension in primary care and GPs have an important role in actively screening for this specifically treatable cause of hypertension.

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Improving the Diagnostic Accuracy and Treatment Decisions in the Face of Changing Epidemiology of Hypogonadism in Men

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Testosterone levels decline gradually with advancing age; the trajectory of age-related decline in testosterone levels is influenced by adiposity, co-morbid conditions, and genetic factors. Low testosterone levels in men are associated with low sexual desire and erectile dysfunction; reduced muscle mass and strength, and impaired physical function; decreased bone mineral density (BMD) and increased risk of osteoporotic fractures. Low testosterone as well as SHBG levels are each independently associated with increased risk of type 2 diabetes mellitus (T2DM) and all-cause mortality. it is possible that low testosterone level is a marker of poor health.

Testosterone treatment of older men with low libido and low testosterone levels improves sexual activity, sexual desire, and erectile function. Testosterone treatment increases muscle mass, muscle strength and leg power, and modestly improves stair climbing power, aerobic capacity, and self-reported mobility. Testosterone modestly improves depressive symptoms and corrects unexplained anemia of aging. Testosterone treatment of older hypogonadal men increases areal and volumetric BMD and estimated bone strength in the hip and spine. Testosterone administration reduces whole body and visceral fat mass. In the T4DM Trial, testosterone treatment administered in conjunction with a lifestyle program for 2 years was more efficacious than placebo in reducing the proportion of men with diabetes.

The adverse effects of testosterone treatment include erythrocytosis, growth of metastatic prostate cancer, reduced sperm production, and increased risk of detection of subclinical prostate cancer. Testosterone treatment does not worsen lower urinary tract symptoms. However, no adequately-powered trial of sufficiently long duration has been conducted to determine the effects of testosterone on the risk of prostate cancer or major adverse cardiovascular events (MACE). An ongoing

randomized trial (TRAVERSE Trial) in hypogonadal men, 45-80 years, at increased cardiovascular risk, will provide definitive information on the effects of long-term testosterone treatment on MACE and other efficacy and safety outcomes.

Because of the lack of evidence of long-term safety and limited evidence of long-term efficacy, testosterone treatment of *all* older men with low testosterone levels is not justified. Instead, an expert panel of the US Endocrine Society suggested that *testosterone therapy should be offered on an individualized basis...in men >65 years who have symptoms or conditions suggestive of testosterone deficiency (e.g., low libido or unexplained anemia) and consistently low testosterone".* The decision to offer testosterone treatment to older men with testosterone deficiency should be guided by an individualized assessment of potential benefits and risks, the burden of symptoms, and patient preferences. A shared decision to initiate testosterone treatment should be accompanied by a standardized monitoring plan.

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Improving outcomes with diabetes technology from early childhood to young adulthood: 4T Study

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Almost 30 years ago the Diabetes Control and Complications Trial demonstrated the benefit of intensive diabetes management to improve vascular outcomes and mortality. However, Pediatric Diabetes clinics have not achieved these HbA1c outcomes in practice. Advances in diabetes technology have been dramatic, but inequity in access to these technologies has increased disparities in diabetes outcomes in the US. Healthcare systems and processes of care can be challenges to achieving optimal outcomes. The Stanford Pediatric Diabetes team developed the Teamwork, Technology, and Targets for Tight Control: 4T Study to improve care and outcomes for children and adolescents newly diagnosed with type 1 diabetes (T1D). Background on outcomes including HbA1c and hypoglycemia, role of quality improvement, diabetes technology, and then methods and outcomes from the 4T Study will be reviewed. A goal of the 4T Study is to share and collaborate with other diabetes clinics materials and concepts developed to improve care and outcome for people with diabetes.

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Activin A levels regulate fetal testicular macrophages in mice

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There is accumulating evidence that immune cells serve central roles in testis development. Our recent work delineated immune cell emergence in human and murine fetal testes documented innate immune cells' early arrival and predominance (Hosseini et al. 2022, and unpublished). In human pregnancies, activin A levels can be altered in conditions such as maternal infection, preeclampsia and pharmaceutical ingestion, and this could significantly impact macrophage function and polarization towards pro-inflammatory or anti-inflammatory phenotypes. This study investigates whether activin A levels influence the establishment of fetal testicular macrophages in the mouse by interrogating their number, distribution and phenotype. Whole testes were collected from two knockout (KO) mouse models, *Inhba* (lacking activin A) and *Inha* (high activin), with wildtype (WT) littermates at embryonic day (E)13.5, E15.5, and postnatal day 0 (PND0). Samples were fixed in 4% paraformaldehyde and paraffin-embedded for immunohistochemistry (IHC F4/80, to detect macrophages; n=4-8 per age per genotype) and snap-frozen to measure transcripts encoding 33 proteins found in immune cells using Fluidigm qRT-PCR (n=3/age genotype). Total macrophage numbers per cross-sectional area were significantly higher in testes of *Inha* KO mice (high activin A) than in *Inhba* KO (no activin A) at E13.5 and E15.5. Macrophages in *Inhba* KO testes were preferentially in the testis perimeter at all ages. Fluidigm revealed a significant association between activin A and mRNA levels for immune mediators that induce a pro- or anti-inflammatory microenvironment and proteins involved in migration. A reciprocal dose-dependent effect of activin A levels was identified for *CX3CL1, IL-4* and *IL-10* receptors, *MHC class II, CCL17, CXCR7, CXCR4* and *Marco* transcripts. RNAseq data validated this outcome. In summary, this study demonstrates that activin A can significantly regulate testicular macrophage number and distribution pattern in the fetal testis. This may occur by modulating the synthesis of factors regulating macrophage functions.

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Genome-wide epigenetic reprogramming of the marsupial germline

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During germline development in eutherian (placental) mammals, gamete precursors (primordial germ cells) undergo genome-wide erasure of epigenetic marks (1,2). This process, termed epigenetic reprogramming, safe-guards against precocious germline differentiation until the gonadal niche is sufficiently developed to dictate male or female fate, at which point methylation levels are restored (3).

While this process is critical to development of the eutherian germline, its role in other vertebrate groups is largely unknown. Evidence from zebrafish (4) suggests that reprogramming is not shared by non-mammalian vertebrates and arose somewhere in the mammalian lineage. Marsupials—the sister group to eutherian mammals—are ideally placed to test this hypothesis.

Since marsupials have short pregnancies and are born highly altricial, germline development occurs post-natally (5). We used post-bisulfite adaptor tagging (PBAT; 6) and deep sequencing to assess global levels of DNA methylation in PGCs isolated from brushtail possum pouch young (*Trichosurus vulpecula*) across early development (2 to 80 days post-partum (dpp)). Previous work has established that at least one imprinted region is reprogrammed in a marsupial (7), but ours is the first study to investigate genome-wide methylation dynamics.

We found that global levels of DNA methylation decreased from 62 to 41% between 2 and 13 dpp, developmentally equivalent to humans and mice (1,2). PGCs remained hypomethylated for approximately 10 days and methylation was restored (65%) by 38 dpp. Methylation loss occurred primarily at CpG islands while repeat elements retained almost all methylation, as for eutherian mammals (1).

Our findings demonstrate that broad patterns of reprogramming are conserved in both mammalian groups. However, since marsupial PGCs retain substantial levels of global methylation (>40% compared with 14-7% in mice) the marsupial pattern involves global methylation reduction, not complete erasure. Like non-mammalian vertebrates, the marsupial germline may thus hold greater potential for transgenerational epigenetic inheritance (4).

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Gonadal development and primordial germ cell migration in the fat-tailed dunnart (*Sminthopsis crassicaudata)*

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We are developing the fat-tailed dunnart as a novel laboratory model in which we can develop advanced genetic engineering and assisted reproductive technologies for marsupials. My project defined the process of early gonad formation in this species. Typically, the process of gonadal sex determination begins around the time of birth or shortly thereafter in marsupials. As in all mammals, the testis begins to differentiate before the ovary, with SOX9 expression in developing Sertoli cells being one of the first indications that the gonad has passed the indifferent stage. We examined gonadal development in the fat-tailed dunnart using histology and immunofluorescence staining. We found that testis differentiation begins at 2 days post-partum (d.p.p.) with expression of SOX9 in the testis primordia. Clear ovarian differentiation was evident by day 8 d.p.p. These processes seem to occur around the same time relative to birth as seen in less altricial marsupials.

In mammals, primordial germ cells arise outside of the embryo and must undergo migration in order to reach the developing gonad. Once in the gonad, the surrounding somatic environment dictates their differentiation into sperm or eggs. We saw arrival of putative primordial germ cells (PGCs) in the gonads of both sexes at 2 d.p.p. The primary route for PGC migration in eutherian mammals is often through the hindgut and dorsal mesentery. In marsupials, the migratory path of PGCs is variable. For example, in the bandicoot PGCs migrate through the hindgut and dorsal mesentery, however in the tammar and brushtail possum, PGCs are excluded from the hindgut entirely. We are aiming to characterise the PGC migration pathway in the fat-tailed dunnart to help better understand early developmental process in the dunnart. Improving our knowledge of this species' biology will support its establishment as a practical and robust and laboratory model for marsupial research.

Quintessential requirement of maternal PRDM10 for female fertility and successful embryonic development

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Publish consent withheld

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Exposure to diethylstilbestrol causes transgenerational effects on female fertility and reproductive development

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Significant decreases in both male and female fertility have been observed over the past 50 years, with female conceptions rates dropping by 44% and male sperm counts decreasing by over 50%. This dramatic decrease in fertility can be attributed in part to our increasing exposure to endocrine disrupting chemicals (EDCs). Diethylstilbestrol (DES) is an estrogenic EDC that was prescribed to millions of pregnant women between 1940-1970 and resulted in reproductive defects in the offspring that were exposed *in utero*. Women who were exposed to DES *in utero* experienced higher rates of infertility, pregnancy complications and reproductive cancers. Alarmingly, there is evidence to suggest that these effects may persist in the grandchildren and great grandchildren of exposed women. To define the transgenerational reproductive impacts in females following exposure to DES, gestating F0 female mice were exposed to 1, 50 or 100ug/kg of DES. The effects of DES were monitored in the F1-F4 female descendants. Reductions in pregnancy rate and fertility index were observed up until the third generation and the onset of puberty was significantly affected, with the timing of vaginal opening occurring significantly earlier in DES descendants. The anogenital distance (AGD) was also impacted in DES descendants with all concentrations resulting in a significantly smaller AGD up until the third, unexposed generation. Furthermore, alterations to the reproductive tract were also observed, with DES descendants presenting higher rates of urethral-vaginal fistulae. These results indicate a transgenerational effect of DES on multiple reproductive parameters including fertility, timing of puberty, AGD and reproductive tract development. These data have significant implications for the >50 million DES descendants worldwide as well as raising concerns for the ongoing health impacts caused by exposures to other estrogenic EDCs which are pervasive in our environment.

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MEK1/2 regulates male germline development in an FGF independent manner

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Male or female germline development depends on sex-specific somatic signalling in the developing testis or ovary. Disrupted testis and germline development is strongly associated with testis cancer in humans. In mice, *Sry* and *Sox9* promote testis and male germline development by inducing genes, including *Fgf9,* that promote testis formation and inhibit ovarian development. FGF9 is required for testis development and has been implicated as a key determinant of male germline differentiation, however, the mechanisms through which it signals are unknown. As FGFs signal through Mitogen-Activated Protein Kinase (MAPK) in other tissues, we explored whether FGF9 regulates male germline development through MAPK using an *ex vivo* organ culture model. Embryonic day (E)11.5-12.5 Oct4GFP transgenic mouse testes were cultured with FGF receptor or MEK1/2 inhibitors for 24, 72 or 96 hours, with impacts on germ cell development determined using flow cytometry, immunofluorescence and RNA sequencing. Inhibition of MEK1/2 blocked mitotic arrest and broadly disrupted the transcription of male germline markers and upregulation of key male germline proteins DPPA4 and DNMT3L. Surprisingly, despite FGF signalling inhibition from E12.5 for 72 hours, germ cells entered mitotic arrest normally and expressed the male specific transcriptional program, although mitotic arrest was mildly disrupted following inhibition from E11.5 for 96 hours. RNA sequencing in isolated germ cells identified 25 and 1403 genes that were not properly expressed after 24 and 72 hours of MEK1/2 inhibition, but only 43 genes and 1 gene after 24 and 72 hours of FGF receptor inhibition. Together, these data indicate essential roles for MEK1/2 signalling in male germline differentiation, but a surprisingly limited role for FGF signalling. Our data strongly indicate that additional ligands acting through MEK1/2 play a significant role in

male germline differentiation and highlights a need for further understanding of the mechanisms underlying male germline development.

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Impact of discrete wavelengths of light on embryo development

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Embryo development in vivo occurs within a tightly regulated microenvironment. However, this is not the case when undergoing IVF. In this instance, embryos are exposed to light sources that are not normally present in vivo, e.g. during microscopic inspection. Previous studies have indicated a potential negative impact of such light exposure on embryo health. For the first time, we conducted a study that carefully accounted for uniform light illumination across the sample, so that the overall optical energy dose applied was consistent between wavelengths and that the wavelengths were narrow band sources in the visible range, thus mimicking light sources commonly used in fluorescence microscopy (470 – 620 nm).

Preimplantation mouse embryos were exposed daily to blue (470 nm), green (520 nm), yellow (590 nm) or red (620 nm) wavelengths and compared to embryos that were not exposed. We assessed embryo development, DNA damage, and postnatal outcomes. We found exposure to the yellow wavelength significantly impaired embryo development to the blastocyst stage ($P < 0.05$). While exposure to blue, green and red wavelengths resulted in significantly higher levels of DNA damage when compared to unexposed embryos ($P < 0.05$). The pregnancy rate was significantly lower when embryos were exposed to the red wavelength ($P < 0.05$). Interestingly, resultant offspring were significantly heavier when derived from red or yellow light exposed embryos compared to those derived from unexposed embryos ($P < 0.01$). Towards understanding the effect on offspring weight we assessed intracellular lipid abundance in the embryo. We found lipid abundance to be significantly elevated following exposure to yellow wavelength (1.8-fold, $P < 0.0001$) but not red. Red and yellow wavelengths are widely considered benign and utilized clinically in time-lapse equipped incubators within IVF clinics. Our results demonstrate the need to re-evaluate these assumptions.

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Sex chromosome dynamics during meiotic prophase I in the Marsupial germ line

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During spermatogenesis, genetically variable haploid gametes are generated in a tightly regulated process called meiosis, which includes homologous chromosome pairing, synapsis and recombination. Errors in these processes may lead to aneuploidy and infertility problems. The presence of partially or completely unsynapsed regions induces a transcriptional silencing checkpoint, including the meiotic sex chromosome inactivation (MSCI), to avoid the premature expression of genes that would induce meiotic arrest at pachytene[1,2]. In marsupials, sex chromosomes lack a pseudo-autosomal region, so the association via a marsupial specific structure called the dense plate (DP) ensures faithful segregation of sex chromosomes in the absence of synapsis and recombination[3]. Due to their key basal position in the mammalian evolutionary tree, marsupials offer a unique opportunity to explore previously uncharacterized meiotic features, from sex chromosome pairing strategies to X chromosome transcription dynamics. Here, we combine cytological analysis and single-cell RNA sequencing to study the sex chromosome dynamics during meiotic progression in the Australian marsupial tammar wallaby. Our results show that sex chromosomes pair forming the so-called dense plate following different sex chromosome pairing strategies in marsupial species, which correlates with differential sex chromosomes architecture and transcriptional patterns. Remarkably, we detected that the tammar X chromosome is partially transcribed during meiosis and escapes MSCI for much of pachytene, which has important implications for sex chromosome evolution.

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From trash to treasure: *Inha*KO testis tumours produce growth factors that support SSC renewal

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Testicular somatic cell tumours represent ~5% of tumours in human adults and ~40% in children (1). Adult *Inha*KO mouse testes feature elevated (>10-fold) activin A bioactivity and develop focally invasive tumours thought to be derived from Sertoli cells (2, 3). I have used this model to study how elevated activin affects spermatogonial stem cells (SSCs) in newborn and adult testes. Unexpectedly, I discovered increased numbers of SSCs (GFRA1+/SALL4+) in *Inha*KO 'normal' tubules, with SSCs clusters (GFRA1+/EOMES+/LIN28-) present in tubules adjacent to tumours. Additionally, immunofluorescence (IF) staining of *Inha*KO tumours in adult testes revealed they are predominantly negative for the Sertoli cell marker, SOX9. These intriguing results formed the basis of this study which aims to identify (1) growth factors produced by *Inha*KO tumours important for SSC renewal, and (2) delineate the cellular origin of *Inha*KO tumours.

Adult testes (PND53-55) from *Inha*WT and KO mice (n=3/genotype) were decapsulated. Tubules with 'normal' spermatogenesis, tumour regions, and tumour-associated tubules were microdissected for RNA extraction. RNA integrity was confirmed prior to whole transcriptomic RNAseq (MHTP Medical Genomics Facility) and bioinformatic analysis.

Proximity to tumours was associated with increased DEGs compared to WT ('normal', 38; tumour associated tubules, 4939; tumours, 12,983 DEGs). Transcripts encoding growth factors important for SSC self-renewal (*Gdnf*, *Lif*, *Fgf2*) were elevated in tumour-associated tubules and in tumour regions. Tumour regions exhibited reduced germ (*Ddx4*) and Leydig (*Insl3*), and increased immune (*Cx3cr1*, *Csf1r*) cell transcripts. Some Sertoli cell transcripts were decreased (*Sox9*, *Wt1*) reflective of IF data, while others were increased (*Gja1*, *Clu*, *Vim*), indicating these tumours comprise Sertoli cells with a modified transcriptomic state.

This demonstrates the *Inha*KO mice are an elegant model with which to identify new factors important to SSC biology, while offering new insights into the origins and transcriptomic landscape of these somatic cell tumours.

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Defining the core sperm proteome; Highly conserved targets for reproductive biology research

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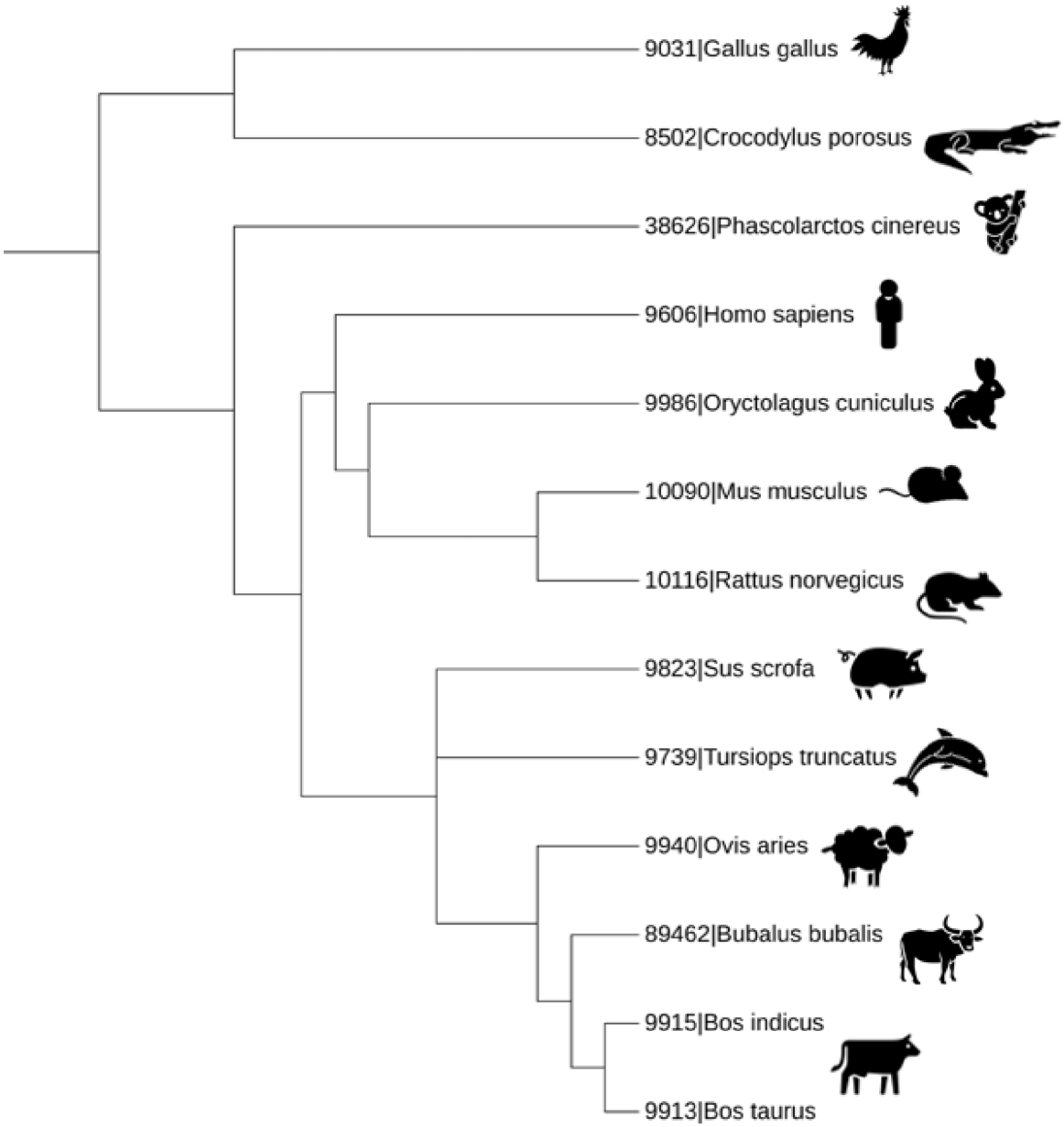
Reproductive biology is often considered in the three siloed areas of humans, domesticated animals and wildlife. There are common needs across these species, notably treatment of infertility, development of assisted reproductive technologies and effective contraception. To efficiently develop solutions applicable to all species, we must develop a better understanding of the common biology underpinning reproductive processes. To this end, we performed an *in-silico* analysis of publicly available sperm proteomic data to define the core sperm proteome; a collection of highly conserved proteins which are critical for sperm structure and function. >2TB of RAW spectral data was sourced from ProteomeXchange and processed through a strict, uniform search and ID validation in-house pipeline. Sperm proteome data was available for 18 vertebrate species, however due to data quality, only 12 species were analysed (Figure 1). A combined total of 12,144 unique proteins were identified, highly biased towards human (9186 IDs total) and mouse (4462 IDs total). In some species >90% of the proteins identified were only predicted or inferred from homology, indicating that experimental evidence for the existence of most proteins remains poor in non-model species. While the mouse sperm proteome captured almost all proteins present in other species (e.g. 93.1% of koala, 89.8% of pig), less than half (42.0%) of the mouse sperm proteome was common to the extensive human sperm proteome. A total of 27 proteins were conserved across all species, with significant enrichment for proteins involved in acetylation and phosphorylation, proteins contained within secretory granules, chaperones, proteasome function and glycolysis. A total of 85 proteins were conserved across all orders, showcasing additional enrichment for mitochondrial respiration. These early results suggest that there are key conserved pathways likely to be critical for sperm function in all species, particularly post translational modification, protein folding and recycling, acrosome function and energy generation.



Male mammalian meiosis is critically dependent on the shared functions of the katanin proteins KATNA1 and KATNAL1

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Katanin microtubule-severing enzymes are key microtubule regulators. Previously, we showed the katanin regulatory B-subunit, KATNB1, is essential for male meiosis and spermiogenesis. While our data suggests the katanin enzymatic A-subunit KATNAL2 mediates KATNB1 function in spermiogenesis, the enzyme(s) mediating KATNB1 meiosis functions remain unclear. Herein, we sought to characterise the role of katanin A-subunits KATNA1 and KATNAL1 in male germ cells.

To study this, we used *Stra8-Cre* to generate *Katna1* and *Katnal1* germ cell-specific knockout (*Katna1*$^{GCKO/GCKO}$ and *Katnal1*$^{GCKO/GCKO}$) models, in addition to a *Katna1* and *Katnal1* double GCKO (*Katna1/al1*$^{GCKO/GCKO}$) model. While single GCKO of *Katna1* revealed it is not essential for spermatogenesis, single *Katnal1* deletion revealed germ cell-

KATNAL1 is required for optimal male fertility. *Katnal1*$^{GCKO/GCKO}$ males were subfertile, exhibited a 42.3% reduction in daily sperm production and, due to spermiation failure, a more dramatic 73.7% reduction in epididymal sperm number. Of the sperm present, they were abnormal with reduced motility. Analysis of *Katnal1*$^{GCKO/GCKO}$ meiosis, revealed chromosome alignment, segregation, and cytokinesis defects, however most cells completed meiosis. During spermiogenesis, we found KATNAL1 regulates axoneme and head-to-tail coupling apparatus formation and manchette-dependent head shaping. More interestingly however, double *Katna1* and *Katnal1* GCKO resulted in complete male sterility and a phenotype much worse than in *Katnal1*$^{GCKO/GCKO}$. *Katna1*/*al1*$^{GCKO/GCKO}$ daily sperm production and epididymal sperm count were reduced by 86.9% and 96.2%, due to catastrophic meiosis and spermiogenesis defects. *Katna1*/*al1*$^{GCKO/GCKO}$ spermatocytes frequently stalled and underwent apoptosis in metaphase and anaphase. Moreover, of the few spermatids produced, they exhibited abnormal vesicle trafficking during acrosome biogenesis, followed by global microtubule dysregulation, ultimately becoming pyknotic by step 13. This study establishes KATNA1 and KATNAL1 as collective mediators KATNB1 meiosis functions and reveals they function in a compensatory manner to regulate microtubule dynamics and bulk during multiple aspects of male germ cell development.

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Importins in male germline differentiation and function: localization using Structured Illumination Microscopy

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To learn why fertilization failure occurs, we seek to delineate the processes which underpin normal sperm formation. Importins are soluble proteins that selectively mediate active protein cargo transport from the cytoplasm into the nucleus. Their levels and actions control transcription, embryonic stem cell pluripotency, cellular stress adaptation, nuclear envelope assembly, and spindle formation. These highly conserved genes are essential for gamete formation in many species. We examined the distribution of four importins in adult mouse testis (C57BL/6J strain) and mature sperm, a2, a3, a4 and b3, that are encoded by genes and proteins with different expression profiles in individual germ cell populations during spermatogenesis. Using Western blots probed with commercially-supplied antibodies, these proteins were identified as present in mouse sperm (from cauda epididymal/vas deferens). These antibodies were applied in three individual experiments to fixed, paraffin-embedded testis sections and sperm captured (n=15) by Structured Illumination Microscopy (SIM) and immunofluorescent imaging. Importin subcellular localization in adult mouse testis was mapped in relationship to germ cell differentiation status and acrosome formation, with results reinforcing their potential for individual functions in sperm differentiation. We compared differences of their localization in acrosome-intact and acrosome-reacted (AR) sperm before and after the AR, which could suggest potential scaffolding and/or subcellular delivery roles. Of particular interest was the different relocation behavior between the importins aand b3 after the AR. Although all were present in the apical acrosome of the acrosome-intact sperm head, after the AR, importins a2 and a4 relocated into the sperm connecting piece, in contrast to a3, which stayed static. Further, b3 initially relocalized to equatorial segment and later covered the whole sperm head. These findings bring novel information about behavior of importins during the AR, a critical final step of sperm maturation that determines the ability of sperm to fuse with the egg.

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Examining the developmental contributions of the phospholipidome and proteome to stage-specific oxidative stress sensitivity in the male germline

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Spermatozoa are known to be amongst the most environmentally responsive cells in the body, which grants them the capacity to sense critical cues in the female reproductive tract that signal readiness for fertilisation. However, this sensitivity also culminates in a mature sperm cell that is highly vulnerable to exogenous oxidative stress. While a notable lack of cytoplasmic antioxidant content underpins a degree of this stress sensitivity, mammalian spermatozoa have been historically charted to possess a phospholipidome replete with an abundance of polyunsaturated fatty acids (PUFA-PLs) that are key substrates for oxidative attack. Despite this long-held view, we have no clear information regarding which stages of germ cell maturation are specifically enriched in PUFA-PLs and which enzymes contribute to these membrane characteristics.

In this study, we examined the phospholipidome and lipid modulating-proteome of three distinct mouse germ cell stages, pachytene spermatocytes (PSc), round spermatids (RSt) and spermatozoa, through high-resolution tandem mass spectrometry approaches. These approaches demonstrated that spermiogenesis results in an enrichment of ester and ether-linked phosphatidylethanolamines (PE), the latter of which may indicate an intrinsic sensitivity to the cell death modality ferroptosis ($P<0.05$). While spermiogenesis gave rise to widespread and significant ($P<0.05$, 1.5-fold change) changes in phospholipid abundance, synthesis/remodelling of lipids was more profound during the maturation of PSc to RSt. Examining lipid structural characteristics revealed a significant increase ($P<0.01$) in PUFA-PLs containing six or more double bonds in RSt compared to PSc, indicating a profound increase in oxidation potential in RSt. In accounting for this enrichment in PUFA-PLs, our proteomic data revealed the presence of several PL remodelling enzymes, including lysophosphatidylcholine acyltransferase 3 (LPCAT3), in RSt that were absent or lowly expressed in PSc. Further examination of these data will permit the design of novel stage-specific strategies to fortify germ cell membranes during periods of environmental stress.

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CEP76 is a centriole and transition zone protein required for sperm tail formation and male fertility

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Sperm tail development is a complex process that requires the selective transport of thousands of proteins through the ciliary gate/transition zone and then to precise locations within the ciliary compartment (tail). We have recently identified a damaging point mutation in the centriole gene *CEP76* in an infertile man. *Cep76* is highly testis expressed and is enriched within spermatids. To test the role of CEP76 in sperm transition zone function and male fertility we generated a knockout mouse model. *Cep76* knockout males were sterile due to the absence of progressive sperm motility. In addition, sperm were on average 15% shorter than sperm from wild type littermates ($p < 0.0001$). Further, sperm head morphology was abnormal in 40% of knockout cells ($p < 0.0001$) and decapitation was three-fold more frequent ($p < 0.001$). We used electron microscopy to further explore these structural defects, which revealed several significant tail abnormalities – the absence of inner dynein arms in the tail axoneme, the absence of a clear annulus structure, abnormal mitochondrial morphology, and duplicated neck structures in knockout sperm. A proteomic analysis of wild type and *Cep76* knockout sperm revealed 32 differentially regulated proteins, including several mitochondrial proteins and DNAH2 – a key component of inner dynein arms. Localisation of DNAH2 via immunofluorescence revealed it to be ectopically localised at the sperm neck in knockout cells, consistent with impaired DNAH2 loading into the sperm tail. Collectively, our data identify CEP76 as a key gatekeeper of protein entry into the developing sperm tail.

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Phosphoproteomic analysis of the adaption of epididymal tissue to corticosterone challenge

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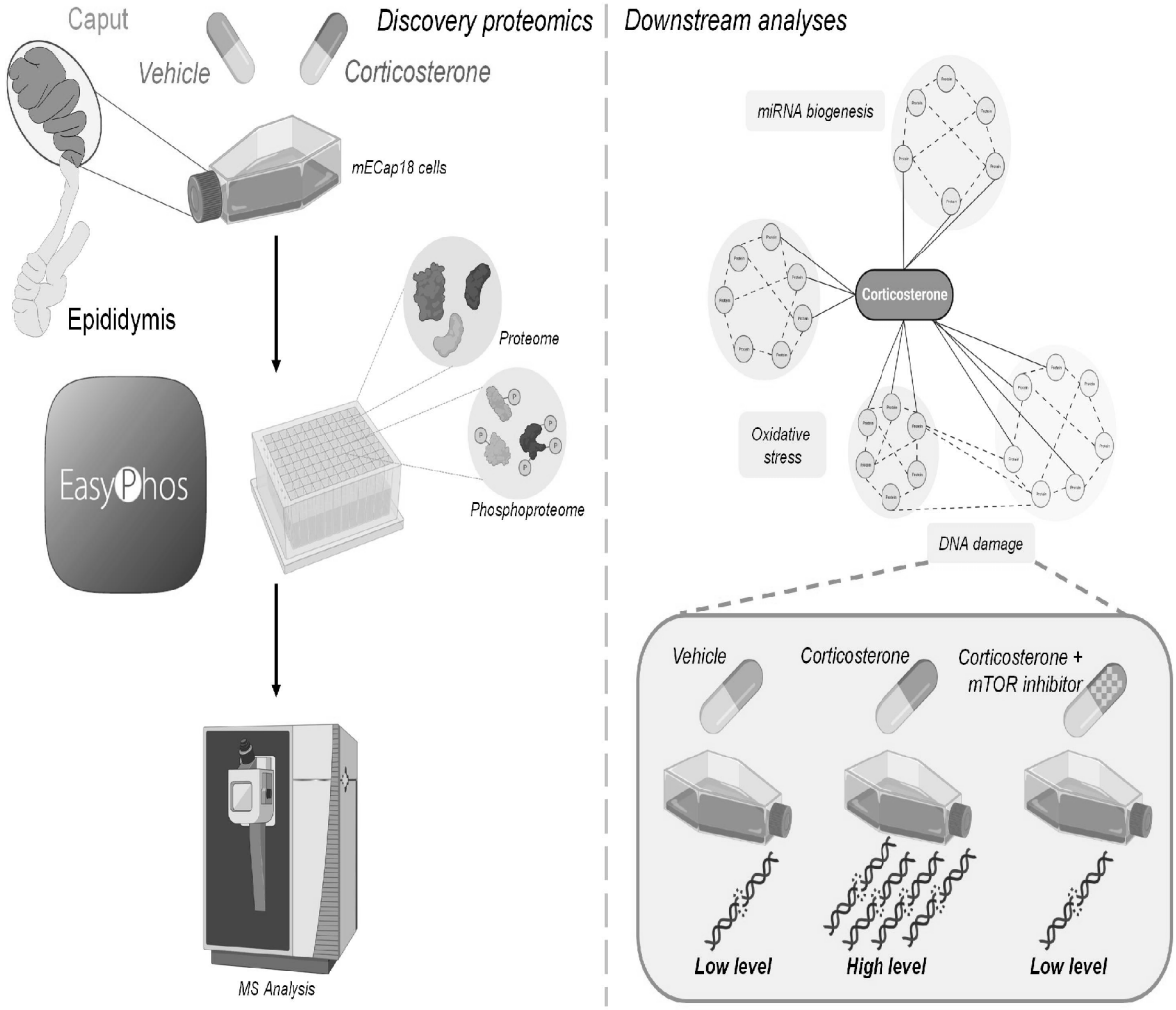
Preconception paternal health has increasingly been linked to a spectrum of offspring health outcomes. Indeed, preclinical models have demonstrated a periconception legacy of paternal trauma that manifests in offspring as impaired stress-relevant behavioural and physiological responses. Our group previously reported that a chronic low-dose corticosterone challenge of male mice produced increased anxiety-relevant behaviours in offspring. Although sperm passage through the male reproductive tract (epididymis) is proposed to be a putative staging point for relaying stress signals to the male germline, the intricacies of the molecular pathways responsible for this form of communication remain to be fully elucidated. To address this important knowledge gap, here we have capitalised on recent advances in phosphoproteomic analyses to investigate the impact of corticosterone supplementation and consequential corticosteroid receptor downstream signalling in a tractable epididymal epithelial tissue culture system (mECap18 cells). In agreement with no overt change in glucocorticoid receptor protein (NR3C1) levels, we detected only subtle adaptation of the global proteomic profile of mECap18 cells to

corticosterone challenge (i.e., 73/4171 proteins). By contrast, ~10% of the mECap18 phosphoproteome was substantially altered following corticosterone exposure. *In-silico* analysis of the dysregulated parent proteins revealed an activation of pathways linked to DNA repair and oxidative stress responses as well as a reciprocal inhibition of those associated with organismal death. Notably, corticosterone also induced the phosphorylation of several proteins linked to the biogenesis of regulatory microRNAs. Accordingly, orthogonal validation strategies confirmed an increase in DNA damage and an altered abundance profile of a subset of microRNAs in corticosterone-treated cells. Further, we demonstrated the DNA damage burden incurred by corticosterone can be ameliorated via prior and selective kinase inhibition. Together, these data confirm that epididymal epithelial cells are reactive to corticosterone challenge and that their response is tightly coupled to the opposing action of cellular kinases and phosphatases.



Loss of EED in the oocyte causes initial fetal growth restriction followed by placental hyperplasia and offspring overgrowth

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Germline epigenetic programming, including genomic imprinting, substantially influences offspring development. Embryonic Ectoderm Development (EED) is an essential component of Polycomb Repressive Complex 2 (PRC2), which catalyses Histone 3 Lysine 27 trimethylation to repress a range of developmental genes and regulate developmental patterning. PRC2 also regulates H3K27me3-dependent imprinting, loss of which leads to placental hyperplasia in mammalian offspring generated by somatic cell nuclear transfer (SCNT).

To determine the role of PRC2 in programming inherited impacts on offspring, we deleted EED only in growing mouse oocytes and analysed fetal growth, placental and pregnancy outcomes. Oocytes lacking EED had severely depleted H3K27me3 and widespread gene derepression, including *Plac1*, an X-linked gene essential for placental and embryonic development (n=4-6, *P*=6.7E-13; FDR=3.19E-09). Moreover, embryonic offspring from EED-deficient oocytes initially had low blastocyst cell counts and low mid-gestation body weights (n=15-38, p<0.0001), demonstrating growth restriction. However, this initial developmental delay was followed by striking late-gestational placental hyperplasia (n=32-68, ****P<0.0001), and subsequent rapid fetal catch-up growth and overgrowth demonstrated by increased body weights at birth suggesting a role for placental hyperplasia in remediating fetal growth restriction (n=32-68, p<0.0001). Remodelling of the placenta involved expansion of fetal and maternal tissues, including conspicuously increased glycogen enriched cells in the junctional zone (n=10, ****P<0.0001). Genome-wide analyses identified extensive transcriptional dysregulation in affected placentas, including imprinted and non-imprinted genes. Effects on pregnancy were evident through decreased litter size (n=12-29, p<0.0001) and increased gestational length in litters derived from oocytes lacking EED (n=7-13, p=0.0068).

Ultimately, this work reveals a critical role for EED in the oocyte for regulating fetal and placental growth and function in offspring via complex intrauterine mechanisms that are independent of genetic background. As early development can mediate effects that persist into adulthood, this model provides a novel paradigm for studying inherited impacts on offspring health and disease.

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Placental extracellular vesicles: a novel link of preeclampsia to future cardiovascular disease

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Cardiovascular disease (CVD) is the leading cause of death in women[1] and the risk increases significantly following preeclampsia (PE), a hypertensive disorder of pregnancy. Extracellular vesicles (EVs) are lipid bilayer-enclosed structures that are extruded from cells to control the function of remote cells/organs. During pregnancy, the placenta extrudes vast numbers of EVs into the maternal circulation and there is increasing evidence that EVs trigger preeclampsia[2]. We hypothesized that preeclamptic placental EVs cause increased CVD risk following preeclampsia.
EVs were isolated from late-onset preeclampsia (LOPE), early-onset preeclampsia (EOPE), or normotensive placentae and administered to female spontaneously hypertensive rats (SHR) via tail veins. Systolic blood pressure (SBP) was recorded by tail-cuff. The vasoreactivity of SHRs mesenteric arteries was measured using a wire myograph. The artery remodeling was determined by histological analysis.
SBP in the LOPE group was significantly increased, with an average of 43.58 mmHg 12-month post-EVs injection. In the EOPE group, there was a larger increase in SBP after 12 months (63.37±12.25 mmHg). In contrast, SBP in the normotensive group showed a significantly smaller increase, with an average of 31.99±4.72 mmHg 12 months after exposure to placental EVs. Wire myography revealed a significant increase in vasoconstriction in response to phenylephrine and U46619 in vessels exposed to LOPE placental EVs. Vessels exposed to EOPE EVs also showed increased vasoconstriction to U46619 and endothelin-1 compared to the normotensive group. Additionally, the relaxation response of mesenteric arteries from the EOPE group was significantly decreased in response to ACh. Small artery thickening was markedly increased in LOPE and EOPE groups.
Our data support the concept that placental EVs have long-lasting effects on the maternal cardiovascular system and altered SBP is associated with abnormal vascular reactivity induced by placental EVs. Preeclamptic EVs may be a mechanistic link between preeclampsia and later CVD.

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The lipoprotein receptor chaperone LRPAP1 is reduced in early pregnancy placenta and maternal serum from pregnancies that subsequently develop late-onset preeclampsia.

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Preeclampsia is a leading cause of maternal and fetal morbidity and mortality. Late-onset preeclampsia (>34 weeks gestation), accounts for >80% of preeclampsia but due to our poor understanding of the underlying etiology there is no predictive test and no preventative treatments. Whether there is an early pregnancy placental defect in late-onset preeclampsia has never been tested experimentally.

Mass spectrometry on early pregnancy placental biopsies (chorionic villous samples [CVS] collected 11-13 weeks gestation) compared placental protein production between uncomplicated and late-onset preeclamptic pregnancies (n=6-8/group). Placental LDL-receptor-related protein-associated protein (LRPAP1) expression across gestation was demonstrated by qPCR and immunohistochemistry/fluorescence. Circulating LRPAP1 in serum at 11-13 weeks gestation from uncomplicated and late-onset preeclamptic pregnancies (n=9-12/group) was quantified by ELISA. The effect of loss of LRPAP1 was determined by siRNA knockdown in the HTR8 trophoblast cell line (n=5).

Proteomics identified 48 significantly dysregulated proteins in CVS from late-onset preeclamptic pregnancies, which were enriched for molecular functions associated with protein binding and catalytic activity. LRPAP1 was significantly down-regulated in late-onset preeclamptic CVS (0.66-fold) and maternal serum (0.23-fold). Immunostaining revealed LRPAP1 expression by syncytiotrophoblast and cytotrophoblast in placental villous and extravillous trophoblast in decidua. Villous production (mRNA) of LRPAP1 fell significantly at 13-16 weeks gestation compared to 8-10 and 18-21. In vitro, LRPAP1 regulated production of the cholesterol metabolism modulators NCEH1 and CAV1, and loss of LRPAP1 impaired trophoblast adhesion, proliferation and invasion.

This is the first study to demonstrate that the early pregnancy placenta is altered in pregnancies that go on to develop late-onset preeclampsia. LRPAP1 may be central to the placental dysfunction of late-onset preeclampsia: LRPAP1 regulates clearance of multiple lipoproteins; lipoproteins including cholesterol show excess placental accumulation in preeclampsia. This study provides much-needed insight into the placental dysfunction of late-onset preeclampsia, which will facilitate the development of predictive tests and preventative treatments.

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Viral mimetic-induced immune activation during periconception has adverse effects on pregnancy outcome and programs impaired immune tolerance in adult female offspring

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The maternal immune profile during pregnancy is implicated in programming fetal development to impact neonatal and long-term health, and the periconception period is particularly vulnerable. In this study we aimed to develop a model of maternal viral mimetic-induced immune activation during periconception to investigate the impact on pregnancy success, offspring development and immune parameters. Polyinosinic:polycytidylic acid (poly I:C) is a synthetic dsRNA, widely used to mimic viral infection. Female mice received 10 mg/kg poly I:C or control on gestational days (gd) 0.5 and 2.5. Flow cytometry analysis of the uterus, uterine-draining lymph nodes and spleen on gd3.5, identified a significant increase in pro-inflammatory T conventional cells and T regulatory cells, confirming maternal immune activation. This periconception inflammation resulted in a significant 18.5% decrease in fetal weight on gd17.5 and a significant 32.5% increase in placental:fetal weight ratios ($p<0.0001$), demonstrating fetal growth restriction and placental insufficiency. Postnatally, male and female offspring from poly I:C treated dams exhibited alterations in development, with elevated weight gain compared to offspring from control treated dams ($p<0.05$ & $p<0.01$ respectively) across a 12-week period. Flow cytometry was performed on 12-week-old offspring following lipopolysaccharide immune challenge. Strikingly, our preliminary data indicates that female offspring from poly I:C treated dams exhibit a less tolerogenic T cell profile with approximately a 10% reduction in the proportion of anti-inflammatory T regulatory cells in the spleen and mesenteric lymph nodes ($p=0.0463$ & $p=0.0101$ respectively). These results confirm the vulnerability of the periconception period and demonstrate that maternal inflammation around the time of conception is a critical determinant of immune system programming evident in adult female offspring. A less tolerogenic immune phenotype increases susceptibility to a range of inflammatory and autoimmune conditions and thus education on pre-conception health warrants further attention.

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Upconversion-based Lateral Flow Assay for Sensitive Preeclampsia Detection

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Preeclampsia is a heterogenous, multiorgan cardiovascular disorder of pregnancy. An early and reliable diagnostic test for preeclampsia would facilitate targeted surveillance and timely delivery, improving the odds of preventing major maternal complications and death. Here, we report the development of novel strip-based lateral flow assay (LFA) using Lanthanide-doped upconversion nanoparticles (UCNPs) conjugated to antibodies targeting two vascular biomarkers; FK506-binding protein like (FKBPL) and its target protein, cluster of differentiation 44 (CD44) [1]. We first used conventional ELISAs to measure circulating plasma FKBPL and CD44 protein concentrations from women with early-onset preeclampsia (EOPE, delivering <34 weeks) and gestational age-matched healthy controls. We confirmed that the CD44/FKBPL ratio is reduced in early-onset preeclampsia (EOPE 160±134, n = 38, vs control 245.2±106.7, n = 13, p = 0.0004) with a good diagnostic potential (AUC=0.81, p=0.0007). Using our rapid FKBPL and CD44-LFA prototypes, we achieved improved lower limit of detection: 10 pg/ml for FKBPL and 15 pg/ml for CD44. The FKBPL signal was significantly higher in early-onset preeclampsia (EOPE 2,685±459.2, n=42 vs control 2,142±324, n=15, p<0.0001), while the CD44 signal was significantly lower in early-onset preeclampsia (EOPE 2646±432, n=42 vs control 4235±1286, n=15, p<0.0001), compared to controls. Aligned with our ELISA results, this translated to a lower CD44/FKBPL ratio in early-onset preeclampsia compared to controls (EOPE 1.01±0.19, n=42 vs control 2.09±0.84, n=15, p<0.0001). Based on the ROC curve (AUC=0.98, p<0.0001), a cut-off point of 1.24 for CD44/FKBPL ratio (sensitivity of 90.48% and specificity of 100%) provided positive predictive value of 100% and the negative predictive value of 91%. This work demonstrates that our CD44/FKBPL LFA has the potential to be developed into a new low-cost, rapid and highly sensitive point-of-care test for preeclampsia.

Reference:

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Prenatal alcohol exposure is associated with sex-specific alterations to placental methyl donors and reduced cerebroplacental ratio

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Aims

Prenatal alcohol exposure (PAE) increases the risk of adverse pregnancy outcomes including miscarriage, low birthweight, and neurodevelopmental abnormalities, which may be mediated by altered placental development. PAE is associated with alterations to placental vasculature, gene expression, and DNA methylation (DNAm) (1). Choline and folate are dietary micronutrients that serve as methyl donors for DNAm, however their availability may be compromised by alcohol consumption (2). This study investigated the impact of PAE on maternal and placental methyl donors, placental growth and the cerebroplacental ratio (CPR).

Methods

Data and samples were collected from women from the Queensland Family Cohort and classified into PAE (n=302) or abstinent/control (n=109). Maternal diet, placental measurements and Doppler ultrasound measures were examined during the 3rd trimester. Plasma folate was measured using a COBAS analyser. Plasma choline and placental methyl donors were measured by mass spectrometry. Placental gene expression was quantified by qPCR. Placental data were analysed separately by fetal sex.

Results

PAE was reported by 73.5% of participants. Most women (77%) took supplements containing ≥400µg folic acid, while few (27%) met the adequate intake for choline. This was consistent across control and PAE groups. Placental content of folate and choline were not altered by PAE, however, in female placentas, the methyl donor s-adenosylmethionine was increased in the PAE group (P<0.05), as were expression of the choline transporter, *CTL1,* and placental growth factor (*PlGF*) (P<0.05). In male placentas, expression of DNA methyltransferases (*DNMTs*) and (*PlGF*) were reduced in the PAE group (P<0.05), and placental thickness was decreased (P<0.05). CPR was lower in the PAE group (<0.05), with 11% of male fetuses, but no female fetuses, falling below the normal range, indicative of placental insufficiency.

Conclusion

PAE is associated with sex-specific alterations in the placenta, which may result in male fetuses being more vulnerable to adverse outcomes.

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Thinking outside the flock: exposure to melatonin *in-utero* alters twin-lamb cognition

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Compared to their single-born counterparts, twin-born lambs are more likely to experience hypoxia and restricted placental blood flow *in-utero*. This can lead to life-long impairment of cognitive function. We investigated whether the neurohormone melatonin, a vascular growth mediator and potent antioxidant, could mitigate this if lambs were exposed at different developmental stages *in-utero*. Twin-bearing ewes (n=150/group) were implanted with slow-release melatonin either during early, late or early + late gestation. Non-implanted twin-bearing ewes were used as controls. Lambs (n=10/group) were culled within 2 hours of birth for tissue collection. Another subset of lambs (n= 12/group) were tested for cognitive function at 5-6 weeks of age, via the maze and novel object tests. At birth, plasma melatonin levels were higher in lambs exposed in late gestation (control 5.7, early 6.6, late 28.7, early + late 39.8 pg/mL, P<0.001). Compared to controls, melatonin in early or late pregnancy resulted in a 1.4-fold increase in the number of accessory vessels to the umbilical cord (P<0.05). Vessel number decreased 0.7-fold if lambs were exposed in early + late gestation (P<0.05). Though brain dimensions were not different between groups at birth, lambs exposed to melatonin during late gestation had heavier brains relative to body weight (P<0.05). By 5-6 weeks of age, lambs exposed to melatonin at any point *in-utero* were able to learn the maze test more rapidly compared to non-exposed lambs, with lambs exposed in early + late gestation demonstrating the greatest learning ability (P<0.001). Lambs from all melatonin-exposure groups had a greater number of interactions with novel objects, and were more vocal in both behavioural tests (P<0.001). We find that exposure to melatonin *in-utero* lead to superior learning, increased vocalisation and inquisitiveness in lambs. Late gestation appears to be most promising period for *in-utero* melatonin exposure to promote improvements in offspring cognitive function.

Glucocorticoid signalling and inflammatory changes in response to maternal asthma and betamethasone treatment in near-term lambs

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Maternal asthma is associated with neonatal respiratory morbidity, indicating lung immaturity at birth. Lung maturation can be stimulated by glucocorticoids and inflammation. Maternal asthma reduces glucocorticoid signalling and modulates the inflammatory response in the fetal lung, potential mechanisms contributing to respiratory morbidity in babies of asthmatic mothers. Antenatal betamethasone is routinely used to induce lung development in preterm babies. We therefore hypothesised that betamethasone would mature the lungs of lambs of asthmatic ewes.

Ewes were sensitised to house dust mite (HDM) and an asthmatic phenotype induced by fortnightly HDM lung challenges; controls (n=11) received saline. Pregnant asthmatic ewes were randomised to antenatal saline (n=9) or betamethasone (n=8, 12 mg i.m.) at 138 and 139 days of gestation (dG). At 140 dG (term, 150 dG), lambs (control: n=16, asthma: n=14, asthma+betamethasone: n=12) were delivered by Caesarean section and ventilated (45 minutes) prior to lung tissue collection for RT-qPCR. Data were analysed using mixed models.

Compared to controls, asthma lambs had lower glucocorticoid receptor (p=0.017) and *IL-6* (p=0.042) gene expression. Expression of surfactant protein *(SP)-A*, *SP-B*, and *SP-C* was higher but expression of *IL-6* (p=0.014), *11βHSD1* (p=0.010), and *KDR* (p=0.008) was lower in the asthma+betamethasone lambs compared to controls. *TNFα* (p=0.001), mineralocorticoid receptor (p=0.002), and *11βHSD1* (p=0.010) gene expression was reduced with betamethasone compared to asthma lambs, while *SP-B* and *SP-C* expression was increased. Gene expression of *SP-D*, *11βHSD2*, hypoxia-related genes (*HIF1α, HIF2α, HIF3α, FLT1*) and inflammatory cytokines (*IL-1β, IL-5, IL-8*) did not differ between groups.

Impaired fetal lung development in response to maternal asthma in pregnancy may be the result of lower glucocorticoid receptor expression and IL-6 driven lung maturation. Betamethasone improved lung maturity by increasing SP gene expression, despite downregulation of upstream genes. Further exploration of these pathways may highlight targets to improve lung outcomes in babies of asthmatic women.

Infertile human endometrial organoid apical protein secretions are dysregulated and impair trophoblast progenitor cell adhesion

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Embryo implantation failure leads to infertility and remains a challenging problem for IVF. As an important approach to regulate implantation, endometrial glands produce and secrete factors apically into the uterine cavity in the receptive phase to prepare the initial blastocyst adhesion and implantation. Organoids were recently developed from human endometrial glands and show long-term expandability, genetic stability and maintenance of their hormone responsiveness. Importantly, organoid exhibits similar apical-basal polarity compared to endometrial gland making it an ideal model to study glandular secretions. We established organoids using endometrial biopsies from women with normal fertility and primary infertility (referred to as fertile and infertile organoids). Organoids from both groups were treated with hormones to model the receptive phase of the endometrial glands and intra-organoid apical fluid (IOF) was collected to compare the apical protein secretion profile. Our data show that infertile organoids were dysregulated in their response to estrogen and progesterone treatment. Proteomic analysis of IOF identified 131 decreased and 19 increased proteins in infertile group compared to fertile (>1.5-fold change). Many of the proteins were similarly differentially regulated in organoid cells at the mRNA level. To determine the effect of organoid apical secretion on blastocyst adhesion, we first developed epithelial monolayers using fertile organoids and compared them with previously established primary human endometrial epithelial monolayers. Using miR-29c as an example, we confirmed that both models respond similarly to microRNA overexpression. IOF was collected after hormone stimulation to treat trophoblast progenitor spheroids (blastocyst surrogates) and their adhesion on the organoid-derived endometrial cell monolayer determined. Incubation of infertile IOF significantly reduced trophoblast spheroid adhesive capacity compared to fertile IOF ($P < 0.0001$) and medium control ($P < 0.01$), respectively. Together, this study paves the way to determine the molecular mechanisms by which endometrial glandular apical released factors regulate blastocyst initial attachment.

A Role for Steroid 5 alpha-reductase 1 in Vascular Remodelling During Endometrial Decidualisation

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Decidualisation is the hormone-dependent process of endometrial remodelling that is essential for fertility and reproductive health. Deficits in decidualisation are implicated in disorders of pregnancy such as implantation failure, intra-uterine growth restriction, and pre-eclampsia. Androgens are key regulators of decidualisation that promote optimal differentiation of stromal fibroblasts and activation of downstream signalling pathways required for endometrial remodelling [1,2]. We have shown that androgen biosynthesis, via 5α-reductase-dependent production of dihydrotestosterone, is required for optimal decidualisation of human stromal fibroblasts in vitro [1], but whether this is required for decidualisation in vivo has not been tested. This aim of this study was to assess the impact of androgens on decidualisation and vascular remodelling in androgen-deficient mice.

This study used mice lacking Steroid 5α-reductase type 1 (Srd5a1-/- mice) and a validated model of induced decidualisation to investigate the role of SRD5A1 and intracrine androgen signalling in endometrial decidualisation. We measured decidualisation response (weight/proportion), transcriptomic changes, and morphological and functional parameters of vascular development. These investigations revealed that 5α-reductase deficiency impaired decidualisation responses which were significantly reduced compared to wild type mice ($p<0.001$). Furthermore, vessel permeability ($p<0.01$) and transcriptional regulation of angiogenesis signalling pathways, particularly those that involved vascular endothelial growth factor (VEGF), were disrupted in the absence of 5α-reductase. In Srd5a1-/- mice, injection of dihydrotestosterone co-incident with decidualisation restored decidualisation responses, vessel permeability, and expression of angiogenesis genes to wild type levels.

Androgen availability declines with age which may contribute to age-related risk of pregnancy disorders. These findings show that intracrine androgen signalling is required for optimal decidualisation in vivo and confirm a major role for androgens in the development of the vasculature during decidualisation through regulation of the VEGF pathway. These findings highlight new opportunities for improving age-related deficits in fertility and pregnancy health by targeting androgen-dependent signalling in the endometrium.

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Role of uterine aging in female fertility

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Mammalian reproductive potential declines with age. Several studies show a significant association of advancing maternal-age with stillbirth, reduced fecundity, and elevated risks for pregnancy complications. This loss in reproductive potential in humans is primarily attributed to ovarian aging owing to the postmenopausal decline in ovarian follicular reserve. However, multiple lines of evidence from donor oocyte programs and assisted reproduction programs involving gestational surrogacy suggest that irrespective of the ovarian functional status, uterine age-related changes are, at least partly, responsible for the loss of reproductive potential. How aging influences endometrial homeostasis, and reproductive potential is currently unclear.

Here, we studied extensively the age-related changes in human endometrium both at the cellular and extracellular level, using a wide array of human endometrial samples, patient-derived xenografts, and co-culture organoid models. We used MALDI-MSI, Micromatrix arrays, and Second Harmonic Generation imaging to define the proteomic profile and decipher the extracellular matrix (ECM) dynamics of aged endometrium. Finally, we functionally validated our findings using transgenic mouse models and our novel recellularization model and deciphered molecular signaling pathways that disrupt endometrial homeostasis during aging.

Our results show that aging induces pathological changes in the endometrial stroma that phenocopy tumour microenvironment. These changes are characterized by loss in stromal Hand2 expression, enhanced secretion of growth-promoting factors, and differentiation of stromal fibroblasts into myofibroblasts which alter the ECM dynamics resulting in hyperactive Wnt signaling and endometrial hyperplasia, a precursor for endometrial cancer development. When we ablated *Efemp1* genetically and inhibited lysyl oxidase pharmacologically, two of the most upregulated ECM proteins in aged matrisome, from aged murine endometrium, both these interventions independently rescued some of these age-induced changes and restored the endometrial homeostasis. These data provide an integrated view of how aged microenvironment, irrespective of ovarian functional status, disrupts endometrial homeostasis and predisposes postmenopausal women to endometrial cancer development.

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Human foetal reproductive organoids and their potential clinical applications

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Background and Aims

Müllerian duct anomalies (MDAs) are developmental disorders of the female reproductive tract organs, and their prevalence is 5% in fertile women and ~ 6-15% in patients with infertility worldwide [1-3]. Studies using mouse models and human genome sequencing has revealed that the WNT signalling pathway is altered in patients with MDAs [4]. However, due to restricted access to foetal tissues, there is very little understood about human reproductive development and diseases. Therefore, we aimed to establish human fetal female reproductive organoids that represent a unique platform for understanding human female reproductive tract development and diseases.

Methods

In this study, we first performed a comparative histopathology analysis to differentiate between human fetal and adult female reproductive tract epithelium. We then developed human fetal female reproductive tract organoids and compared them to their adult counterparts using proteomic analysis and histology validation. Further, we established an *in vitro* transplantation model to regenerate adult tissues using fetal organoids to assess the differentiation potential of the fetal organoids. Finally, the transplanted tissue scaffolds were treated with Wnt inhibitors to understand the functional consequences of Wnt signalling suppression.

Results

The histopathological and proteomic analysis of the epithelial cell types revealed significant differences in protein expression and signalling pathways between fetal and adult tissues. We showed the successful establishment of culture conditions for fetal organoid growth and long-term maintenance. Our transplantation model provided evidence that fetal organoids represent a transplantable source of cells and have the capacity to regenerate adult organs. We further highlight the requirement of Wnt signalling in the self-renewal and differentiation of the fetal epithelium.

Conclusion

To conclude, we developed an organoid-scaffold model from the fetal female reproductive tissue to understand the basis of human female reproductive tract development and associated disorders.

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WD-repeat containing protein-61 dysregulation in the endometrial luminal epithelium impairs human endometrial receptivity

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Endometrial receptivity is a hallmark of successful blastocyst implantation in early pregnancy. A major challenge to successful IVF treatment is recurrent implantation failure (RIF) whereby impaired endometrial receptivity is a major contributor. However, there is no way to identify or treat abnormalities in the endometrium. Recently, we developed endometrial organoids from women with infertility and with normal fertility. We identified that WD-repeat-containing protein-61 (WDR61) was abnormally reduced in apical endometrial epithelial organoid secretions from primary infertile women (N=7). WDR61 is a transcriptional cofactor of the Wnt pathway, which has known roles in endometrial function. We hypothesised that WDR61 plays a role in endometrial receptivity and implantation during the window of implantation (WOI). Our immunohistochemistry data demonstrated WDR61 immunostained in endometrial glands, stroma, and luminal epithelium (N=7). WDR61 was significantly higher during the receptive phase than in the non-receptive, proliferative phase of fertile women (N=8, $P<0.05$), despite minimal difference in immunostaining levels between fertile and infertile tissues (N=6). To assess function, siRNA knockdown of WDR61 in Ishikawa cells (endometrial epithelial cell-line) was used to assess adhesion and proliferation by xCELLigence real-time monitoring. WDR61 siRNA treatment reduced adhesion and proliferation of Ishikawa cells compared to respective controls ($P<0.05$). Qualitative-PCR was employed to determine the changes in the expression of genes implicated in blastocyst implantation and development, cell proliferation, and Hox and Wnt pathways. Several pivotal genes such as *HOXD10*, *MMP2*, *CD44* and *CXCR4* were significantly diminished compared to controls ($P<0.05$). Our data suggests that WDR61 is involved in cyclic changes within the endometrium to prepare for blastocyst implantation during the WOI. When it is dysregulated *in vivo*, WDR61 may contribute to RIF due to the impaired proliferative and adhesive capacity of the luminal epithelium. These findings could be translated to the clinic to enhance current IVF in women with implantation failure.

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Prevention of miscarriage in mice by boosting maternal Treg cells in pregnancy with an IL2-antibody complex

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T regulatory (Treg) cells are essential for maternal immune tolerance in healthy pregnancy. They are generated at conception and act to suppress effector responses against fetal alloantigens and facilitate uterine vascular adaptations required to support placental development. Treg cell insufficiency in women is associated with infertility, recurrent miscarriage, and preeclampsia. In the present study, we utilised an abortion-prone mouse model to evaluate the efficacy of IL-2 complexed with JES6-1 anti-IL-2 antibody (IL-2/JES6-1) to increase uterine Treg cells and improve reproductive success. CBA/J female mice were mated with DBA/2 males to generate abortion-prone pregnancies, which were treated with three injections of IL-2/JES6-1 at 24h intervals on days 0.5-2.5 post coitum (pc). Treg cells, measured as the proportion of CD4+ T cells expressing Foxp3, were increased >3-fold and >2-fold on day 3.5 pc in the uterus and draining lymph nodes following treatment with IL-2/JES6-1 but not IL-2/IgG or PBS control (all 9-16 dams/group), and the ratio of Foxp3+ Tregs to Foxp3- Tconv cells was increased in both tissues. Elevated expression of Ki67 and suppressive function markers CTLA4, CD25, and Foxp3 were observed for both thymic-derived and peripheral Treg cells. IL-2/JES6-1 treatment reduced fetal loss from 31% to 10%, a rate comparable to non-abortion prone CBA/J female x Balb/c male matings (all $P<0.05$, ANOVA). A small decline in fetal weight at day 18.5 pc after IL-2/JES6-1 treatment was partly attributable to larger litter size, while no abnormalities were observed in placenta development or spiral artery remodelling. These experiments demonstrate the efficacy of boosting uterine Treg cells through targeting IL-2 signaling for mitigating immune-mediated fetal loss. The results

are relevant for designing clinical interventions targeting Treg cells to manage immune-mediated infertility and pregnancy disorders in women.

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Beta-defensin 22 in seminal fluid promotes female fecundability through effects on maternal receptivity to implantation in mice

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In many mammalian species, components of seminal plasma and sperm interact with the female reproductive tract after mating to elicit an immune response that promotes reproductive success. Beta-defensins are a diverse group of antimicrobial and immune-modulatory peptides carried by sperm, which interact with female reproductive tract cells after mating, potentially via TLR4 binding. In men, the major beta-defensin moiety is DEFB126, and reduced expression of *DEFB126* is associated with impaired fertility. To investigate the physiological significance of DEFB126 in fertility, we utilised CRISPR technology to generate C57Bl/6 mice with null mutation in *Defb22* (*Defb22*$^{-/-}$), the murine ortholog of human *DEFB126*. *Defb22*$^{-/-}$ males sired pregnancies with poorer outcomes than *Defb22*$^{+/+}$ males when mated to wild-type Balb/c females, with reduced implantation rates, increased post-implantation fetal loss, and fetal growth restriction in late gestation (n=24-30 dams/group,$P<0.05$). However, this was not attributable to reduced fertilisation, as there was no detectable difference in blastocyst numbers or development in females mated to *Defb22*$^{+/+}$ or *Defb22*$^{-/-}$ males on day 3.5 post-coitum (d3.5pc) (n=10-12), implicating an embryo implantation defect. qPCR analysis of the uterine endometrium collected 8h after mating showed that unlike *Defb22*$^{+/+}$ males, mating with *Defb22*$^{-/-}$ males failed to induce expression of endometrial cytokine genes *Il6* and *Tnf*, and microRNAs *miR155* and *miR223* (n=10-12,$P<0.05$), likely impacting the uterine immune environment. RNAseq analysis of the uterine endometrium on d3.5pc (n=4/group) showed altered expression of genes associated with implantation-related immune-regulatory and tissue remodelling pathways, with downregulation of *Mmp7*, *Wnt7b*, *Il11*, *Fosl1*, *Lifr*, *Ptgs2*, *Cebpb*, *Cd55*, *Csf1*, and *Atp6v0d2,* when females were mated with *Defb22*$^{-/-}$ males. These data demonstrate that DEFB22 is required for optimal fertility in males, through a role in enabling seminal fluid to interact with the uterine endometrium to stimulate a permissive female immune response promoting endometrial receptivity to support healthy embryo implantation and optimal reproductive outcomes.

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Periconception regulatory T cell deficiency results in late gestation pregnancy pathology and susceptibility to preterm birth in mice

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Regulatory T (Treg) cell insufficiency in pregnancy is associated with inflammatory disorders including spontaneous preterm birth (PTB). Emerging evidence suggests periconception events are critical in determining the number and function of Treg cells in later gestation. To assess the importance of early gestation Treg cell responses in PTB susceptibility, we developed a model of Treg cell deficiency in periconception using Foxp3DTR mice, which undergo transient depletion of Treg cells when administered diphtheria toxin (DT).

Foxp3DTR females mated to BALB/c males were administered low dose DT (5-25 ng/g, or vehicle) on gestational day (GD)0.5 and 2.5. Treg cell depletion was tracked by flow cytometry on GD3.5, 6.5, 9.5, and 16.5 (n=4-7/group). Effects on fetal and placental weights and pregnancy viability were evaluated on GD17.5 (n=16-20/group). Treg-depleted and control dams were administered 0, 4, or 8 µg of lipopolysaccharide (LPS) on GD16.5 (n=12-19/group) and video-monitored to measure time of birth.

A dose-dependent effect of DT on Treg cell depletion was demonstrated. Dams given 10-25 ng/g DT exhibited extensive (~95%) systemic Treg cell depletion resulting in reduced pregnancy rates ($p=0.011$), fewer viable fetuses/dam ($p=0.036$), growth restricted fetuses ($p=0.009$), and a decreased fetal:placental weight ratio ($p=0.021$) on GD17.5. Heightened CD25 expression by CD4+Foxp3- T cells and aberrant lymphocyte proliferation were observed throughout gestation. Greater sensitivity to LPS-induced PTB was seen, such that following 4 µg LPS, Treg-depleted dams had a 20% incidence of early (<GD18) PTB, and a 25% reduction in viable pups/dam, while control dams were unaffected ($p=0.027$). Both groups exhibited PTB and fetal loss following 8 µg LPS, but this was more pronounced in Treg-depleted dams.

We conclude that periconception Treg cell deficiency impairs late gestation outcomes in mice and increases susceptibility to inflammatory challenge resulting in PTB. This model will increase our understanding of the upstream inflammatory processes causing PTB.

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Reproductive Influences on Health and Ageing

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Adult health and ageing are influenced by a variety of environmental factors, including social interactions and engaging in reproduction. Sensory cues perceived during these interactions are detected by sensory systems, and these inputs may be a key factor linking altered health to social conditions. In this presentation I will show that specific social stimuli are responsible for the changes in metabolism and ageing that occur with reproduction in mice. In females, pregnancy and lactation have been assumed to exert the major metabolic and life course changes occurring with reproduction. However, our research suggests that exposure to stimuli during the act of mating causes major changes in female growth and aspects of ageing. In males, olfactory cues detected by specific olfactory sensory neurons are important in driving metabolic and life course changes that occur with reproduction. Exposing males to just female olfactory cues causes metabolic changes, reducing body weight and adiposity when nutrients are provided in excess. Exposure to female odours can also influence male mortality, reducing survival when provided in addition to actual mating opportunities. This research highlights that sensory cues and stimuli from the opposite sex can have a major effect on the metabolism and life-course of mammals, explaining some of the well-established links between engaging in reproduction and effects on health.

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Biological Strategies to promote Tissue Repair in Regenerative Medicine

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The science of regenerative medicine aims to replace living tissues. Translational scientific advances offer great promise in many areas of medicine especially those presented by our aging population. Novel interdisciplinary approaches to health care such as tissue engineering have the potential to improve the surgical outcomes of tissue repair through the collaboration of biologists, bioengineers, surgeons, patient groups, and commercial interests.

Understanding the cellular and molecular responses of cells to enable *in vitro* evaluation of biocompatible scaffolds for use in tissue engineering is essential. Providing a scaffold that encourages appropriate cell attachment, growth, and ultimately tissue regeneration could improve the clinical outcomes from injuries and diseases. Cellular and molecular responses of cells grown in traditional 2-dimensional tissue culture plates compared to simple 3-dimensional cultures can be used to identify mechano-sensors, mechanically-mediated switches in cell commitment, focal adhesions, and cell-to-cell communication to better understand the biocompatibility of potential tissue engineering products. Much time and expense can be saved by such *in vitro* evaluation of biomaterials prior to embarking on *in vivo* studies.

The FDA receives millions of reports of medical device failure annually suggesting that pre-clinical assessment of these novel products is shaky. The majority of adverse events are related to immune rejection, thus a greater understanding of how these materials interact with the immune system is imperative, with a focus on the macrophage response being particularly important. Uptake of *in vitro* immune response assessment will allow for substantial reductions in experimental time and resources, including unnecessary and unethical animal use, with a simultaneous decrease in inappropriate biomaterials reaching the clinic.

Regenerative medicine can include the use of growth factors as well as cell and gene therapies, however recently, global stem cell opportunities for unregulated therapies have been challenged. This improvement in bench-to-bedside safety is paramount to reducing patient harm.

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Bone health and fracture outcomes in the ageing population

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While fracture has long been regarded as the terminal outcome of osteoporosis, we now know that almost all fractures lead to further fractures, functional decline and premature mortality. In Australia, it has been estimated that more than one in three women and one in five men aged 50+ will suffer fractures. The relationship between fracture and subsequent adverse outcomes depends on both fracture site and underlying general health.

The vast (95%) majority of people with osteoporosis have at least one and > 66% of people have at least two comorbidities. Indeed, multimorbidity associated with increased risk of subsequent fracture, even after accounting for the competing risk of mortality. Concerningly, multimorbidity was also associated with lower risk of being treated or investigated for underlying bone health after a fracture.

We have also demonstrated that underlying co-morbidities independently add to increased post fracture mortality. Furthermore, we have found that comorbidities naturally group into specific clusters that are strongly associated with post-fracture premature mortality. These clusters are based on disease severity and co-existence of related conditions. These clusters differentiated fracture patients who had uncomplicated comorbidity or were unlikely to have comorbidity from those with either multiple or/and advanced comorbidities. Indeed, the contribution of these clusters to fracture risk was at least as great, and mostly greater, than the individual comorbidities themselves.

As comorbidities both exacerbate the adverse post-fracture outcomes and lower treatment rates, there is an urgent need to identify the highest risk patients and prioritise their care. In this presentation, I will discuss the evidence surrounding the role of comorbidities and underlying health on fracture outcomes and suggest alternatives to our current model of care.

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Metabolism in ageing: cellular and physiological ageing and unravelling the riddle of the Sphinx.

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Addressing the health conditions associated with advancing age has become a global issue, including dementia and conditions that accelerate it, such as diabetes mellitus, vascular disease and the metabolic complications associated with insulin-resistant obesity. Endocrine and metabolic factors contribute to cellular ageing, which manifests as progressive functional loss in a number of organ systems where cellular rejuvenation is limited, including neurological tissues, the beta-cell and the myocardium.

This presentation will examine the pre-clinical and clinical evidence on how metabolism contributes to ageing, the contribution of nutrient toxicity and advances in our knowledge.

For millennia, humans have been fascinated by the myth of the fountain of youth. The evidence for potential repurposing of medications that may assist in slowing cellular ageing and the effects of caloric restriction (and the myriad forms of fasting) will be discussed.

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Six-month randomised trial of open-source automated insulin delivery in Type 1 Diabetes

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Aims: Open-source automated insulin delivery (AID) predated the availability of commercial systems and is used by thousands with type 1 diabetes (T1D) despite no regulatory approval. We examined efficacy and safety of an open-source AID system.

Methods: A 24-week, multi-center randomised (1:1) controlled trial in children (7–15 years) and adults (16–70 years) with T1D, comparing open-source AID (OpenAPS algorithm within a modified version of AndroidAPS in a smartphone, pre-production DANA-i™ insulin pump, and Dexcom G6® CGM), to sensor augmented pump therapy (SAPT). The primary outcome was percent time in target sensor glucose range (TIR; 3.9–10.0 mmol/L), between AID and SAPT during days 155-168 (final 2 weeks of the study).

Results: Ninety-seven participants (48 children, 49 adults) were randomised (44 to open-source AID and 53 SAPT). The mean adjusted difference in TIR between AID and SAPT at end of study was 14% (95% confidence interval (CI), 9.2 to 18.8; P<0.001), with no treatment effect by age interaction (p=0.56). AID users spent 3 hours 21 minutes (95% CI, 2h 12m to 4h 30m) more in target range per day. In the AID arm, mean TIR (±SD) increased from 61.2±12.3% to 71.2±12.1%, and decreased from 57.7±14.3% to 54±16% in the SAPT arm. More participants achieved TIR >70% using AID (60% vs. 15%). No severe hypoglycemia or diabetic ketoacidosis occurred in either arm. Two participants withdrew from AID due to connectivity issues.

Conclusion: Open-source AID using the OpenAPS algorithm within a modified version of AndroidAPS, a widely used open-source AID solution, is efficacious and safe.

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Association between maternal hyperglycaemia in pregnancy and offspring anthropometry in early childhood the PANDORA Wave 1 study

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Exposure to *in-utero* hyperglycaemia influences later cardiometabolic risk, although few studies include women with type 2 diabetes (T2D) or assess maternal body mass index (BMI) as a potential confounder. This study explored the association of maternal T2D and gestational diabetes mellitus (GDM) with childhood anthropometry, and the influence of maternal BMI on these associations.

The PANDORA birth cohort comprises 1138 women and 1163 children, women with GDM and T2D recruited from a hyperglycaemia in pregnancy register, and women with normoglycaemia from the community. Wave 1 follow-up included 423 children, aged 1.5-5 years (median 2.5 years). Multivariable linear regression assessed associations between maternal antenatal variables with offspring anthropometry (weight, height, BMI, skinfold thicknesses, waist, arm and head circumferences).

Greater maternal BMI was associated with increased anthropometric measures in offspring independent of maternal glycaemic status. After adjustment, including for maternal BMI, children exposed to GDM had lower mean weight (-0.54kg, 95% CI -0.99, -0.11), BMI (-0.55kg/m^2, 95% CI -0.91, -0.20), head (-0.52cm, 95% CI -0.88, -0.16) and mid-upper arm (-0.32cm, 95% CI -0.63, -0.01) circumferences, and greater mean suprailiac skinfold (0.78mm, 95% CI 0.13, 1.43), compared to children exposed to normoglycaemia. Children exposed to T2D had smaller mean head circumference (-0.82cm, 95% CI -1.33, -0.31) than children exposed to normoglycaemia. Adjustment for maternal BMI strengthened the negative association between GDM and child weight, BMI and circumferences. Maternal T2D was no longer associated with greater mean skinfolds (p=0.14) and waist circumference (p=0.18) in children after adjustment for maternal BMI.

Compared to children exposed to normoglycaemia, children exposed to GDM had greater suprailiac skinfold thickness, despite having lower mean weight, BMI and mid-upper arm circumference, and both GDM and T2D were associated with smaller head circumference. Future research should assess whether childhood anthropometric differences influence lifetime cardiometabolic and neurodevelopmental risk.

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Australian and New Zealand Paediatric Surveillance Units X-linked Hypophosphataemia survey: estimate of prevalence and characteristics of paediatric X-linked Hypophosphataemia in Australia and New Zealand.

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Australian and New Zealand Paediatric Surveillance Units (APSU/NZPSU), which facilitate active surveillance of rare childhood diseases, conducted prospective data collection to review prevalence and characteristics of paediatric XLH in Australia (Aust) and New Zealand (NZ).

Seventy-five cases in Aust and 16 cases in NZ were identified with 60 completing a follow-up survey in Aust. Of the 91 cases overall, 46% were on burosumab therapy.

Clinical, demographic and management data are summarised in the Table. These demonstrate that despite a family history in the majority of cases, delayed diagnosis is common and there is a high rate of complications of nephrocalcinosis and hyperparathyroidism in this cohort. Additionally, while guidelines stress the importance of multidisciplinary care, many do not have access to recommended health professionals, with only 3% seeing a psychologist and 68% seeing a dentist. This is despite the high psychological burden of XLH and a significant proportion of this cohort having dental issues (tooth abscess, dental capping, tooth extraction).

There was a significant number of orthopaedic interventions performed. Of the 60 follow-up cases, 31 had undergone at least one orthopaedic procedure. Three cases reported cancellation of orthopaedic surgery due to improvement in lower limb deformity after commencement of burosumab.

Consistent with clinical trials, those on burosumab had a higher phosphate ($p<0.001$) at most recent follow-up.

Conclusions: These data suggests the estimated minimum prevalence of XLH <18y is 1.31 and 1.6 per 100,000(95%CI 1.02 – 1.64) in Aust and NZ respectively. Burosumab is a promising new alternative to conventional therapy, but does not negate the need for multidisciplinary care. These data highlights the lack of access to many health professionals, especially psychological support, for many children with XLH.

Table: Demographic and clinical characteristics			
Demographic features	**Aust (n=75)**	**NZ (n=16*)**	**Aust and NZ (n=91)**
Gender % Female (n=)	57% (43)	67% (11)	59% (54)
Family history of XLH % (n=)	60% (45)	88% (14)	65% (59)
Age at onset Months, median (range)	26.5 (0-205)	8.8 (0.6-143.7)	24.0 (0-208)
Clinical features[a] present at any time % (n=)			
Bowing of legs	95% (71)	71% (10)	89% (81)
Bone of joint pain	73% (55)	54% (7)	68% (62)
Abnormal gait	60% (45)	50% (7)	57% (52)
Muscle pain	51% (38)	29% (4)	46% (42)
Flaring of wrists	49% (37)	25% (3)	44% (40)
Short stature (height <3rd percentile)	49% (37)	67% (8)	49% (45)
Dental manifestations or interventions**	44% (33)	25% (4)	41% (37)
Motor delay or reduced activity levels	41% (31)	7% (1)	35% (32)
Nephrocalcinosis	**32% (24)**	**33% (5)**	**32% (29)**
Hyperparathyroidism	**17% (13)**	**53% (8)**	**23% (21)**
Rib deformity/ rachitic chest	16% (12)	0% (0)	13% (12)
Craniosynostosis	11% (8)	14% (2)	11% (10)
Myopathy/muscle weakness	11% (8)	0% (0)	9% (8)
Scoliosis	5% (4)	7% (1)	5% (5)
Hearing impairment	3% (2)	0% (0)	2% (2)
Fractures	3% (2)	0% (0)	2% (2)
Use of mobility aid	3% (2)	14% (2)	4% (4)
Kyphosis	2% (1)	21% (3)	4% (4)
Management % (n=)			
Burosumab % (n=)	56% (42)	0% (0)	46% (42)
Conventional therapy % (n=)	44% (33)	100% (16)	54% (49)
Growth Hormone % (n=)	3% (2)	0% (0)	2% (2)
Health professionals involved % (n=)			
Paediatrician	89% (67)	75% (12)	87% (79)
Orthopaedic surgeon	81% (61)	50% (8)	76% (69)
Dentist	72% (54)	50% (8)	68% (62)
Physiotherapist	55% (41)	25% (4)	49% (45)
Physician	35% (26)	14% (2)	30% (28)
Geneticist	28% (21)	28% (4)	27% (25)
Occupational therapist	25% (19)	14% (2)	23% (21)
Psychologist	3% (2)	7% (1)	3% (3)
Other[+]	24% (18)	38% (6)	26% (24)

***** data not available for 2 of the 18 cases in NZ

****** Includes tooth abscess, dental caries, toothache or malocclusion and/or dental procedures such as dental capping or tooth extractions

[a] Clinical features with n=1 included ongoing vitamin D deficiency with high PTH, language disorder, hepatic steatosis (NASH), Coxa Vara, Congenital ptosis, diabetes mellitus, and in NZ cohort one with renal failure (secondary to unmonitored conventional therapy), learning difficulties, asthma, allergic rhinitis, alopecia, obesity, and menorrhagia.

[+] "Other" includes: Nephrologist, paediatric endocrinologist, osteopath, chiropractor, ophthalmologist, general practitioner, neurosurgeon, craniofacial surgeon, plastic surgeon, audiologist, otorhinolaryngologist, immunologist, and sports medicine.

What do adolescents with Type 1 Diabetes and their healthcare professionals think of a self-compassion chatbot to improve wellbeing?

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Introduction: Adolescents with type 1 diabetes (T1D) experience psychological distress at greater rates than their peers (1) and are also at greater risk of complications and sub-optimal glycemic control compared to younger children and adults (2). With the COVID-19 pandemic adding restrictions to traditional psychological supports, chatbots offer a unique modality to remotely deliver psychological tools. Building from previous work (3-5), our research group developed a novel self-compassion chatbot (called COMPASS) for adolescents aged 12 to 16-years-old with T1D.

Objectives: A qualitative focus group study evaluated the acceptability and clinical usability of the self-compassion chatbot among adolescents with T1D and their healthcare professionals.

Methods: Nineteen adolescents and eleven diabetes team healthcare professionals took part in qualitative Zoom interviews in March and April 2022. Transcripts were analysed using directed content analysis to examine the features and content of greatest importance across groups.

Results: Findings highlighted what adolescents with T1D and their healthcare professionals see as the advantages of a self-compassion chatbot intervention and desired future additions, including a safe peer-to-peer sense of community, personalisation, self-management support to reduce diabetes-related burdens, clinical utility, and breadth and flexibility of tools.

Conclusions: The study suggests that a self-compassion chatbot for adolescents with T1D is acceptable, relevant to common difficulties, and offers clinical utility. Common desired features across both groups included appropriate peer support elements, integration with diabetes technologies, assistance with problem-solving, and broadening the representation of different cultures and lived experience stories to further improve the chatbot. Based on these findings, the COMPASS chatbot intervention is currently being adapted to be tested in a future feasibility study.

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Dental consequences of vitamin D deficiency during pregnancy and early infancy

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Background: Vitamin D (25OHD) is important for mineral balance in early childhood, optimizing absorption of minerals such as calcium and phosphorus. Deficiency during pregnancy is common in New Zealand and correlated with foetal and new-born 25OHD. Late pregnancy and birth are critical periods for dental development. Severe 25OHD deficiency can result in bone and tooth mineralisation defects. The effects of milder 25OHD deficiency on oral health are unclear.

Aim: To investigate dental consequences of vitamin-D deficiency during pregnancy and infancy.

Methods: Dental examinations were conducted, and exfoliated primary teeth analysed for mineral and protein content using Micro-Computed-Tomography (Micro-CT), Energy-Dispersive-X-ray analysis (EDX) and Raman Spectroscopy. Pregnancy and birth data, including 25OHD status, were available for participants and their mothers through a previous study. Enamel structure, dental caries and developmental enamel defects, and associations with 25OHD were investigated.

Results: 81 children participated, and 64 provided an exfoliated primary tooth for analysis. The mean age was 6.6 years, 52% were male, and 80% resided in areas of low or medium deprivation. Two thirds of participants had at least one tooth affected by an enamel defect, and half had experienced dental caries. 25OHD insufficiency was not associated with hypomineralisation, but an increased caries risk (IRR of 3.55) was observed by age six. EDX and Micro CT analysis found no differences in mineral or protein content by 25OHD status, however, Raman spectroscopic data revealed subtle structural differences between those with sufficient, insufficient, and deficient levels of 25OHD.

Conclusions: Enamel defect and dental caries prevalence in this sample was high relative to national and international data. Maternal 25OHD insufficiency during third-trimester was associated with greater caries experience in the primary dentition. No association was found between early life 25OHD and enamel defect prevalence. Subtle differences in enamel quality according to 25OHD categorisation were identified.

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Adolescents with type 2 diabetes have poor emotional wellbeing and unmet needs in relation to their diabetes care

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INTRODUCTION: Type 2 diabetes (T2D) is increasingly prevalent amongst adolescents. Diabetes distress is common in Type 1 diabetes, but there is no data about diabetes distress in adolescents with T2D. Research is also limited examining how diabetes teams can better support emotional wellbeing in adolescents with T2D.

AIMS: We aimed to evaluate diabetes distress and emotional well-being; and to qualitatively examine the lived experiences and barriers to healthcare system engagement in adolescents with T2D.

METHODS: Adolescents with T2D (negative diabetes antibodies) were recruited from the WCH (PCH governance pending). They were asked to complete three questionnaires (diabetes distress scale (DDS), World Health Organisation-5 wellbeing index (WHO-5) and patient health questionnaire-2 [PHQ-2]) and two open-ended questions related to their experience living with T2D and whether they would like additional healthcare system support. Basic demographic and clinical data were also collected.

RESULTS: Eleven participants completed the study (6 males, 1 Indigenous, mean±SD age 16.1±1.4 years, diabetes duration 1.8±1.1years, body mass index 31.9±7.3 kg/m^2 and median [IQR] HbA1c 6.3%[5.7-11.3]). Three participants had clinically significant diabetes distress (DDS ≥3), 6 experienced suboptimal emotional wellbeing (WHO-5 score 28-50) and 7 had a PHQ-2 of 2+, indicating an increased risk of depression. Qualitative data revealed that whilst few participants were satisfied with their diabetes care, others desired more frequent appointments with their endocrinologist, access to T2D-specific support groups, and/or more open discussions about the link between T2D and mental health during their appointments. One participant reported wishing her healthcare team understood “the reality of going back to everything normally after diabetes is diagnosed” and another one “I'm young and none of my friends have it.”

CONCLUSIONS: Adolescents with T2D experience significant diabetes distress, poor emotional wellbeing and depressive symptoms. They have also unmet needs in relation to diabetes care wanting better mental health and wellbeing support.

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Retractions in paediatric endocrinology: are we failing to regulate the literature?

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Aims: The quality of the published literature relies heavily on peer-review. Retractions are another method of managing the evidence base and can be used to deal with fraud, scientific misconduct, and serious errors. We aimed to describe the characteristics and rate of retractions in paediatric endocrinology articles.

Methods: All publications for the 40 highest ranked endocrinology and diabetes journals in the SCImago Journal Rank database were retrieved from PubMed up to 01/08/2022. The publications were restricted using the paediatric filter (0-18yr) and combined with publication types ‘Retraction of Publication’ OR ‘Retracted Publication’.

Results: There were 227,652 publications during this period, of which 35,058 (15.4%) related to paediatrics. Of this cohort, there were 221 retractions in total, of which six were relevant to paediatrics.(1-6) This gives a retraction rate of 9.7 retractions per 10,000 publications for the endocrinology literature and a retraction rate of 1.7 retractions per 10,000 paediatric endocrinology publications. The retracted publications dated between 2002-2015 and together they had been cited a total of 256 times. The corresponding authors came from the USA (n=2), Brazil (n-1), Israel (n=1), Switzerland (n=1), and Turkey (n=1). The reasons for retraction were unreliable data/results (n=3), duplication of data (n=2), misconduct by the author (n=2), methodological error (n=2), and self-plagiarism (n=2).

Conclusion: Retractions are an important method of policing the quality of the scientific literature. Compared to the standard 4 retractions for every 10,000 papers in the general medical literature the rate in paediatric endocrinology (1.7/10,000) is below average.(7) This may reflect increased scrutiny and higher standards at the peer-review stage or reduced post-publication peer review.

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Survey of Australasian clinicians to assess current care provision models and the use of genetic testing in Differences of Sex Development (DSD)

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Pathways of care for management of DSD are evolving, with poorly described models of care and limited evidence-based guidelines. This study aimed to explore current care models for individuals with DSD in Australasia, to identify perceived gaps/strengths/barriers in current practice and explore the diagnostic approach of clinicians, particularly in regards to genetic testing.

An anonymous REDCap online survey was undertaken, recruiting clinicians involved in the diagnosis/management of DSD. The survey questions included demographics, terminology, perceptions of changes/barriers, benefits/downsides to genetic testing, the DSD multidisciplinary team (MDT), availability of genetics and opinions on the role/utility of genetic testing in DSD. Results are reported as a percentage of respondents for that question, with branching logic used.

There were 59 partial and 53 complete responses to the survey, from all states and territories of Australia/New Zealand. 35/56(63%) respondents had an established MDT at their centre, with only 7/35(20%) having psychology involved. 41/56(73%) identified changes to DSD diagnosis/management over the past 5 years, predominantly an increase in genetic testing(54%), introduction of an MDT(51%) and decrease in surgical intervention(44%). The benefit in having a genetic diagnosis in DSD was almost unanimous (96%) with 44% of respondents reporting barriers in genetic testing. Respondents perceived genetic testing in DSD to be underutilised (mean 37 on a sliding scale, with 0=underutilised and 100=overutilised). Approaches to genetic testing when faced with four different clinical scenarios varied across respondents (Table 1).

Responses to the survey identifies gaps and barriers to DSD care across Australasia, in particular the lack of psychosocial supports (an internationally recognised standard of care). There is a discrepancy between the perceived benefit of genetic testing and application to clinical care. Lack of consensus in management highlights the need for further education,

nationwide clinical guidelines and improving access to both MDT care and genetic testing in DSD.

Table 1:

	Chromosomal testing (karyotype/microarray)	Endocrinology	Urology	Genetics (clinical genetics/counsellor)	Other teams	Further genomic testing if XY and normal biochem?
Scenario 1: Proximal hypospadias	20/53 (37%)	15/28 (54%)	1/11 (9%)	1/5 (20%)	3/9(33%)	3/20 (15%)
Scenario 2: Micropenis, bifid scrotum	44/53 (83%)	25/28 (89%)	1/11 (9%)	2/5 (40%)	7/9(78%)	22/44 (50%)
Scenario 3: Primary amenorrhoea, uterus, 46,XY						34/53 (64%)

Other teams: gyaecology, general paed, nursing, neonatology

Does substantial preconception weight loss in women with obesity modify the epigenetic signature of the offspring?

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One in five Australian women are obese (Body Mass Index (BMI) >30) at initial antenatal presentation(1). The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Follow-Up Study demonstrated the metabolic state of the mother during pregnancy, resulted in offspring with greater risk of metabolic disease in late childhood (2).

We aim to determine if substantial pre-conception weight loss in women with obesity alters the epigenome of the offspring. This would support the concept of bi-directional metabolic programming, and the importance of pre-conception weight management.

A two arm, parallel group, non-blinded randomized control trial was conducted at four hospitals in Melbourne, Australia. Women with obesity (BMI 30-55) who were planning pregnancy were randomised in to one of the two 12-week weight loss intervention - a standard lifestyle program and a Very Low Energy Diet (VLED) - followed by a 4 week weight maintenance program and 12 month observation period while trying for pregnancy. Study protocol and pregnancy outcomes have previously been published (4, 5). In a sub-group of consenting participants, buccal swabs (x2) were collected from neonates within 72 hours of delivery. DNA was extracted from these swabs and methylation analysed using the Human Genomics Facility at Erasmus MC. Methylation patterns will be analysed as discrete data according to group allocation and as a continuous variable according to preconception weight loss achieved.

Mean preconception weight loss in the standard lifestyle program and VLED was 3.2kg and 13.0kg ($p<0.01$) respectively. Singleton livebirth rate was 22/79 (28%) and 37/85 (44%) respectively. DNA was extracted from 29 neonates (7 from mothers randomised to the lifestyle arm and 18 from mothers allocated to the VLED arm); 4 samples were deemed unsuitable.

At the time of writing, epigenetic results are pending. It is anticipated that results will be available and analysis complete within 3 months of abstract submission.

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Efficacy and safety of an open-source automated insulin delivery system over 48 weeks of use in the CREATE randomised controlled trial

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Aims: To assess long-term efficacy and safety of open-source automated insulin delivery (AID) in children and adults with type 1 diabetes (T1D) in a 24-week continuation phase following a 24-week multi-site randomised controlled trial.

Methods: Two arms from a 24-week randomised (1:1) controlled trial (RCT) that compared open-source AID (OpenAPS algorithm within a modified version of AndroidAPS in a smartphone, pre-production DANA-i insulin pump, Dexcom G6 continuous glucose monitor), to sensor augmented pump therapy (SAPT), entered into a 24-week continuation phase where the SAPT arm (SAPT-AID) crossed over to join the open-source AID arm (AID-AID). A hardware switch occurred in the majority of participants in the continuation phase, where the pre-production DANA-i insulin pump was substituted with a pre-production YpsoPump.

Results: In the SAPT-AID group (n=52), mean percentage of time in range (TIR; 3.9-10mmol/L) increased from 54.5±16% using SAPT during the RCT to 67.4±10.6% using AID (Δ+12.9%, 95% confidence interval (CI) 9.6 to 16.2; p<0.001), with 44% achieving TIR >70% compared to 15% using SAPT (p<0.001). In the AID-AID group (n=42), mean TIR increased from 61.2±12.3% pre-randomisation to 71.2±12.1% during the RCT and remained stable at 69.3±12.5% during the final two weeks of the continuation phase (Δ-1.9% from the RCT, 95% CI -5.6 to 1.8; p=0.310). By the end of the continuation phase mean TIR was almost identical between treatment groups (p=0.92). No episodes of diabetic ketoacidosis or severe hypoglycaemia occurred in either group. Four participants in the SAPT-AID group withdrew; 1 due to infusion site skin irritation, 1 due to a hardware issue, 2 preferred SAPT.

Conclusion: Further evaluation of the CREATE trial to 48 weeks (24 weeks post RCT) confirms open-source AID using the OpenAPS algorithm within a modified version of AndroidAPS is efficacious and safe with various hardware, and demonstrates sustained glycaemic improvements without additional safety concerns.

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Diabetic ketoacidosis at onset of type 1 diabetes and long-term HbA1c in 7961 children and young adults in the Australasian Diabetes Data Network (ADDN)

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Aims

The reported relationship between diabetic ketoacidosis (DKA) at diagnosis of type 1 diabetes (T1D) and long-term glycemic control varies between studies. We aimed firstly to characterise the association of DKA and its severity with long-term HbA1c in a large contemporary cohort, and secondly to identify other independent determinants of long-term HbA1c.

Methods

Participants were 7961 children and young adults diagnosed with T1D by age 30 years from 2000-2019 and followed prospectively in the Australasian Diabetes Data Network (ADDN) until 31/12/2020. Linear mixed effect models were used to study the relationship between HbA1c and other variables.

Results

DKA at diagnosis was present in 2647 (33.2%) participants. Over a median 5.6 (IQR 3.2, 9.4) years follow-up, participants with severe, but not moderate or mild, DKA at diagnosis had a higher mean HbA1c (+0.23%, 95% CI: 0.11, 0.28; p<0.001) compared to those without DKA. Use of continuous subcutaneous insulin infusion (CSII) was independently associated with a lower HbA1c over time than multiple daily injections (MDI) (-0.28%, 95% CI: -0.31, -0.25; p<0.001), and this relationship was more pronounced in participants with severe DKA at diagnosis. Indigenous status was associated with higher HbA1c (+1.37%, 95% CI: 1.15, 1.59; p<0.001), as was residing in postcodes of lower socioeconomic status (most versus least disadvantaged quintile +0.43%, 95% CI: 0.34, 0.52; p<0.001).

Conclusion

Severe DKA at diagnosis (but not moderate or mild) was associated with a marginally higher HbA1c over follow-up of ADDN children and young adults with T1D, an effect which was modified by use of CSII. Indigenous status and lower socioeconomic status were independently associated with higher HbA1c, and CSII with lower HbA1c, further emphasising the need for equity of access to health services and modern diabetes technology for all patients with T1D.

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Outcomes of genomic testing for 46, XY Differences of Sex Development

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Genomic sequencing of DSD-related genes commenced at Victorian Clinical Genetics Service (VCGS) in March 2018. The DSD panel comprises >70 genes with validated gene-disease association listed on PanelApp Australia (https://panelapp.agha.umccr.org/panels/99/). Our objective was to review the outcomes of DSD-related genetic testing performed at VCGS between March 2018 and Dec 2021.

A retrospective review was conducted. Clinical and demographic information provided at referral for genomic testing were extracted. Outcomes of interest included the range of pathogenic (Class 5), likely pathogenic (Class 4) variants and variants of unknown significance ([VUS] Class 3a)[1] reported, the overall diagnostic yield in the cohort and associations with clinical features.

Data from 155 individuals with 46,XY DSD were included. Pathogenic (n=28) or likely pathogenic (n= 8) variants consistent with DSD phenotype were found in 36/155 (23%) individuals, across sixteen different genes. *AR*(n=8; all assigned female), *NR5A1*(n=6) and *SRD5A2*(n=5) variants were most common. Diagnostic variants were identified in n=15/36(41%) and n=22/118(19%) assigned females and males respectively. Additional extra-genital clinical features were reported in 7/36(19%). DSD-relevant Class 3a VUS were also reported in 6/155(4%), with segregation testing recommended. A further

six individuals had one copy of a DSD-relevant pathogenic / likely pathogenic variant but no 'second-hit' to explain the diagnosis.

Genomic testing has confirmed a DSD-diagnosis in ~1/4 of individuals tested over the initial 4 years at VCGS. Higher diagnostic rates were found in those with (predominantly-) female genital phenotype; however, a majority of this subset remains undiagnosed. No individual with 46,XY DSD and male-appearing genitalia had an AR variant, highlighting the relative infrequency of a diagnosis of partial androgen insensitivity syndrome in 46XY DSD in the era of genomic testing. These findings assist with optimising clinical decisions (e.g. gonadal management - *AR*, dihydrotestosterone replacement - *SRD5A2*) and along with longitudinal clinical follow-up will improve our understanding of DSD.

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Which is the better newborn screening marker for classical CAH - 21 Deoxycortisol or 17α-hydroxyprogesterone?

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The NSW Newborn Screening Programme commenced screening of newborns for classical congenital adrenal hyperplasia (CAH) in May 2018. All newborns born in NSW and ACT are screened for CAH using a two-tier protocol, the first tier measuring 17α-hydroxyprogesterone (17αOHP) using immunoassay followed by second-tier steroid profiling via liquid chromatography-tandem mass spectrometry (LC-MS/MS) measuring 17αOHP (MS17αOHP), androstenedione, and cortisol [1, 2]. This screening algorithm gave a CAH screening positive predictive value (PPV) of 71.4%[3]. There were 16 (from 388,416 screened) proven cases of CAH giving an incidence of 1:24,276

The CAH screening efficacy with the addition of 21 deoxycortisol to LC-MS/MS steroid profiling was investigated to determine if it is a better disease marker compared to 17αOHP for CAH. The top 98th centile of daily immunoassay 17αOHP measurement from June 2021-February 2022 were selected (n=2762).The positive predictive value (PPV) for 17αOHP and 21 deoxycortisol as a sole indicator and part of a ratio were calculated.

There was no statistically significant effect of birth weight or gestational age (GA) on 21 deoxycortisol compared to 17αOHP.

The calculated PPV, using a 95th centile concentration cut-off, showed no significant improvement when comparing 21 deoxycortisol (10.9%), against MS17αOHP (11.5%), as a sole disease marker without birth weight and GA stratification.

By combining the use of MS17αOHP concentrations of 40.1 nmol/L whole blood (WB) and MS17αOHP+A4)/cortisol of 1.2, MS17αOHP/cortisol of 1.6, and (MS17αOHP+21 deoxycortisol)/cortisol of 1.9 all newborns with CAH with gestational age (GA) >259 days were correctly identified. Screening efficacy for newborns with GA <259 days improved when the cut-off MS17αOHP WB concentration of 58.2 nmol/L in combination with (MS17αOHP + 21 deoxycortisol)/cortisol of 3.4 was used.

This study showed that the screening efficacy improved with GA stratification. No significant statistical improvement was detected by using 21 deoxycortisol as a disease marker for CAH.

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Participant experiences of open source automated insulin delivery during the CREATE study; a 6 month randomised controlled trial.

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Aims: Open-source automated insulin delivery (AID) is perceived to be technologically complex, and data to date has limited generalisability due to reporting in self-selected populations. This sub-study investigated participants' experiences in the CREATE study, a multi-site randomised controlled trial of an open-source AID algorithm.
Methods: The CREATE study compared an open-source AID system—consisting of an app on an Android phone, with CGM and a Bluetooth enabled insulin pump—versus sensor-augmented pump therapy. Study participants completed age appropriate psychosocial surveys (HFS-II, DTSQs, PSQI, EQ-5D) at the beginning and end of the 24 week trial. Survey data were summarised by treatment group, and differences estimated using analysis of covariance. A representative sample of adults, children, and parents of children from the intervention group were selected for in-depth, semi-structured interviews. Interviews were recorded and transcribed. Thematic analysis was undertaken using Nvivo. The sample was completed at the point of thematic saturation.
Results: 97 participants completed surveys. Survey data are presented in table 1. Treatment satisfaction improved for children. Most other scales favoured AID but differences were not statistically significant. 8 Adults (4f, mean age 41.6), 6 children (2f, mean age 10.1y) and 8 parents (5f) participated in interviews. All interview participants reported improvements with AID, including improved quality of life and glycaemic control, reduced diabetes burden and, for parents, improved family dynamics. Most participants found the phone-based system convenient; technical problems were present but manageable with study team support; participants were able to use the system to fit their lifestyle needs.
Conclusion: Use of open-source AID improves quality of life in multiple domains and reduces treatment burden.

Table 1. Survey results for CREATE study participants.

		Baseline		Study end		AID - Control	
Outcome	**Age**	**Control**	**AID**	**Control**	**AID**	**Diff. (95% CI)**	**P value**
DTSQ - total	7 to 17 yrs	28, 79.5 (12.0)	22, 81.4 (10.0)	27, 78.0 (10.2)	21, 85.9 (9.5)	6.1 (0.4, 11.7)	0.036
	18+ yrs	25, 76.7 (15.5)	22, 80.2 (10.9)	25, 80.0 (15.5)	21, 87.3 (13.6)	6.2 (-1.9, 14.2)	0.131
EQ5D - health	8 to 15 yrs	24, 86.5 (11.3)	21, 85.3 (12.5)	23, 79.1 (17.4)	20, 85.2 (8.1)	6.1 (-3.0, 15.1)	0.184
	16+ yrs	26, 74.4 (17.5)	23, 70.4 (18.5)	26, 78.3 (12.8)	22, 77.9 (15.2)	1.0 (-6.8, 8.8)	0.797
HFS-II (SF) - Behaviour	7 to 17 yrs	28, 48.1 (14.7)	22, 46.1 (13.3)	27, 50.9 (16.4)	21, 46.1 (13.6)	-3.4 (-11.0, 4.3)	0.376
	18+ yrs	25, 67.4 (16.7)	22, 68.0 (17.2)	25, 69.6 (13.5)	21, 69.5 (17.7)	0.4 (-8.0, 8.8)	0.921
HFS-II (SF) - Worry	7 to 17 yrs	28, 74.8 (15.3)	22, 73.3 (15.1)	27, 71.0 (17.0)	21, 75.1 (15.1)	5.2 (-3.2, 13.6)	0.219
	18+ yrs	25, 78.4 (21.4)	22, 68.6 (23.4)	25, 76.6 (19.7)	21, 80.0 (18.9)	8.3 (-1.5, 18.1)	0.096
PSQI - total	13+ yrs	35, 72.8 (13.8)	37, 71.3 (15.9)	37, 72.8 (14.8)	35, 73.3 (16.1)	0.6 (-5.1, 6.2)	0.841

Results presented as number of respondents, raw mean (SD) and mean difference (95% CI). All outcomes have been rescaled from 0 to 100, representing the worst and best possible outcomes respectively. Mean differences were estimated using analysis of covariance and adjusted for stratification factors (site, HbA1c). Participants with missing data have been excluded from the analysis. The PSQI was not collected for children aged less than 13 years.

Impact of oil-soluble contrast medium hysterosalpingography on thyroid function in the offspring

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Hysterosalpingography (HSG) using oil-soluble contrast medium (OSCM) improves pregnancy rates[1], but results in severe and persistent iodine excess[2,3] potentially impacting the fetus and neonate. The aim of this study was to determine the incidence of thyroid dysfunction in newborns conceived within six months of OSCM HSG.

A prospective newborn study was undertaken in Auckland region, New Zealand from 2020-2022. The cohort consisted of 57 newborns conceived within 6 months to a group of women who underwent OSCM HSG as part of SELFI (Safety and efficacy of Lipiodol in Fertility Investigations) study[4] that assessed iodine excess and thyroid dysfunction of women post-HSG. All newborns had a dried blood spot card for TSH measurement after 48 hours of birth (newborn screening). 41 neonates also had a heel prick serum sample at one week for thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3). Maternal urine iodine concentration (UIC) and TSH in the six months after OSCM HSG were retrieved from the SELFI study. The primary outcome was the incidence of congenital hypothyroidism or subclinical hypothyroidism in the newborn.

There was no evidence of primary hypothyroidism on newborn screening (whole blood TSH 2-10mIU/L). All neonates tested at one week had serum TSH, FT4 and FT3 in the age-appropriate reference range. However, increasing maternal peak UIC levels were associated with lower TSH (95%CI -0.5, -0.1; p= 0.029) despite lower FT4 levels (95% CI -2.1, -0.1 pmol/L; p=0.041).

In conclusion, pre-conceptional OSCM HSG in women did not increase neonatal subclinical hypothyroidism or congenital hypothyroidism. However, gestational iodine excess was associated with a paradoxical lowering of neonatal FT4 levels despite lower TSH levels. We speculate these changes reflect iodine excess on the fetal hypothalamic-pituitary axis.

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Mapping the single cell transcriptome of murine adrenal glands

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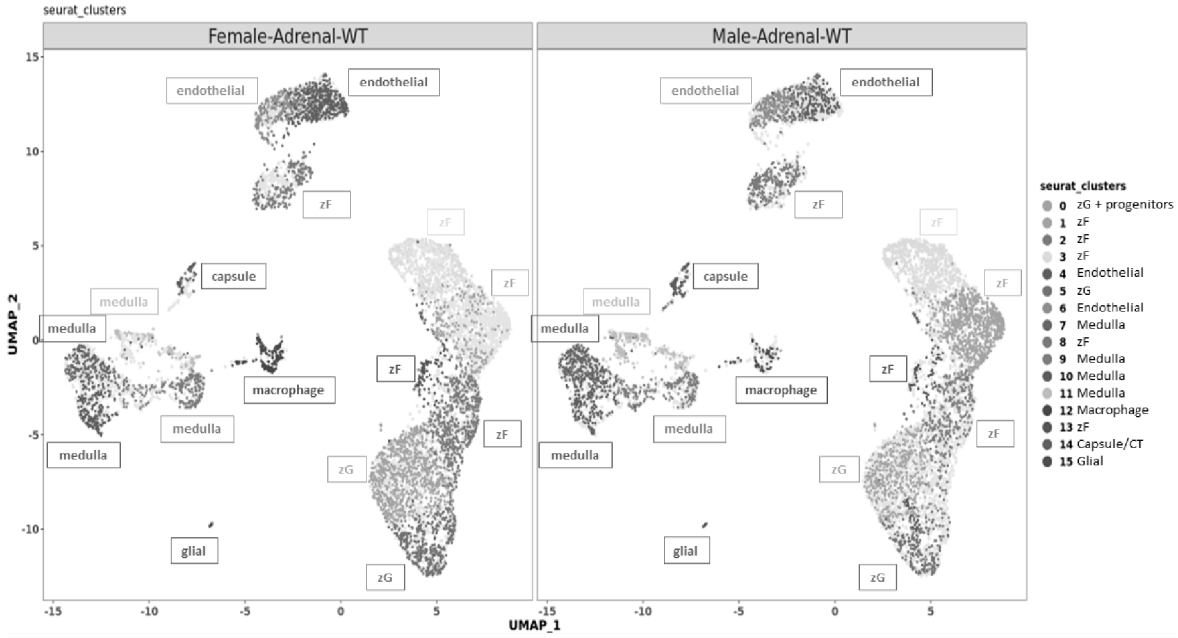
The adrenal cortex undergoes constant renewal(1): capsular stem cells expressing GL1+ give rise to cortical SF1+ progenitor and SF1+ differentiated steroidogenic cells of the zona glomerulosa (zG)(2-4). Despite knowledge of their existence, these populations are yet to be adequately described. Identification is required for the development of durable gene therapy for monogenic disorders of the adrenal cortex. As the progenitor population is very small, they do not form a distinct cell cluster in single cell RNA sequencing (scRNA-Seq) transcriptomic analysis and have not been identified by a previous group(5). The aim of this study was to map the adrenal single cell transcriptome and to identify adrenocortical progenitor cells.

Adrenal glands were harvested from wild-type mice, snap frozen, and dissociated into single nuclei. The scRNA-Seq library was prepared using 10x Chromium technology and sequenced by the Illumina NovaSeq platform. The transcriptome was mapped to the mouse mm10 reference genome using the *CellRanger* pipeline(6), followed by scRNA-Seq analysis using the *Seurat v4.0* package. Cell types were annotated using a combination of the Clustifyr package and manual annotation using gene expression patterns.

The scRNA-Seq clustering pattern is shown in Figure 1. The progenitor population did not form a distinct cluster. However, cells co-expressing *Nr5a1* (encodes SF1 protein), *Shh* and *Ctnnb1* that did not express *Cyp11b1* or *Cyp11b2* (markers of differentiated adrenocortical cells) were detected in cluster 0, consistent with SF1+/SHH+ progenitors. Progenitor gene expression was detected in 0.34% and 0.22% of cells from the female and male, respectively.

In conclusion, a population consistent with adrenocortical progenitor cells was identified. Transcriptomic analysis of adrenals from 21-hydroxylase deficient mice is underway, and the resultant profile will be compared with that of the wild-type mice. Additional ACTH stimulation is postulated to affect these populations and may expand the progenitor pool.

Figure 1. Using the Seurat package, 16 distinct cellular clusters were determined when the wild-type male and female single nuclei transcriptomic data was combined. The Uniform Manifold Approximation and Projection (UMAP) non-linear dimensional reduction technique with annotated clusters is shown with the sexes separated. Clusters 0 and 5 were zona glomerulosa cells (zG) and clusters 1, 2, 3, 8 and 13 were zona fasciculata (zF) cells. All 16 of the clusters are listed within the figure. Progenitor cells were identified as cells that expressed *Shh*, *Nr5a1* and *Ctnnb1* and did not express *Cyp11b1* or *Cyp11b2*. These were located within cluster 0 (zG).



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Personalized modelling of human infertility with human induced pluripotent stem cells

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Infertility is a disease that impacts around 8-10% of the reproductive age population around the world with around 30% of infertility cases being unexplained. Human induced pluripotent stem cell (hiPSC) research enables reproductive diseases such as infertility to be studied when the reproductive status of the research participant is known. In this study, we consent research participants to undergo a procedure known as a skin punch biopsy to generate hiPSC lines following induced reprogramming using non-integrating sendai virus reprogramming technology. Research participants in this study include individuals with proven fertility (live births without assisted reproductive intervention), individuals diagnosed with non-obstructive azoospermia (NOA), as well as individuals diagnosed with primary ovarian insufficiency (POI) including rare sets of monozygotic (MZ) twins with discordant POI. Using various *in vitro* differentiate strategies, our group is currently assessing germ cell induction potential using hiPSC lines generated from each research participant as an individualized approach to understanding infertility in humans. Here, I will discuss our work on MZ twins discordant for the disease of POI, and the use of stem cell-based embryo models to understand germ cell induction and amnion specification. For two of the MZ twin pairs in our study, gestational history indicates the twin pairs shared a single chorion (MC) and amnion (MA). Given germ cells are specified as the amnion forms, we hypothesized that MA twins discordant for POI arise through discordant allocation of germ cells from the single amnion. Here we show that hiPSCs generated from MA twins with discordant POI induce equivalent numbers of germ cells when comparing the infertile twin's cells to her fertile sister, as well as the ability of each twin's cells to generate their own amniotic sac-like structure. Using these hiPSCs together with genome sequencing, our data suggests that the lack of germ cells in the infertile twin is not due to a genetic barrier to amnion or to germ cell formation. Future studies will evaluate germ cell induction potential in the remaining research participants in order to increase our understanding of human infertility using the personalized approach of hiPSC-based modelling.

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The prognostic value of DNA integrity assessments: An ongoing investigation.

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Fertilisation with sperm harbouring damaged DNA can trigger de novo mutations in the embryo, deleteriously affecting embryo development and future offspring health. As such, it is vital that DNA integrity assessments are routinely conducted. Despite the range of DNA integrity assessments available, it remains unclear which assay holds greatest prognostic value. We therefore aimed to assess the ability of common DNA integrity assays to identify ill-fated pregnancies. To this end, dismount semen samples were collected weekly from 47 Thoroughbred stallions during the 2017 (n=486) and 2018 (n=318) breeding seasons. Samples were diluted (2:1,extender:semen), and purified to isolate the high-quality sperm fraction. Sperm concentration and motility were also recorded (iSperm™). Samples were subsequently fixed for 8-oxoguanine (8-oxoG) assessment via flow cytometry, or snap frozen for sperm chromatin structure assay (SCSA) and alkaline comet assessments. Pregnancy data were used to identify samples that were associated with successful pregnancies (healthy offspring produced), and those that were associated with 'lost' pregnancies (pregnancy confirmed at 14 days post-breeding, and subsequently lost). 8-oxoG fluorescence of sperm DNA was significantly higher in samples associated with a lost pregnancy, compared to successful pregnancies (P≤0.05). In support of this, comet tail fluorescence was also significantly higher in samples where the pregnancy was lost (61±2.3%), compared to those that produced a healthy offspring (50±5.1%;P≤0.01). However, no difference was detected via SCSA, with both groups recording a DNA Fragmentation Index of 1% (P≥0.05). These findings indicate the prognostic value of both the 8-oxoG and alkaline comet assays in identifying ejaculates that will likely result in lost pregnancies, alongside the vital importance of ensuring appropriate assessments of sperm DNA integrity are routinely conducted. Further research is required to assess the value of other DNA integrity assays and to understand the heritable genetic risks to offspring.

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Vitrification within a nanolitre volume: oocyte and embryo cryopreservation within a 3D photopolymerised device

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Cryopreservation is an essential assisted reproductive technology widely practiced in the IVF clinic, providing the valuable opportunity for fertility preservation. The procedure is technically demanding, requiring precise handling of cells by an experienced embryologist within a strict timeframe to ensure minimal transfer of potentially cytotoxic cryoprotectants. The embryologist must meticulously trace cells throughout the process and avoid procedural deviations that affect cell survival post-warming, and subsequently fertilisation and embryo development. We hypothesised that minimising direct handling will simplify the procedure, improve traceability and consequently, cell viability. To address this, we present a novel 3D photopolymerised device that houses cells during vitrification and warming. The fabricated device consists of two components: the Pod (670 x 235 x 353 µm; l x w x h) and Garage (1150 x 450 x 345 µm). Individual mouse oocytes or embryos were housed in a Pod, with three Pods docked into a Garage. We assessed the suitability of the device for cryogenic application by examining its structural integrity following repeated vitrification and warming cycles. To understand how the device affected cell viability post-warming, we evaluated oocyte and embryo survival, developmental potential (fertilisation and subsequent embryo development) and metabolic profile by measuring endogenous fluorophores using confocal microscopy. Oocytes or blastocyst-stage embryos were vitrified either using standard practice or within Pods and a Garage and compared to non-vitrified control groups. Vitrification within the device occurred within ~3 nL of cryoprotectant: this volume being ~1000-fold lower than standard vitrification. We demonstrate that vitrification and warming within the device had comparable oocyte and embryo survival, developmental competency, and metabolic profile to that of standard practice. The Pod and Garage device minimised the volume of cytotoxic cryoprotectant at vitrification, improved traceability and reduced direct handling of the sample, paving the way for a major step in simplifying the procedure.

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High-throughput sperm selection using a network of 3D microchannels

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More than 70 million couples around the world are confronted with infertility (1) where male factors contribute to around 45% of the cases (2). Assisted reproductive technologies (ART) have been used to facilitate infertility over the past 40 years. Selection of high-quality sperm is an important step in ART as it influences the treatment success rate, live-birth rate, and offspring health (3). However, current clinical sperm selection methods are highly manual, time-consuming, prone to operator errors and differ significantly from the natural three-dimensional (3D) selections *in vivo*. Lack of technological developments to improve sperm selection in fertility clinics, to provide the required number and volume of high-quality sperm, has been the main barrier to improving treatment methods (4). Here, we present a scalable and clinically applicable technology to select high-quality sperm via a 3D network of microchannels.

This 3D platform mimics the *in vivo* sperm selection process and enables a high throughput selection of over 1.5 million sperm in 15 minutes. The device was originally fabricated using a stereolithography 3D printer (5), but to ensure clinical translation, we have developed a new prototype of the device for fabrication using injection molding out of polystyrene. The device is prefilled with buffer and during the selection time, motile sperm swim through a network of microchannels to reach the outlet while debris and non-motile cells remain in the inlet. The device achieves the state-of-the-art retrieval efficiency of 41%, significantly outperforming previous technologies by at least 10%, selecting sperm with more than 65% improvement in both DNA integrity and morphology.

In conclusion, we present a high throughput 3D sperm selection device that provides novel practical opportunities to improve sperm selection practices in assisted reproduction.

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Folic acid food fortification and pregnancy health

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To reduce risk for neural tube defects, pregnant women in Australia are advised to supplement with 400µg folic acid (FA) daily, at least one month prior to conception and during first trimester. In September 2009 the Australian government implemented mandatory fortification of bread-making flour with FA, targeting women of reproductive age who may conceive without supplementing. Soon after, manufacturers commenced voluntary fortification of numerous food products with FA, exposing the population to high levels of FA with unknown consequences for pregnancy health.

We have extensive databases and biobanks from two large prospectively recruited pregnancy cohorts from the Lyell McEwin Hospital in Adelaide, Australia prior to (SCOPE 2005-2008) and post (STOP 2015-2018) FA food fortification. Gestational diabetes mellitus (GDM) incidence has increased post (STOP, 15.2%) compared to prior to (SCOPE, 5%) FA fortification, mirroring national trends. We previously showed that in early pregnancy SCOPE women who were later diagnosed with GDM had higher serum folate than those with uncomplicated pregnancies. Here we evaluated data from uncomplicated SCOPE (n= 604) and STOP (n=711) pregnancies to identify factors that may contribute to GDM risk. We measured serum folate, vitamin B12 and homocysteine, as well as placental hormones, hPL, GH-V and prolactin, that are secreted into maternal circulation to stimulate insulin resistance and beta cell expansion.

Compared to SCOPE, STOP women with uncomplicated pregnancies were slightly older (Mean [SD]: 23.6 [5.0] vs 25.6 [4.9]) and in early pregnancy had significantly higher serum folate (Mean±SD 42.5±28.9nM vs 52.9±34.9nM), B12 (Mean±SD 263.9±101pM vs 298±121pM), homocysteine (Mean±SD 4.6±1.1µM vs 5±1.1µM) and hPL (Estimated Marginal Mean±SE 89.5±2.07 vs 100.7±1.78). Interestingly, in women <35years, all three hormones were significantly lower in obese compared to non-obese women.

Considering FA food fortification, high intake of FA during pregnancy warrants further evaluation of potential adverse effects on placental function and pregnancy health.

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Human *INHBB* Gene Variant (c.1079T>C:p.Met360Thr) is disruptive for pregnancy and labouring in female mice

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Ovarian-derived inhibin A and inhibin B (α/β dimers) act in an endocrine manner to suppress pituitary production of follicle-stimulating hormone (FSH), by blocking the actions of activins (β/β dimers). This hypothalamic-pituitary-gonadal (HPG) loop is integral to reproductive function, and consequently, imbalances in inhibin/activin can impact fertility. In a recent study, we showed that a human *INHBB* gene variant (c.1079T>C:p.Met360Thr), identified in an infertile man, significantly reduced serum activin B levels and altered testis germ cell content in corresponding *Inhbb*$^{M364T/M364T}$ male mice. This study aimed to determine if the identified *INHBB* gene variation also had consequences for female reproductive function. To address, we examined ovarian and uterine function in *Inhbb*$^{M364T/M364T}$ adult female mice. As seen *Inhbb*$^{M364T/M364T}$ male mice, female *Inhbb*$^{M364T/M364T}$ mice displayed reduced levels of circulating activin B, and also activin A relative to wildtype littermates. Despite this, ovarian folliculogenesis and ovulation rates were comparable. Interestingly, *Inhbb*$^{M364T/M364T}$ females had a greater number of implantation sites at late gestation, and a significant pre-disposition to dystocia, with extended gestation periods and labours relative to wildtype litter mates. Dystocia in these females appears to be attributed to disrupted uterine contractility. This study provides evidence that reduced circulating levels of activins A/B are detrimental to pregnancy outcomes in females.

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Lipopolysaccharide induced inflammation in fetal membranes can be mitigated by treatment with Angiotensin-(1-7)

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Inflammation has long been implicated as a key mediator in the onset of parturition in both preterm and term births. Tissue renin-angiotensin systems (RASs) can regulate inflammation. The angiotensin converting enzyme 2 (ACE2)/Angiotensin-(1-7)/Mas receptor anti-inflammatory pathway, has been shown to inhibit the pro-inflammatory cytokines interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α levels and enhance the anti-inflammatory cytokine, IL-10, in pancreatic acinar cells. We aimed to investigate whether activation of the ACE2/Angiotensin-(1-7)/Mas receptor pathway protects against lipopolysaccharide (LPS) induced inflammatory cytokine expression in term fetal membranes.

Term non-labouring choriodecidual explants were treated with AVE0991 (100 μM, Mas receptor agonist) for 24h after which lipopolysaccharide (0.2μg/ml, O55:B5) was introduced for 6h. Tissue and culture medium was collected for analysis (n=12). Levels of IL-1β, IL-6, and IL-10 and TNF-α were measured via qPCR and ELISA.

Low dose LPS treatment of choriodecidual explants enhanced the expression of TNF-α, IL-6, IL-1β and IL-10 relative by 4-15-fold relative to vehicle control explants (all P<0.0001). Similarly, LPS induced a significant increase in the secretion of TNF-α and IL-6 from explants relative to vehicle control explants (P<0.0001 and P=0.001 respectively). Pre-treatment of choriodecidual explants with 100 μM AVE0991 was able to mitigate LPS induced mRNA expression of pro-inflammatory cytokines IL-6 and IL-1β compared with explants pre-treated with 0 μM AVE0991 (P=0.01 and 0.02 respectively). This effect however was not seen with pre-treatment with 10 μM AVE0991. Pre-treatment with both 10 and 100 μM AVE0991 was unable to mitigate the effects of LPS induced inflammation on the mRNA expression and protein secretion of TNF-α or IL-10 mRNA.

These data suggest that the ACE2/Ang-(1-7)/MasR pathway may play a role in regulating inflammation within the choriodecidua during pregnancy and thus pose as a novel therapeutic target to prevent inflammation induced preterm birth.

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Metformin has pleiotropic effects on key molecules dysregulated in placenta affected by preeclampsia

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Preeclampsia is a serious complication of pregnancy and there is no medical treatment. Metformin has been identified in laboratory studies and a clinical trial as a potential treatment for preeclampsia [1]. Here in, we explore its potential mechanism of action in treating preeclampsia.

We have previously shown metformin likely reduces sFlt-1, an antiangiogenic molecule upregulated in preeclampsia, by inhibiting complex I of the electron transport chain [2, 3]. In treating trophoblasts with metformin (1-5mM) we showed a reduction in sFlt-1 secretion (*p<0.001*). We rescued sFlt-1 secretion by adding succinate, a substrate for complex II, which switches the electron transport chain on in the presence of metformin [3].

Metformin is known to upregulate AMP activated protein kinase (AMPK) in the liver. AMPK is a regulator of energy homeostasis that coordinates metabolic function and mitochondrial dynamics. We discovered that AMPK protein was higher in metformin (1-5mM) treated trophoblasts (n=5, *p<0.05*). Similarly, AMPK is increased in placentas from patients with preterm preeclampsia treated with metformin, compared to placebo (n=21, *p<0.01*).

Reactive oxygen species (ROS) are upregulated in preeclampsia. In other fields metformin increases ROS. Reassuringly, we demonstrated treating trophoblasts with metformin (1-2mM) significantly reduced ROS under both normoxic (n=5, *p<0.01*) and hypoxic conditions (n=5, *p<0.01*), compared to vehicle treated controls (as determined by DCFDA kit).

We have shown metformin likely has pleiotropic effects in trophoblasts to quench preeclampsia. It inhibits complex I of the mitochondrial electron transport chain reducing sFlt-1 secretion. It upregulates AMPK, the master regulator of cellular metabolism. It also reduces reactive oxygen species. By understanding its mechanism of action we might develop targeted therapies which may be more efficacious at treating preeclampsia.

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Combined IAP inhibition with rigosertib leads to synergistic anti-tumour responses in vitro in papillary and anaplastic thyroid cancer

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Background: Limited therapeutic options exist for radioiodine-refractory thyroid cancers. Overexpression of Inhibitors of Apoptosis (IAP) in thyroid cancer is associated with adverse clinicopathological features.[1,2] IAP inhibition holds a promising role in combination treatment strategies for other malignancies, however is yet to be fully elucidated in thyroid cancer.[3]

Objective: To determine efficacious partners for combination therapy with IAP inhibition in papillary thyroid carcinoma (PTC) and anaplastic thyroid carcinoma (ATC).

Methods: High-throughput screening (HTS) using established drug libraries was performed in the PTC cell line, TPC-1 (RET-CCDC6), following pre-treatment with the IAP inhibitor, Compound A (CmpdA; 500nM).[4] Drug combination hits were defined as >80% reduction in viability compared to CmpdA alone. Validation studies of prioritised combination treatments were expanded to K1 ($BRAF^{V600E}$ PTC) and SW1736 ($BRAF^{V600E}$ ATC) cell lines. Effects on cell viability, apoptosis, cell cycle progression and cell migration capacity were studied.

Results: HTS after CmpdA pre-treatment identified 179 hits. Rigosertib, a multikinase inhibitor, was selected for further study. Dose-response evaluation of cell viability demonstrated that CmpdA treatment alone had only modest effect, but established synergistic efficacy in combination with Rigosertib. Synergy quantification[5] revealed optimal responses with 500nM Rigosertib. The combination induced apoptosis evidenced by increased caspase 3/7 activity. Rigosertib led to G2/M cell cycle arrest. There was no difference in cell cycle progression in combination with CmpdA. Scratch wound assays demonstrated that Rigosertib reduced cell migration capacity at 24 hr, whilst no difference was seen with CmpdA.

Conclusion: IAP inhibition has synergistic potential for therapy in PTC and ATC *in vitro*. IAP inhibitor therapy had significant synergistic effects when used with Rigosertib. Effects are mediated primarily by enhanced apoptosis as well as G2/M phase arrest. Reduced cell migration capacity was also observed. Further study investigating optimal treatment combinations involving IAP inhibition including *in vivo* models is required.

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Analysis of ciclesonide, a novel glucocorticoid receptor agonist, as a potential novel treatment for the consequences of preterm birth

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Glucocorticoid (GC) signalling is essential for normal fetal lung development. During late gestation a surge of endogenous GCs rapidly matures the lung by thinning the mesenchymal tissue and driving an increase in alveolar gas exchange surface area. Currently in situations of imminent preterm birth potent synthetic GCs such as betamethasone or dexamethasone (Dex) are administered antenatally to accelerate fetal lung maturation and reduce the risk of respiratory distress syndrome. There are however growing concerns that systemic exposure to powerful synthetic GCs is associated with detrimental side effects, particularly in the developing fetal brain. We are currently assessing novel activatable and selective partial agonists of the glucocorticoid receptor (GR) as new potential antenatal steroid treatments of preterm birth. One such GR agonist is a steroid prodrug called ciclesonide (Cic) that is activated in vivo by a family of intracellular serine-esterase enzymes, called the carboxylesterases (Ces), that are predominantly expressed in peripheral organs but are virtually absent in the brain. We have compared the effect of inactive and activated ciclesonide to dexamethasone for the regulation of key GR-regulated respiratory genes. Ciclesonide activity in the fetal lung was assessed by culturing primary mouse fetal fibroblast cells from wild-type and GR-null mice and treating them with Dex, Cic and its active compound, Des-Cic, for six hours. Changes in the fibroblast transcriptome was assessed by microarray analysis and RT-qPCR. Analysis of the top 30 induced and repressed genes illustrate that Dex and Des-Cic have similar activity profiles. Additionally, analysis of induced mRNA levels for four known GR induced genes, Fkbp5, Crispld2, Tgm2 and Zbtb16, showed that the active GR agonist Des-Cic strongly induced expression of these genes, and utilises functional GR to exert their effects. The data suggests that Cic regulates important respiratory genes in a similar fashion to Dex.

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Temporal changes in bone turnover and microarchitecture following withdrawal of RANKL inhibition

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Denosumab is an effective osteoporosis treatment, preventing bone loss by inhibiting RANKL. However, stopping denosumab leads to rebound bone mineral density(BMD) loss. This is due to accelerated bone resorption by osteoclasts. Serum bone turnover markers have been utilised to guide sequential therapy following denosumab discontinuation, however an optimal strategy has not been established. Understanding the temporal changes in osteoclast activity will guide safe, effective sequential therapy following denosumab discontinuation. We hypothesised that serum TRAP, a marker of enzymatic activity of osteoclasts, would be a more useful marker in this context.

Seven-week-old female C57BL/6 mice were treated with 2-weeks of thrice-weekly saline (vehicle) or OPG:Fc (10mg/kg) to inhibit RANKL. Longitudinal BMD and serum TRAP5b were measured throughout the study. Serum CTX and microCT were performed at weeks 2, 11 and 13.

Following OPG:Fc, BMD peaked at week 8 in treated mice and normalised to vehicle levels by week 13 (A). MicroCT analysis showed significantly higher trabecular volume (BV/TV) at the diaphysis in OPG:Fc treated mice at all timepoints (B).

Serum TRAP was significantly higher in treated mice at week 11 though serum CTX was equivalent to vehicle levels. Both serum TRAP and CTX were significantly higher in treated mice at week 13 by which bone loss had occurred and BMD reached vehicle levels (C). Longitudinally, serum TRAP was fully suppressed by week 2 and remained suppressed until week 8, following which serum TRAP levels rose 64% above vehicle levels at week 12. This rise in TRAP between weeks 8 to 10 preceded the decline in BMD (D).

Our findings show that rebound decline in BMD has already occurred by the time CTX rises above vehicle levels. A significant rise in serum TRAP occurs earlier and may be a better marker to guide sequential therapy following denosumab discontinuation.

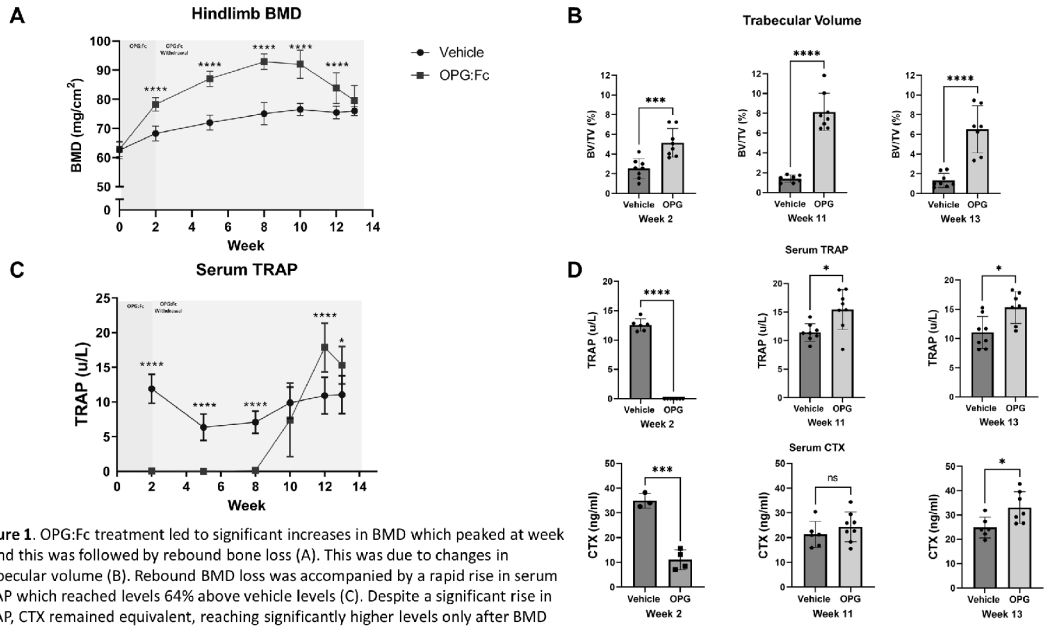

Figure 1. OPG:Fc treatment led to significant increases in BMD which peaked at week 8 and this was followed by rebound bone loss (A). This was due to changes in trabecular volume (B). Rebound BMD loss was accompanied by a rapid rise in serum TRAP which reached levels 64% above vehicle levels (C). Despite a significant rise in TRAP, CTX remained equivalent, reaching significantly higher levels only after BMD had reached vehicle levels (D).

Epigenome-wide association study of autoimmune thyroid disease highlights novel loci

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Autoimmune thyroid disease (AITD) demonstrates familial clustering, with both Graves' disease (GD) and Hashimoto's disease (HD) often present within the same family. The aetiology of AITD is incompletely understood, but genetic factors may account for up to 75% of phenotypic variance[1] and epigenetics (including DNA methylation (DNAm)) probably contributes to the remaining variance. We aimed to perform an epigenome-wide association study (EWAS) comparing DNAm in GD versus HD, to identify differentially methylated positions (DMP) associated with AITD.

We measured whole blood DNAm using the Illumina EPIC array in 32 Australian participants with GD treated with antithyroid medications only, within 1.5 years from diagnosis and 30 with HD on levothyroxine replacement, within 8 years of diagnosis. We performed an EWAS, using linear models to test for associations between quantile normalised beta values of DNAm in GD and HD. Results were then replicated in 32 participants with GD and 32 HD from Denmark using the same methodology.

We identified epigenome-wide significant differences (p <9E-8) in 5 novel DMPs between GD and HD in the discovery study: cg00049440 in *KLF9* was associated with reduced DNAm and cg06315208 in *MDC1*, cg03633435 near *MTMR3*/*HORMAD2-AS1*, cg27064684 near *LINC01581* and cg13843840 in *ZMIZ1* were associated with increased DNAm in GD compared with HD. In the replication study, data from all 5 DMPs provided additional evidence for the relevance for these CpGs and 2 DMPs were replicated (p <0.05).

Reduced DNAm in cg00049440 in *KLF9* has been shown to be associated with free T3 by our group in a previous EWAS[2]. We provide further evidence of a close relationship between thyroid hormone and DNAm. These data highlight additional DMPs of potential clinical significance. Our other findings provide a basis for further larger well-designed EWAS of AITD and for functional studies to investigate the relationship between DNAm and AITD.

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Blockade of both canonical and alternate androgen biosynthetic pathways reveals a third androgen-production pathway in mice

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Androgen deficiency in males is associated with disorders of sexual development, infertility, increased risk of cardiovascular disease and diabetes and premature mortality[1]. Exogenous testosterone therapy is widely prescribed, yet side-effects persist, and long-term risk remains unclear. An alternative approach would be to develop safer strategies supporting endogenous androgen production in men, that remains self-regulating under negative feedback control[2]. Testosterone is essential for spermatogenesis and is synthesised by testicular Leydig cells via the “canonical” pathway of androgen biosynthesis, however, testosterone can be converted to the more potent androgen dihydrotestosterone. Dihydrotestosterone can also be produced via the “alternate” pathway, utilising androsterone as a substrate instead of testosterone. Both pathways are essential for human male sexual development, yet how these pathways co-operate, especially to support androgen action in adulthood, remains unknown[3,4].

To dissect the roles and interactions of these pathways, we generated a double knockout (KO) mouse model, where genes essential for the canonical (*Hsd17b3*) and alternate (*Srd5a1*) pathways were disrupted. We validated these mutations at the DNA, transcript and protein level.

Surprisingly, double KO male mice showed normal development of reproductive organs and remained fertile. Precursor steroidogenic enzyme mRNA and protein levels in testis were increased, suggesting disrupted Leydig cell function and steroidogenic compensation. This increase was apparent in *Hsd17b3* single KO mice, but was further increased in double KO mice. Circulating and intratesticular hormone levels are currently being measured.

We conclude a third compensatory androgen production pathway may exist in mice that maintains androgen production in absence of HSD17B3 and SRD5A1. We have identified candidate steroidogenic enzymes that could support this pathway and are testing their involvement using similar approaches. Our mouse models are defining the pathways that co-operate to maintain androgen production and will in turn enable us to define therapies to support healthy androgen production across the life course.

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Transcriptomic profiling of monocytes to seek a potential biomarker for primary aldosteronism

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Primary aldosteronism (PA), the most common endocrine cause of hypertension, is associated with increased cardiovascular risk compared with essential hypertension. The diagnosis of PA currently relies on measurements of plasma aldosterone and renin levels which can be compromised by patient- or assay-related issues and relies on arbitrary thresholds. A cellular marker that reflects excess endogenous aldosterone activity could enhance the diagnostic process. Mineralocorticoid receptor (MR) activation in macrophages has previously been shown to play a key role in mediating cardiovascular injury[1]. Macrophage precursors, the monocytes, also express MR and can be easily isolated from circulation. We therefore sought to identify unique transcriptomic markers of aldosterone excess in peripheral blood monocytes.

Monocytes were isolated from nine male patients with unilateral PA before and 3 months after curative adrenalectomy. Each patient’s post-adrenalectomy samples served as the control for comparison with the pre-adrenalectomy samples. RNA

sequencing (RNA-seq) was performed on monocyte RNA samples to identify differentially expressed genes (DEGs), followed by evaluation using a second method by real-time quantitative PCR (RT-qPCR).

Distinct clustering of gene expression was observed between the “before” and “after-surgery” samples (Figure) with 1679 significantly differentially expressed (false discovery rate <0.05). The top 26 DEGs were selected for evaluation by RT-qPCR. Differences were observed in the relative expression of phosphoglycerate mutase 1 (*PGAM1)*, ferrochelatase (*FECH)*, and nuclear enriched abundant transcript 1 (*NEAT1)* genes pre- and post-surgery. The ratios of the relative expression of these genes accentuated the difference between the before- and after-surgery samples.

In conclusion, differential gene expression was found between monocytes of patients with unilateral PA before and after adrenalectomy. Further validation studies are needed in female patients and those with bilateral PA and essential hypertension. These transcriptomic markers or their ratios have the potential to serve as novel biomarkers of aldosterone excess.

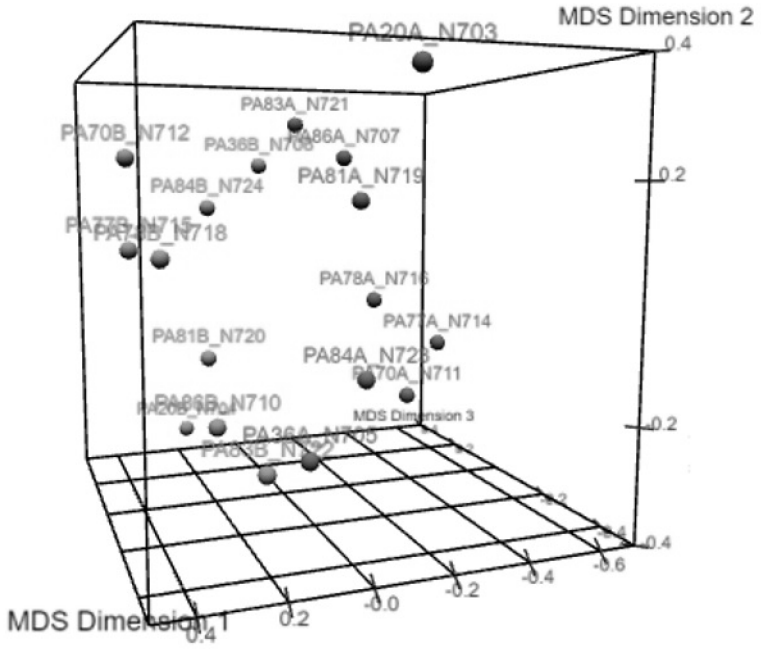

Figure: Multi-dimensional scaling plot demonstrating distinct clustering of gene expression in pre-adrenalectomy (blue dots, labelled PAxxA) versus post-adrenalectomy (orange dots, labelled PAxxB) samples of 9 patients with unilateral PA.

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Leydig Cell Glucocorticoid Receptor is Required to Maintain Steroidogenesis in Adulthood

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Androgens are essential for life-long health and well-being[2]. Disruptions to the production or action of androgens are associated with many chronic pathologies, and metabolic disorders. Glucocorticoids, exerting their action via the glucocorticoid receptor (GR), can regulate androgen biosynthesis by blocking key enzymes required for their production in the androgen-producing cells in the testis, Leydig cells[3]. Despite glucocorticoid action on androgen production being well documented, how they regulate testis function is unknown.

To establish the role of GR-signalling in the testis in adulthood, we utilised a novel technique to rapidly generate cell specific knockouts using adeno-associated virus to deliver Cre recombinase to Leydig cells specifically. Leydig cell GR knockout mice were generated via injection of Adeno-Associated Virus serous type-9 (AAV-9)[4] carrying either GFP (control) or Cre recombinase into the interstitial compartment of the testis of GR floxed mice. Mice were injected in adulthood and were collected following one round of spermatogenesis.

Our preliminary data demonstrate that blockade of GR signalling in Leydig cells shows markedly suppressed luteinizing hormone receptor (*Lhcgr*) and steroidogenic enzymes required for normal androgen production. Furthermore, an increase in

DHT in these mice demonstrates that Leydig cells are in a compensatory state following GR ablation. This important new data suggests that GR-signalling plays a physiological role in normal testis function and potentially fertility. These novel findings provide a timely and previously unavailable opportunity to elucidate the role of GR-signalling in testis function and its ability to influence LC function and androgen production.

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Term side-population trophoblasts can be differentiated to mature trophoblast populations and form organoids

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Trophoblast stem cells (TSC) give rise to all mature trophoblast lineages of the human placenta (cytotrophoblast, syncytiotrophoblast (STB) extravillous trophoblast (EVT)). The side-population technique allowed the isolation of TSCs directly from first-trimester placentae[1]. A similar trophoblast side-population can be isolated from term placentae, and are reduced 10-fold in fetal growth restriction. Our prior work established that term side-population trophoblasts require different culture conditions to first-trimester TSCs, in line with morphological/transcriptomic placental differences across gestation. Here, we aimed to use these refined conditions to determine whether term side-population trophoblasts exhibit similar functional capacity as their first-trimester counterparts.

Term side-population trophoblasts[1] were seeded at 6000 cells/well in a 96-well plate coated with Laminin-521. Differentiation to STB/EVT was undertaken by modifying prior TSC differentiation protocols[2]. Undifferentiated controls were maintained in TSC-Medium containing 25ng/mL decorin and 50ng/mL IL-8. Organoid formation was undertaken in Matrigel domes[3], with halved dome volume for term side-population trophoblasts, and decorin and IL-8 medium supplementation. All experiments were run in triplicate. Data are mean±SEM.

Term side-population trophoblasts could be differentiated into; **a)** STB as shown by up-regulation of Syncytin-1 (66.09% ±9.428 in STB-Medium; 23.37%±2.307 in undifferentiated controls, $p<0.05$) and hCG, (25.28%±11.09 in STB-Medium, no expression in undifferentiated controls,), or **b)** EVT as shown by up-regulation of HLA-G (29.05%±11.42 of cells in EVT-Medium; no expression in undifferentiated controls, $p=0.0637$). First-trimester side-population trophoblasts formed organoids (90.53±29.93µm diameter at 28 days), demonstrating self-renewing capacity. Term side-population trophoblasts formed smaller organoids (38.57±5.568µm diameter at day 28). Organoids could not form from pure FACS-sorted first-trimester cytotrophoblasts that had side-population trophoblasts excluded, demonstrating that side-population trophoblasts are required for organoid formation.

These data indicate term side-population trophoblasts exhibit the differentiation potential expected of a TSC population. Formation of the first term trophoblast organoids opens up their potential use as disease models.

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Chronic excess of activin A perturbs lipid profile and steroid production in the mouse gonads and adrenal gland

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The versatile TGF-B superfamily member Activin A (AA) is important in organogenesis and maintenance of the reproductive system; transient elevation occurs in fetal testes during masculinisation, and its signalling inhibition is characteristic of ovarian development. AA absence results in abnormal testicular lipid droplet composition and steroid production in fetal mouse testes, while chronic elevation promotes tumour formation in adult mouse gonads. Inappropriate AA elevation in humans is associated with preeclampsia, medications, and postnatal pathologies including infection. We hypothesize altered steroid synthesis is a key mechanism underpinning effects of chronic AA excess.

Aim: define the effects of chronic AA elevation on gonadal and adrenal lipid and steroid profiles.

In adult mice with excess AA (Inhibin-alpha knockout, *Inha*KO), Oil-Red-O staining identified gonadal and adrenal lipid droplets, and mass spectrometry imaging (MSI) profiled testicular lipids. Transcript analyses and liquid chromatography mass spectrometry (LC-MS) defined alterations in steroid production, and tumour-microenvironment effects (tumour, associated seminiferous tubules, and morphologically normal regions) investigated by RNA-sequencing and immunohistochemistry.

*Inha*KO mice exhibit increased lipid droplet size and number in proximity to testis and ovarian tumours, with no effect apparent in the adrenal cortex. MSI identified a cohort of lipids differentially present in the *Inha*KO testis, including cholesterol ($[m\text{-}H_2O+H]^+$ ion *m/z* 369.3489 elevated). Statistically significant changes in steroidogenic enzyme transcript levels support abnormal metabolism of cholesterol to steroids (testes: reduced *Star*, *Cyp17a1*, *Hsd17b3*, elevated *Hsd3b1*, *Cyp19a1*; ovary: reduced *Cyp11a1*, *Srd5a1*, elevated *Hsd17b1*; adrenal: *Cyp11a1* elevated) with local effects of tumour microenvironment detected at the transcript and protein level (HSD3B1 and CYP11A1). LC-MS confirmed abnormal steroid production in *Inha*KO gonads, including reduced testicular testosterone and DHT.

This study identified abnormal gonadal lipid and steroid profiles, with localised effects of the tumour-microenvironment, highlighting aspects of steroidogenesis from foundation to fruition that are vulnerable to excess AA bioactivity.

Thyroglobulin antibody positivity in pregnancy does not induce maternal glucose intolerance but affects fetal sex ratio and alters markers of placental angiogenesis

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Autoimmune thyroiditis (AIT) is the most common autoimmune disease impacting up to 20% of women of reproductive age [1, 2]. The disease is characterised by the presence of thyroid antibodies (TAs), and lymphocytic infiltration of the thyroid gland [1]. TAs in pregnancy increase risk of miscarriage, recurrent pregnancy loss, pre-term birth and gestational diabetes mellitus (GDM) in the absence of changes to thyroid hormones . Therefore, we aimed to explore how AIT impacts risk of maternal and fetal complications in pregnancy.

Thyroglobulin antibody positivity (TgAb+) was induced before pregnancy in Lewis rats by immunisation with porcine thyroglobulin in Freund's adjuvant and exposure to sodium iodide in drinking water. We then explored how TgAb+ affects estrous cycling prior to pregnancy, maternal glucose tolerance on embryonic day 16 (E16), maternal thyroid parameters, placental development, and fetal survival.

Maternal TgAb+ increased maternal plasma T4 concentration by E20. However, given there was no change to thyroid stimulating hormone (TSH) concentration and no overt thyroid pathology, this may indicate reduced peripheral T4 utilisation. TgAb+ increased maternal estrous cycle length and reduced survival rate of male fetuses but did not affect pregnancy success rate or litter size. Maternal glucose tolerance on E16 was unaffected by TgAb+, however maternal random blood glucose prior to pregnancy and on E20 was increased, suggesting that TgAb+ does not increase risk of GDM in the absence of changes to TSH or presence of other pathogenic TAs. Placentas were significantly larger which was associated with increased junctional zone glycogen accumulation and altered labyrinth zone (LZ) expression of genes that regulate angiogenesis and syncytialisation. Genes important for achievement of term gestation were also reduced in the LZ.

Maternal TgAb+ should be monitored in pregnancy as it may increase risk of maternal and fetal complications in the absence of overt thyroid pathology.

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Spastin is an essential regulator of male meiosis, acrosome formation, manchette structure, and nuclear integrity

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Microtubule structures and dynamics drive many key processes during spermatogenesis, however, their regulation during spermatogenesis remains poorly understood. Here, we sought to investigate the role of spastin, a microtubule-severing enzyme that is yet to be explored during spermatogenesis but was preliminarily reported to be essential for male fertility in mice.

Using a *Spast*$^{KO/KO}$ mouse model, we reveal that spastin loss resulted in complete absence of functional sperm. We show spastin plays a critical role in the assembly and function of the meiotic spindle, and in its absence, spermatocytes frequently stalled at metaphase and anaphase and germ cell apoptosis was significantly increased. Spastin has previously been identified as essential for the poleward movement of chromosomes during mitosis. Consistent with an essential role for spastin in chromosome segregation, of the round spermatids that were produced in *Spast*$^{KO/KO}$ mice their nuclei were frequently abnormally large. During spermiogenesis, our data reveals essential roles for spastin in regulating acrosome development. In *Spast*$^{KO/KO}$mice we observed the mis-trafficking of pro-acrosomal vesicles resulting in the formation of supernumerary acrosomes. Additionally, we observed extreme abnormalities in manchette structure, characterised by abnormally long and dense manchettes that failed to shape the nucleus. This indicates an essential role spastin in pruning the microtubules of the manchette. Finally, we found that loss of nuclear integrity occurred, leading to increased DNA damage and the vast majority of spermatids becoming pyknotic and dying. The loss of nuclear integrity is likely due to failure to remove microtubules that cross the nuclear envelope as it reforms following meiosis, a role that requires interactions with the ESCRT-III machinery.

Our results demonstrate multiple novel and essential roles for spastin in spermatogenesis and together with our previous data, reveals that the testis requires a 'toolbox' of different microtubule severing enzymes each of which have unique subspecialised roles.

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Chemogenetic Inhibition of AgRP neurons Improves Metabolic PCOS traits in a Hyperandrogenic PCOS Mouse Model

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductively aged women, and associated with a wide range of reproductive, metabolic, and endocrine traits. Hyperandrogenism is a PCOS key trait, and many women with PCOS also exhibit luteinizing hormone hypersecretion due to disruptions in the hypothalamic-pituitary-ovarian (HPO) axis. Hypothalamic agouti-related protein (AgRP)-expressing neurons are key mediators of energy homeostasis and HPO axis regulators, and studies have shown an increased AgRP population in a PCOS sheep model. Therefore, we aimed to investigate if chemogenetic inhibition of AgRP neurons can ameliorate PCOS-like traits in a PCOS mouse model. PCOS was induced in peripubertal AgRP-Cre mice using dihydrotestosterone (DHT) implants. AgRP neurons were chemogenetically inhibited using designer receptor exclusively activated by designer drugs (DREADDs). The development of PCOS traits was then assessed in mice treated ± DHT and ± AgRP inhibition. As expected, DHT exposure induced reproductive and metabolic PCOS-like features. AgRP inhibition had no effect on acyclicity in PCOS mice. A main effect of DHT exposure caused an increase in total body weight, inguinal, parametrial and retroperitoneal fat pads (all $P<0.05$). Parametrial ($P<0.05$) and inguinal ($P<0.05$) fat pad weights were significantly decreased in PCOS mice following AgRP neuron inhibition. A DHT main effect led to an increase in food intake in PCOS mice ($P<0.05$). A trend towards decreased food intake was observed in PCOS mice after AgRP neuron inhibition. Basal glucose levels and insulin tolerance were not significantly different between any treatment group. In summary, chemogenetic inhibition of AgRP neurons ameliorated the development of some adverse metabolic PCOS features in a PCOS mouse model. These results suggests that AgRP neurons are likely implicated in androgen driven neuroendocrine disruption of PCOS associated metabolic features, and thus are a potential target for the future development of therapeutics for PCOS associated weight gain.

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Paternal Expressed Gene 10 (PEG10) is decreased in preeclampsia

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Preeclampsia is a serious pregnancy complication that is attributed to placental dysfunction. Trophoblast Paternal Expressed Gene 10 (PEG10) is crucial for normal placental development, with knockout mice demonstrating impaired

vascular development[1]. This study aimed to characterise PEG10 in preeclampsia and human cytotrophoblast stem cells (hTSCs).

PEG10 messenger RNA (mRNA) expression was significantly reduced in placenta samples from patients with early-onset (<34 weeks' gestation) preeclampsia (p=0.04, n=78 vs n=18 controls). Immunohistochemistry staining localised PEG10 to the cytotrophoblast layer within the placental villi. Semi-quantitative, blinded assessment of this staining (n=5) confirmed reduced PEG10 protein in preeclamptic placentas (p=0.03, n=5 vs n=5 controls). *PEG10* mRNA and protein are not secreted into the maternal circulation and cannot be used as a diagnostic biomarker.

To confirm *PEG10* is enriched in cytotrophoblasts, hTSCs were terminally differentiated into syncytiotrophoblast and extravillous trophoblast (EVT) cells. Differentiation was confirmed by a reduction in cytotrophoblast markers (*TEAD4*, *CDH2*) and increase in syncytiotrophoblast (*SDC1*) and EVT markers (*HLAG*). PEG10 expression was significantly decreased in syncytiotrophoblast (p<0.0001, n=5 vs n=5 controls) and EVT cells (p<0.001, n=5 vs n=5 controls).

Preeclampsia is associated with excessive placental hypoxia and inflammation, therefore we measured *PEG10* mRNA expression when hTSCs were exposed to these conditions. PEG10 was significantly reduced when exposed to hypoxia (1% O_2) (p<0.01, n=5 vs n=5 controls). *PEG10* expression was also reduced in hTSCs treated with inflammatory cytokine tumour necrosis factor alpha (TNFα) (p<0.01, n=5 vs n=5 controls) but not with interleukin-6 (ns, n=5 vs n=5 controls).

This study demonstrated that PEG10 expression is reduced in placentas from women with early-onset preeclampsia. Although PEG10 is not secreted into the maternal circulation, *in vitro* studies confirm that TNFα and hypoxia may contribute to reduced placental *PEG10* expression. Future studies will elucidate how PEG10 knockdown affects cytotrophoblast proliferation, differentiation, and interaction with human endothelial cells.

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Pattern recognition receptors as mediators of seminal fluid signalling in the female reproductive tract

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Exposure of the female reproductive tract (FRT) to seminal fluid provokes an inflammation-like response characterised by cytokine and chemokine induction and subsequent leukocyte recruitment. To date, several signalling mediators involved in eliciting FRT cytokine production have been identified in seminal plasma (SP). However, specific neutralisation of these factors does not completely abolish the observed response, implying additional factors account for the residual activity.

We aimed to identify receptors present on ectocervical epithelial (Ect1) cells, and ligands contained within SP that contribute to eliciting the female response. We performed ingenuity pathway analysis (IPA) on microarray data generated from Ect1 cells treated with either 10% SP or media alone (control) to identify upstream regulators of the cytokine response, and then used neutralising antibodies to inhibit receptor activation of the identified signalling pathways.

IPA identified several potential upstream regulators, including many members of the toll-like receptor family. TLR2 and TLR4 were selected for further investigation. qPCR confirmed the presence of genes encoding TLR2, TLR4, and co-receptor MD2, however, co-receptor CD14 was undetectable. Treatment of Ect1 cells with TLR2 and TLR4 agonists lipoteichoic acid and ultra-pure lipopolysaccharide did not induce cytokine production. When Ect1 cells were treated with 10% SP, significant cytokine production was observed. However, treatment with anti-hTLR4 antibody and TLR4-specific small-molecule inhibitor, TAK-242, failed to reduce cytokine induction. Interestingly, when Ect1 cells were treated with TLR2 and TLR4 agonists in the presence of exogenous soluble CD14 (sCD14), cytokine production was increased.

We conclude that despite Ect1 cells expressing both TLR2 and TLR4, these cells do not possess all the necessary machinery to respond to TLR-ligands and may depend on soluble factors released from other cell lineages. We are now investigating whether the addition of sCD14 can alter the Ect1 cell response to SP and whether inhibition of TLR4 reduces sCD14-enabled SP signalling.

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Assessment of the impact of Gestational and Lactation PFAS exposure on Mouse development.

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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 unsurprising that PFAS has been readily detected in human blood and breast milk, and in some occupationally exposed populations has been found in concentrations1000-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 and the total number of pups born. Intriguingly, however the pups born from PFAS exposed mothers were significantly heavier on day of birth ($P= 0.0256$) and continued to be come weaning (day 21, $P < 0.0001$). Anogenital distance was also significantly increased ($P=0.0033$) in these pups. This study presents new evidence that PFAS chemicals impact offspring health. 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.

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Examining activin and BMP signaling in human testicular cancer cell lines with emphasis on the role of nucleocytoplasmic protein IPO5 in modulating their crosstalk

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The incidence of testicular germ cell tumors (TGCTs) has steadily increased to become the most common malignancy affecting men aged between 15-40 years. Seminomas and non-seminomas (embryonal carcinomas) comprise over 95% of TGCTs [1]. These are considered to arise in the testis from fetal germ cells that fail to differentiate correctly due to genetic and/or environmental factors. Although the exact mechanism is unclear, disruption of TGF-β superfamily signaling is implicated [2]. The well-characterized cell models of seminoma (TCam-2) and embryonal carcinoma (NT2/D1) were used to study the effects of TGF-β superfamily proteins activin A and BMP4 on TGCTs. Transcript changes were measured using RNA-sequencing and RT-qPCR following 6 hours of stimulation with activin A or BMP4. Major signaling pathways and biological processes affected were identified using DAVID analyses. Activin A significantly upregulated genes linked to pluripotency, cancer, TGF-β, Notch, p53, and Hippo signaling, whereas BMP4 altered TGF-β, pluripotency, Hippo and Wnt signaling components. Predicted key upstream regulators were identified using Ingenuity Pathway Analysis software and included pharmaceuticals. Reflecting the presence of both activin and BMP in the testis *in vivo*, we examined the potential for their crosstalk in TCam-2 cells using a dual promoter luciferase assay and documented a dose-dependent antagonism of BMP signaling by activin A. The importin protein, IPO5, levels and subcellular localization change dramatically in fetal and adult male germline cells [3], and IPO5 selectively mediates nuclear localization of the BMP-specific SMADs1/5/9 [4]. Here we show that *IPO5* knockdown significantly impeded BMP4-induced transcription of BMP-regulated genes, providing evidence that *IPO5* could play a crucial role in deciding which of the two arms of TGF-β signaling is more active in TGCTs. These results demonstrate how local activin A and BMP4 levels may modulate the TGCT transcriptome and could themselves be modulated to influence testicular cancer progression.

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Using a quantitative high-throughput screening platform to identify novel molecular targets and repurposable compounds for endometriosis treatment

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Endometriosis is defined as a chronic pro-inflammatory estrogen dependent disease where endometrial-like tissue grows outside of the uterine cavity of reproductive aged women. Endometriosis impacts 11% of Australian women causing chronic pelvic pain, dysmenorrhea and subfertility. Current pharmaceutical and surgical interventions are not always curative highlighting a significant need for the discovery of new compounds that disrupt estrogen-driven endometriosis pathways.

Using quantitative high-throughput screening (qHTS) technology, 3,700 clinically approved compounds were screened in 384 well formats using 3 immortalised endometrial stromal cell lines. Cells were screened under estrogenic conditions to mimic endometriosis environment and after 72 hours, cells were washed, fixed, stained, and imaged using the high Cell-Insight CX7 High Content Imaging system. Compounds with >70% growth inhibition were selected for dose response curve validation where an IC_{50} ≤1 μM triaged a compound for cell-based functional assay validation.

The initial qHTS identified 40 compounds that significantly inhibited cell growth during estrogen treatment compared to vehicle. This demonstrated a specific inhibitory function related to estrogen stimulated signalling. From these compounds, 23 exhibited IC_{50} ≤1μM demonstrating a physiological relevant (non-toxic) concentration. Six compounds with analgesic or anti-inflammatory functions (Cytidine triphosphate disodium, 7-8 Dimethoxyflavone, Hydroxyzine pamoate, Pregabalin, Repaglinide, and Sildenafil HCl) were then characterised in cell based functional assays with each compound inhibiting cell migration, invasion and adhesion along with down regulation of ERK signalling and compound target expression.

New therapeutic discoveries for non-hormonal treatments for endometriosis are essential to improve patient lives and repurposing existing compounds is a faster pathway to clinical translation. This study demonstrates the feasibility of qHTS for therapeutic discovery in endometrial disease and identified several compounds that are prioritised for preclinical endometriosis model investigations and future clinical trials. Future studies using this technology are also being designed to accommodate organoid cell lines, molecular screens and novel compounds.

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FGF signalling is required at different times by the sexes during gonadal development

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During gonadal development in mice, FGFR2 signalling in response to activation by FGF9 is required for male sex determination at embryonic day E11.5. However, whether FGFR2 is required for either testis or ovary development after sex determination remains unresolved. To investigate the role of FGFR2 in the fetal testis, *Fgfr2* was deleted conditionally in Sertoli cells after sex determination at E13.5 by generating XY *Fgfr2$^{fl/fl}$; Amh-Cre* mice. XY mutant gonads exhibited no testicular phenotype when analysed at 15.5 dpc and 5 months of age. Re-examination of FGFR2 protein localisation in XY wildtype gonads using immunofluorescence demonstrated that Sertoli cells lacked FGFR2 expression after E11.5. Intriguingly, in XX wildtype gonads, immunofluorescence analysis showed strong FGFR2 protein expression in somatic cells at E12.5-E15.5. Therefore, we investigated the role of FGFR2 in the developing ovary.

Gene expression analysis of sorted gonadal cells from E12.5 XX Wt1-RG reporter mice indicated that ovarian somatic cells mostly expressed the FGFR2 splice variant; *Fgfr2c*. To investigate the function of FGFR2c in the ovary, we analysed XX gonads from *Fgfr2c$^{-/-}$*fetal mice. At E13.5-15.5, XX *Fgfr2c$^{-/-}$*gonads exhibited normal morphology and granulosa cell differentiation, however a severe loss of germ cells was observed without evidence of increased cell apoptosis or reduced cell proliferation. To determine whether the FGFR2c-dependent germ cell survival involves the FOXL2 pathway, we analysed XX *Fgfr2c$^{-/-}$; Foxl2$^{-/-}$* gonads. Loss of *Foxl2* from XX *Fgfr2c$^{-/-}$* gonads lead to the rescue of germ cell numbers. Together, our results indicate that after the sex determination stage, FGFR2 signalling is not required in Sertoli cells of the testis but plays an important role in the ovary. In particular, the FGFR2C isoform specifically in the pregranulosa cells supports proper germ cell development and prevents germ cell apoptosis.

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Inhibitory/Excitatory balance changes within the cerebellum in a guinea pig model of preterm birth

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Preterm birth is associated with impairment to the inhibitory GABAergic system, affecting inhibitory/excitatory balance, increasing vulnerability to neurobehavioral conditions (ADHD and anxiety). We hypothesize that a premature loss of *in-utero* exposure to inhibitory neurosteroids provided by the placenta, is responsible for GABAergic impairment. This study aims to determine changes in GABA/glutamate balance in the cerebellum following preterm birth.

Time-mated Dunkin Hartley guinea pigs were delivered either preterm (induced at GA62) or term (spontaneous at ~GA69) and allocated to neonatal or juvenile collection. Preterm born neonates were maintained until term equivalent age (TEA) whilst cerebellums of term born neonates were collected 24hrs post-delivery. Tissues from term born juveniles were collected 40 days post-delivery and preterm born juveniles were collected at corrected 40 days post-delivery (TEA+40 days). RT-PCR analysis of cerebellums was performed using the JUNO and BIOMARK instruments to quantify changes in the expression of GABAergic and Glutamatergic pathway components.

Expression of the $GABA_A$ receptor α6 subunit (*GABRA6)* was lower in preterm neonates than preterm juveniles (males p=0.0018) (females p=0.0062) but this difference was not observed in term cohorts. Preterm juvenile females had higher expression of excitatory glutamate receptors (*GRIA4*, *GRM5*, *GRIA1*) than term born (p=0.0004, p=0.0422, and p=0.0199 respectively).

Preterm birth induces changes in receptors involved in excitatory/inhibitory balance in the cerebellum with changes differing between sexes and continuing to the equivalent of childhood. As the cerebellum has important roles in cognitive processes, the ongoing alterations in GABA/glutamate pathways may increase the risk of developing ADHD-like and anxiety disorders in these infants and represent targets for treatment options to improve outcomes.

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The long-acting recombinant human follicle-stimulating hormone (SAFA-FSH) for enhancing spermatogenesis

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It is recommended to replace follicle-stimulating hormone (FSH) in stimulating spermatogenesis in man who are infertile due to secondary hypogonadism whose sperm counts have not responded to human chorionic gonadotropin (hCG) alone. However, FSH have a short serum half-life which requires frequent administration to maintain the therapeutic efficacy. To improve the pharmacokinetic properties, we had developed one of unique albumin-related technology, coined as “anti-serum albumin Fab-associated” (SAFA) technology. We tested the feasibility of applying SAFA technology in creating a long-acting FSH as a therapeutic candidate for secondary hypogonadism patients.

SAFA-FSH was produced in a Chinese hamster ovary (CHO) expression system. To confirm the biological function, production of cyclic AMP and phosphorylation of ERK1/2 and CREB were measured in TM4-FSHR cells. The effect on spermatogenesis in a hypogonadal rat model treated with gonadotropin releasing hormone (GnRH) agonist was investigated.

In cell line experiments, SAFA-FSH treatment increased production of cyclic AMP and increased phosphorylation of ERK1/2 and CREB in a dose dependent manner. In animal experiments, sperm production wasn't restored after hCG alone treatment. However, Sperm production was restored after additional conventional FSH treatment at intervals of 3 times per week or once every 5 days. Further, sperm production was restored even after additional SAFA-FSH treatment at intervals once every 5 days or once every 10 days.

In conclusion, long-acting FSH with bioactivity was successfully created by using SAFA technology. The data support further development of SAFA-FSH in the clinical setting, potentially representing an important advance in treatment of secondary hypogonadism patients.

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Targeted radiotherapy induces changes to the tumour microenvironment in advanced prostate cancer

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Publish consent withheld

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Effects of myocyte-specific deletion on Vitamin D receptor on muscle function in mice

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Vitamin D deficiency is strongly associated with falls, muscle weakness, and sarcopenia. Our previous studies demonstrated whole-body vitamin D receptor (VDR) deletion in mice reduces their grip strength and endurance.

Aim: This project investigates the effects of myocyte-specific Vitamin D receptor deletion (mVDR) on muscle regeneration and function.

Methods: Floxed VDR mice and human skeletal actin Cre-recombinase mice were bred to generate mVDR mice and their floxed control (FC) littermates were used as controls. Ten-month old males (n = 11-13/group) were injected with Notexin in their right tibialis anterior (TA) to induce myocyte death. The left TA was injected with saline-control. Mice were monitored for 28 days to assess regeneration. Treadmill endurance tests (day 14) and forelimb grip strength (day 26) were performed to investigate muscle function. Promethion cages were used to measure voluntary wheel running. Quadriceps and TAs were weighed and collected upon cull.

Results: mVDR body weights did not differ significantly to FC, but they showed decreased grip strength (rmANOVA, $p<0.01$). However, there were no significant differences in endurance-distance or voluntary wheel-running. At 28 days, the notexin-treated TA mass in mVDRs was 25% heavier than their left-TA control ($p=0.031$) and 18% heavier than notexin-treated FC mice TA ($p=0.04$), suggesting possible oedema in the regenerating muscle. Preliminary histological analysis of mVDR notexin-treated muscles showed a high proportion of central nuclei and ongoing myocyte regeneration.

Conclusion: mVDR showed decreased grip strength and increased TA weight after a notexin-model of muscle injury and regeneration. However, there were no significant differences in endurance or voluntary wheel running suggesting muscle function effects were resolved by completion of the study. Further histological assessment will be performed to investigate increased TA mass and muscle morphology.

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Does capsaicin “beige” human white fat that has been transplanted into mice and exhibit improvements in mouse metabolism?

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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. 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 fed mice showed improvements in glucose tolerance and insulin sensitivity. The human fat showed up-regulation of uncoupling protein 1.

CONCULSONS: These results indicate beiging of human white fat is possible and has the potential to improve metabolism. 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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Medullary Thyroid Cancer- new targets for imaging and challenges in treatments

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Medullary Thyroid Cancer is an intrinsically different disease to its DTC counterpart and requires a nuanced personalised approach to management. Early spread to local lymph nodes is a classic presentation in MTC with nodal disease evident in up to 50% of patients at diagnosis. Surgery remains as the mainstay of treatment for the majority of MTC. Calcitonin and CEA doubling time can assist in prognosticating but there have been advances in both histological and imaging staging. A novel histological grading system has recently been developed involving three histological features which divide into low, intermediate, and high grade which correlates with prognosis. Imaging modalities improving detection and characterisation of metastatic disease have also been developed with various radiolabelled somatostatin analogue including 68Ga-DOTATATE and 68Ga-DOTATOC together with 18F-DOPA, 18F-FDG reviewed for MTC patients. Persistent and metastatic disease treatments have evolved with targeted kinase therapy especially for those harboring *RET* germline or somatic variants. Successful phase III trials for multikinase inhibitors (vandetinib and cabozantinib) improved progression free survival but rendered patients with significant adverse effects limiting treatment efficacy. Recently, novel more selective RET kinase inhibitors (selpercatinib and pralsetinib) have by contrast been far better tolerated and have impressive PFS

improvements. Phase III trials are ongoing with selperatinib and pralsetinib vs physicians choice cabozantinib or vandetinib aiming to delineate the best sequence of treatment. Selection pressures from TKIs can lead to secondary somatic mutations and prevailing of specific genetic clones and ultimately clinical progression. In addition acquired, “on-target” resistance to selective RET inhibition have led to solvent front mutations rendering the selective kinase inhibitors less effective. These mechanisms of resistance are one of the new challenges these successfully treated cohorts are facing and novel strategies including analysis of circulating tumour DNA (ctDNA) from liquid biopisies may assist in identifying these resistant clones earlier.

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Active Surveillance Versus Immediate Surgery for Low-Risk Papillary Thyroid Microcarcinoma; Comparison of Outcomes, Quality of Life, and Medical Costs from a Multicenter Prospective Cohort Study of Korea, MAeSTro

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Molecular Profiling of Thyroid Cancer: Current and Future Developments

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Historically, clinicopathologic risk assessment of thyroid cancer (TC) has often been inaccurate, and the overtreatment of inherently low-risk TC and under-treatment of potentially aggressive TC commonplace. However, our knowledge of the molecular makeup of TC, and the genetic determinants of tumorigenesis and progression, has grown considerably over the past decade. This has heralded in a new era of molecular-based risk stratification, based upon the presence/absence of informative cancer mutations, which can more precisely guide clinical management. To date, the most extensively validated TC biomarker is BRAFV600E, which while never being present in benign disease, is detected in about two-thirds of all TC. BRAFV600E is associated with more aggressive disease and is clearly associated with locoregional recurrence, but not with distant metastases or death independently from other clinicopathological risk factors (1-2). More recently, TERT promoter mutations (TPMs) have also be shown of predictive value, occurring with increasing frequency in progressively worsening prognostic TC subtypes (3); and within relatively indolent differentiated TCs, to be correlated with adverse prognostic features such as older age, larger tumour size and male gender (4) Furthermore, several studies have found an interaction between the presence of TPMs and BRAFV600E in predicting recurrence (4). This talk will provide current overview of these and other promising biomarkers, where the field is currently, and the exciting developments that are on the horizon; including novel approaches beyond tumor DNA sequencing, such as transcriptomics, epigenomics and immunophenotyping. We will also review current and potential applications of liquid biopsy-based biomarkers, such as circulating tumor cells and circulating nucleic acids, a new modality which is transforming oncology.

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PRC2 subunits, EZH2 and EED, have differential contributions to epigenetic programming in oocytes

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Epigenetic modifications facilitate cell differentiation partly by regulating transcription of developmental genes. While it has been proposed that epigenetic programming of germ cells is critical for offspring development, the mechanisms are poorly understood. As extensive evidence suggests that environmental factors, including drugs or diet, can alter germline epigenetic programming, understanding these mechanisms is essential. Polycomb Repressive Complex 2 (PRC2), containing the core subunits EED, EZH1/2 and SUZ12, catalyses the repressive modification H3K27me3. We previously demonstrated that oocyte-specific deletion of *Eed* versus *Ezh2* differentially impacts offspring growth, with *Eed* deletion resulting in postnatal overgrowth while *Ezh2* deletion results in growth restriction. As PRC2 is frequently dysregulated in cancer, various EZH2 and EED inhibitors have been developed. To investigate how PRC2 inhibition may impact oocytes, we used our genetic mouse models to delete PRC2 function and examine how H3K27me3 is regulated in oocytes. We identified a key window of transient PRC2 activity that regulates establishment of H3K37me3 at developmentally important genes in growing oocytes. *Eed* deletion resulted in 93% loss of H3K27me3 and de-repressed 343 genes (DEGs) in fully-grown Germinal Vesicle (GV) oocytes. Importantly, many of these genes contained H3K27me3 in human GV oocytes suggesting this PRC2 activity is conserved in humans. Conversely, while *Ezh2* deletion reduced H3K27me3 by 75%, only 34 DEGs were identified in GV oocytes. Together our work identifies differences in the relative contributions of EED versus EZH2 to oocyte epigenetic programming. Our findings suggest that EZH2 ablation may more mildly impact oocytes and may explain different outcomes observed in offspring from *Eed*-null and *Ezh2*-null oocytes. Understanding these processes is critical for determining epigenetic inheritance mechanisms, and how exposure to clinically relevant EZH2 or EED inhibiting drugs may impact on oocyte epigenetic programming, and subsequent health and development of the next generation.

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BMP signalling facilitates meiotic progression in mouse fetal germ cells, *in vivo*

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Essential to sexual reproduction is the generation of haploid gametes from diploid germ cells through meiosis. Germ cells initiate meiosis during fetal life in females, but at puberty in males. This temporal difference is believed to depend solely on the fetal gonadal somatic environment. The signalling molecule retinoic acid triggers meiosis in both sexes, as it stimulates expression of pre-meiotic transcription factors STRA8 and MEIOSIN, which together drive extensive transcriptional change. Despite this progress, the molecular mechanisms underlying mitosis-to-meiosis transition remain poorly understood.

Working with primordial germ cell-like cells (PGCLCs) *in vitro*, researchers recently showed that retinoic acid alone is unable to direct the oogenic fate, and that BMP is also required in their system[1], with ZGLP1 being the critical downstream target essential to ensure progression through oogenesis[2]. To establish whether BMP signalling is also required *in vivo,* we used a mouse model that is deficient for BMP receptor 1A (BMPR1A) specifically in the germ cells, and showed that BMPR1A-mediated signalling is indeed necessary for normal female mouse fetal germ cell meiosis. Germ cell-specific loss of BMP signalling does not affect the initiation of *Stra8* expression, but it does slow meiotic progression and compromises mitotic exit. Interestingly, disruption of BMPR1A-mediated BMP signalling abolishes the expression of *Cdx2* – a homeobox gene crucial for the first cell fate decision in early embryos. CDX2 is transiently expressed in female fetal germ cells just prior to meiotic entry, and slightly earlier than the pre-meiotic factors STRA8 and MEIOSIN.

Our study has demonstrated an *in vivo* role for BMP signalling in fetal germ cell meiosis and identified CDX2 as a potential effector. Understanding how germ cells lose pluripotency and commit to sex-specific fate is relevant to stem cell biology, and our findings will hopefully inform ongoing attempts to generate safe effective gametes, *in vitro*.

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Mouse preimplantation embryo development *in vitro* requires endogenous sphingosine-1-phosphate (S1P)/S1PR signalling

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The addition of specific growth factors to *in vitro* embryo culture medium improves the viability of cultured preimplantation embryos. For example, sphingosine-1-phosphate (S1P) improves oocyte maturation (Cheng et al., 2015; Jee et al., 2011), rate of blastocyst formation (Jee et al., 2011), and decreases rates of apoptosis throughout development (Guzel et al., 2018; Roth and Hansen, 2004). The mechanism by which S1P improves development is poorly understood. The current study aims to determine the expression of the 5 isoforms of S1P receptor (S1PR) in mouse preimplantation embryos and the role of endogenous S1PR1-3 signalling in preimplantation embryo development *in vitro*. Immunofluorescence staining demonstrated that S1PRs 1, 3, and 5 are expressed throughout all stages of preimplantation development but that

localisation differs for each: in the blastocyst, S1PR1 was predominantly nuclear and expressed in the trophectoderm and hypoblast cells, S1PR2 and S1PR3 were expressed in the nucleus and cytoplasm respectively of all cells of the blastocyst, and S1PR5 was localised to the cytoplasm of trophectoderm cells. Treatment of embryos with competitive inhibitors of S1PR1, S1PR2, and S1PR3 decreased the percentage of embryos that developed to the blastocyst stage, with mortality occurring at compaction, cavitation, and cleavage stages, respectively. Together, the differing expression and localisation of S1PR1-5 and differences in the timing of mortality from antagonising S1PR1-3 suggests that, while endogenous S1P/S1PR signalling is required for mouse preimplantation embryo development *in vitro,* individual S1PRs may be part of different axes of S1P/S1PR signalling at different times across mouse preimplantation development. Understanding S1P/S1PR signalling and its role within preimplantation embryo development would facilitate a greater understanding of the molecular mechanisms underpinning embryo development, and potentially allowing us to improve *in vitro* embryo culture for clinical, research, and agricultural purposes.

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Epsilon tubulin is essential for spermatogenesis in the mouse wherein it regulates meiosis, sperm tail structure and nuclear remodelling

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Chasing their own tail: Malate dehydrogenase 1B is a testis-enriched energy protein required for progressive sperm swimming motion and male fertility.

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To reach the site of fertilization, sperm must swim through the viscous fluid of the female tract. This and other processes, such as hyperactivated sperm motility and the acrosome reaction, likely require ATP production. We recently identified a high-confidence loss of function mutation in a previously uncharacterised malate dehydrogenase (MDH1) paralogue, MDH1B. Canonical MDH1 is a cytoplasmic enzyme that translocates electrons generated during glycolysis into the electron transport chain for ATP payoff. To characterise the function of MDH1B, we produced a knockout mouse model. *Mdh1b* knockout males are sterile but have histologically normal spermatogenesis and produced normal numbers of morphologically normal, motile sperm. MDH1B is localised to the mitochondrial sheath in mouse sperm and *Mdh1b* knockout sperm exhibit a 'stiff midpiece' phenotype that results in beat cycle asymmetry, reduced flagellar amplitude and inefficient power dissipation. Further analysis of free-swimming sperm revealed that in fluids of increasing viscosity, sperm lacking *Mdh1b* display precocious rolling suppression and swim in circles compared to wildtype. Both the rolling suppression (3D) and circular swimming (2D) can be rescued with the addition of exogenous ATP, suggesting that MDH1B plays a vital role in the supply of ATP to the sperm in situations of physical challenge. Furthermore, following mating, sperm from *Mdh1b* knockout mice fail to reach the site of fertilisation in the female reproductive tract. Collectively these data reveal MDH1B as an essential regulator of mouse, and likely human, male fertility and suggest that MDH1B plays a vital role in the supply or production of ATP required for sperm motility through viscous fluid.

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Novel insights into mouse sperm membrane proteins and surface remodelling during capacitation

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A critical stage of sperm maturation, termed 'capacitation', occurs while spermatozoa transit the female reproductive tract, and consists of a series of physiological and biochemical changes that ultimately afford spermatozoa the ability to recognise and fertilise an oocyte. Given the translationally quiescent nature of mature sperm cells, capacitation is largely supported by protein remodelling and post-translational modifications, with these events also driving the remodelling of the sperm surface architecture necessary for oocyte adhesion. Despite decades of research, the extent of sperm surface remodelling and the identification of the entities responsible for sperm-oocyte binding are yet to be fully elucidated. In this study, we have utilised a combination of comparative proteomic profiling and *in silico* analysis to identify membrane proteins in mouse spermatozoa immediately upon retrieval from the epididymis (non-capacitated), and after *in vitro* capacitation (capacitated). Using label-free quantification via high-resolution LC-MS/MS, we identified 1,745 and 1,562 insoluble proteins from non-capacitated and capacitated spermatozoa, respectively. Of the 684 membrane proteins identified, subsequent *in silico* analysis revealed a subset of 64 proteins from non-capacitated-, and 58 from capacitated spermatozoa that are universally characterised as 'membrane' proteins, with several not previously described or annotated in the sperm membrane, including carbohydrate binding proteins, such as malectin. These proteins may therefore represent new candidate receptors for binding of the glycoprotein rich zona pellucida matrix that surrounds the oocyte. Notably, we also identified a small number of proteins unique to either non-capacitated or capacitated spermatozoa that offer new insights into the surface changes that precede successful fertilisation. Such changes are of considerable interest in terms of informing rational avenues towards distinguishing fully functional sperm for use in assisted reproductive technologies.

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Novel pharmacology following heteromerisation of the angiotensin II type 2 receptor and the bradykinin type 2 receptor

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Background: Receptor heteromerisation is the phenomenon whereby two different receptors form a functional complex that attains unique pharmacological properties. Consequently, receptor heteromers can be considered novel drug targets, with the potential to achieve greater therapeutic selectivity and specificity. The angiotensin II type 2 (AT_2) receptor and bradykinin type 2 (B_2) receptor are both promising drug targets in their own right (for cardiovascular and other diseases), and the existence of a heteromer that forms between the two could allow improved specificity of targeting. The two receptors have some overlapping pharmacology, such as nitric oxide-mediated vasodilation, and there is some evidence that observed functional interactions may occur as a result of heteromerization (1).

Aim: Investigation of evidence for heteromerization of the AT_2 receptor and the B_2 receptor.

Methods: AT_2 receptor and B_2 receptor pharmacology and potential heteromerisation was investigated in HEK293FT cells using various bioluminescence resonance energy transfer (BRET)-proximity based assays. These assays allow real time, live cell monitoring of proximity between biomolecules of interest, such as receptors and signalling proteins, or receptors and ligands, and therefore produce physiologically relevant, real time pharmacological data.

Results: Heteromerisation of the AT_2 receptor and B_2 receptor was confirmed with assays showing recruitment of various signalling and regulatory proteins proximal to AT_2 receptors only upon B_2 receptor coexpression and activation. Additionally, the close proximity between the two receptors was also demonstrated with the NanoBRET ligand-binding assay (2). Attainment of novel pharmacology (Ga_z coupling) was observed upon heteromerisation, which did not occur with either receptor alone.

Discussion: Our study has confirmed the heteromerisation of the AT_2 receptor and B_2 receptor, and showed that this results in the attainment of novel pharmacology. Further studies will aim to reveal the physiological consequences of this heteromerisation.

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Modulation of mineralocorticoid receptor (MR) signalling with AZD9977 for the treatment of diabetic cardiac myopathies

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Impact of oral contraception on screening for primary aldosteronism

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Primary aldosteronism and oral contraception (OC) can both cause hypertension in young women. However, the effect of OC on the screening test for primary aldosteronism, the aldosterone to renin ratio (ARR), is not clear and has not been studied in a large cohort.

To understand how OC affects the ARR, we analysed data from the female offspring (Gen2) of women enrolled in the Raine Study, a population-based birth cohort, who had blood pressure (BP) measurements, stored blood samples for aldosterone and renin measurements, and information about OC use at age 17y and/or age 27y.

The current analyses included 484 female participants at 17y (333 OC non-users and 151 OC users) and 486 at 27y (251 OC non-users and 235 OC users). Serum aldosterone concentration was significantly higher in OC users than non-users at 17y (median 486 pmol/L vs 347 pmol/L, p<0.001). Renin concentration was significantly lower in OC-users at both 17y (13.4 mU/L vs 20.6 mU/L) and 27y (9.2 mU/L vs 11.8 mU/L), hence the ARR was significantly higher in OC-users compared to non-users at both 17y (31.5 vs 18.3) and 27y (27.3 vs 21.1). The proportion of participants with ARR > 70 pmol/mU (current threshold for PA detection) was significantly higher in OC-users at both 17y (12.6% vs 2.1%) and 27y (6.4% vs 0.4%), however, they had comparable BP to those with ARR < 70 pmol/mU. OC use at any age abolished the relationship between ARR at 17y and BP at 27y that was previously described in non-OC users1.

Overall, our data suggest that OC use can cause a false positive ARR which may lead to unnecessary investigations for primary aldosteronism. Hence, alternative contraception should be considered in women being evaluated for primary aldosteronism, especially if the initial ARR is abnormal.

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Adrenal histopathology, adrenal vein sampling results and surgical outcomes in patients with primary aldosteronism

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Primary aldosteronism caused by unilateral adrenal disease can be identified by adrenal vein sampling (AVS) and treated by adrenalectomy[1]. A recent study suggested that patients whose resected adrenal tissue contains a CYP11B2 (aldosterone synthase) staining adenoma (classical pathology) are more likely to be cured than those whose resected adrenal contains micronodules (non-classical pathology)[2]. We have reported that AVS outcomes before and after the use of ACTH stimulation can be discordant (median lateralisation index ≥ 4 pre-ACTH to <4 post-ACTH), with the loss of lateralisation post-ACTH affecting 18% of patients[3]. We hypothesise that AVS discordance may predict both adrenal histopathology and surgical outcomes.

We performed a retrospective analysis of AVS results, histopathology, and surgical outcomes in 41 patients who underwent AVS and adrenalectomy at Monash Health between 2009-2020.

Of the 32 patients with concordant lateralisation on AVS both pre- and post-ACTH, 9 demonstrated classical pathology, 6 had non-classical pathology and 11 had mixed pathology. 6 did not stain for CYP11B2. 18 patients achieved complete biochemical cure with a normalised aldosterone renin ratio at 3 – 12 months post-surgery, while 8 patients had missing post-operative data. Of the 9 patients with discordant lateralisation on AVS, none had pure classic pathology while 1 had non-classical and 7 had mixed pathology. Among this group, from 5 patients with post-operative biochemical data, 4 achieved complete cure while 1 had partial cure with persistently abnormal aldosterone to renin ratio.

In summary, patients with concordant results pre- and post-ACTH stimulated AVS are more likely to display a CYP11B2 positive adenoma and achieve complete surgical cure. Conversely, patients with AVS discordance are more likely to display non-classical or mixed pathology and lower rates of clinical cure. Hence, ACTH stimulation may be a valuable tool in AVS to identify “falsely lateralising” PA and decrease the risk of unsuccessful surgery.

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Utility of Adrenal Vein Sampling (AVS) with and without ultra-low dose ACTH infusion

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Background
Adrenal vein sampling (AVS) is integral to identifying cases of surgically remediable unilateral primary aldosteronism (PA), however, is technically challenging, limiting its overall success. The administration of intra-procedural synthetic adrenocorticotrophic hormone (ACTH), conventionally as a 250mcg bolus or 50mcg per hour infusion, increases cortisol and aldosterone secretion and can improve successful adrenal vein sampling, however, may mask lateralisation.

Aim
The primary aim of this study was to assess if AVS performed with ultra-low dose ACTH infusion masks lateralisation.

Methods
Retrospective review of results of consecutive AVS procedures (n=21) performed both with and without ultra-low dose ACTH infusion at 1.25mcg per hour (1). Successful adrenal vein (AV) cannulation (the selectivity index) was defined by an AV to peripheral vein cortisol ratio >2.0 pre-ACTH and >4.0 post-ACTH. Lateralisation was defined by an aldosterone-to-cortisol ratio of the dominant to non-dominant adrenal vein >3.0 pre-ACTH and >4.0 post-ACTH (the lateralisation index). ACTH was measured at baseline and immediately following unstimulated AVS and again following stimulated AVS. Histological confirmation of pathology following unilateral adrenalectomy was sought where available.

Results
Bilateral AV cannulation was successful in 86% of procedures pre-ACTH and 90% post-ACTH. The number of studies that lateralised improved from 57% pre-ACTH and 43% post-ACTH to 71% in the combined AVS. Lateralisation results were discordant in 43% of cases, including 29% in which lateralisation was masked by ACTH, and 14% in which lateralisation was identified only with ACTH stimulation. Of these, 22% were confirmed histologically as adenomas and 22% as adrenal hyperplasia, with the remainder awaiting surgery. ACTH levels were highest at baseline and lowest following ACTH-stimulated AVS.

Conclusion
AVS performed with ultra-low dose ACTH masked lateralisation and did not obviate the need for non-ACTH AVS; however, combined AVS both with and without ultra-low dose ACTH improved the overall diagnostic yield, facilitating the identification of additional cases of surgically curable unilateral PA.

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Enhanced user experience and reduced diabetes care burden when transitioning to calibration free sensors in advanced hybrid closed loop devices : a qualitative study

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Continuous glucose monitoring (CGM) systems that require calibration are known to add management burden for people with diabetes. CGM calibration likely also adds additional burden for users of automated insulin delivery, although transition between calibration-requiring and calibration-free systems has not been previously been explored qualitatively. Therefore, we aimed to evaluate the experiences of people with type 1 diabetes who transitioned from a calibration-based to a calibration-free CGM while using an Advanced Hybrid Closed Loop (AHCL) system.

Two semi-structured interviews were conducted among users of an Advanced Hybrid Closed Loop (AHCL) system. The first interview was conducted ≥20 weeks after starting the use of the calibration-requiring sensor, with the follow-up interviews conducted ≥4 weeks following Calibration free CGM use. Interviews were transcribed independently and analyzed using NVivo 12 pro. Thematic analysis was used to identify key themes and subthemes.

Fifteen participants were interviewed to reach thematic saturation. These participants had a mean age of 24.9 years (range 7-65). At baseline mean diabetes duration was 14.5 years (+/-10.9) , mean Hba1c of 54.8 mmol/mol (+/-10.2) and Time in range of 75.4% (+/-11.6). Non-Europeans represented 13% of participants and 67% were female. Participants reported a progressive improvement in digital integration, lifestyle integration and device trust following transition to calibration free CGM. They also reported more convenient calibration ,reduced overall capillary glucose assessment, increased device satisfaction and reduced burden of diabetes care following transition to calibration free devices. Negative aspects reported included perceptions of reduced sensor duration compared with CGM systems utilised in other AHCL devices and impaired integration with mobile devices.
In conclusion, calibration free CGM is an acceptable and trusted component of contemporary automated insulin delivery systems as it reduces diabetes burden while simultaneously supporting improved glycemic outcomes.

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Evaluation of elevated IGF-1 in patients without clinical evidence of acromegaly

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Aims: IGF-1 acts downstream of GH mediating many of its effects. It is the recommended screening test for acromegaly, in evaluating pituitary incidentalomas and possible hypopituitarism. Case-based observations suggest elevated IGF-1 may occur in patients without acromegaly. The aims were to 1) identify the frequency of elevated IGF-1 without evidence of GH

excess, and 2) to examine potential differences in relevant medications and comorbidities between people with an elevated IGF-1 compared to a control group matched for age, sex, gonadal and pituitary status.

Methods: All people whose IGF-1 was measured at a single reference laboratory between Dec 1st 2018 – Dec 1st 2020 were identified. Electronic records of those with at least one IGF1 >1.1x the upper limit of the age-matched reference range were appraised to determine; 1) documentation of acromegalic features, 2) presence of relevant comorbidities and medication use, and 3) further investigation to exclude pathological GH excess. There were 2759 IGF-1 samples measured in 1963 people ≥18 years, over the specified period. Out of these, 204 had IGF-1 >1.1 times the upper limit of the age-matched reference range. 102 cases (61M, 41F) met inclusion criteria, and were matched to 102 controls with a normal IGF-1.

Results: There were significant differences in the frequency of dopamine agonist use (19/102 cases vs 6/102 controls, OR=3.66, 95%CI: 1.45-9.29, P=0.009) and chronic kidney disease (CKD) (14/102 cases vs 4/102 controls, OR=3.90, 95%CI: 1.28-11.14, P=0.024).

Conclusion: Out of 1963 patients having IGF-1 measured, 102 (5.2%) had an elevated IGF-1 where there was no known acromegaly, GH replacement or glucocorticoid excess. There were significant associations with dopamine agonist use and CKD. While assay imprecision, intra-individual biological variability and accuracy of reference ranges probably represent the main influences on the prevalence of elevated IGF-1, we have identified two additional factors which should be considered.

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A retrospective analysis of the prevalence of hypocalcaemia and hypophosphataemia post intravenous ferric carboxymaltose and iron polymaltose in the inpatient setting

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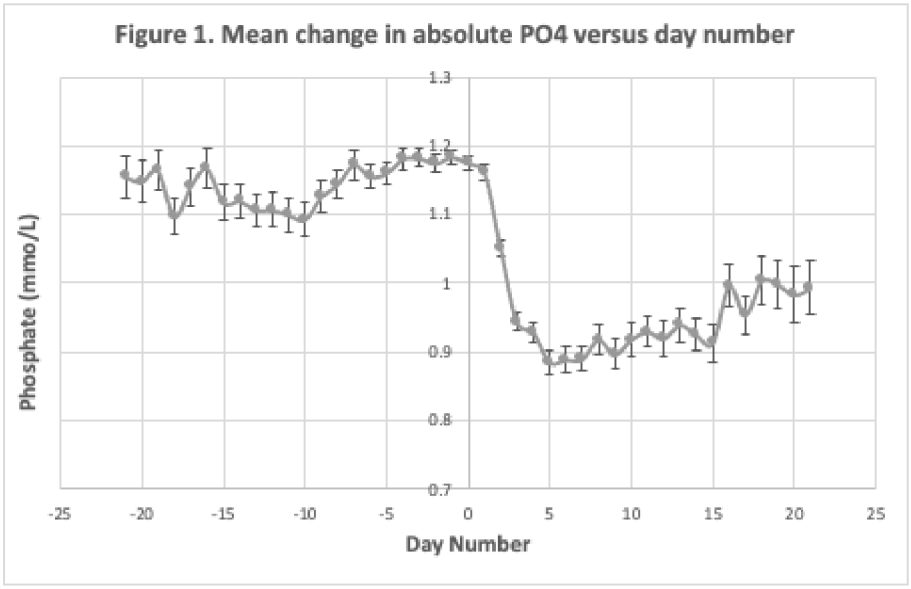
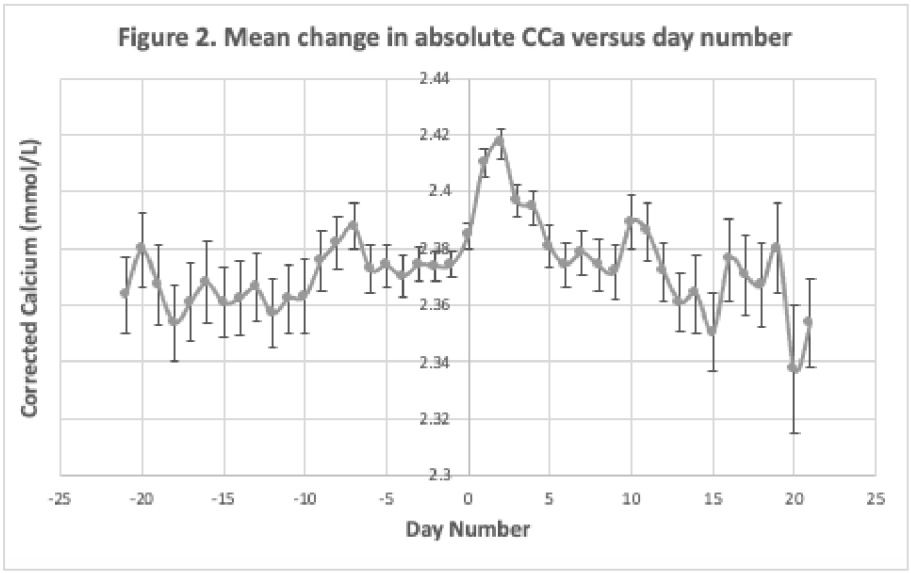
Hypophosphataemia following intravenous iron infusion is increasingly recognised, mediated by inhibition of fibroblast growth factor 23 (FGF23) degradation, renal phosphate wasting and reduced calcitriol (1,2). Hypophosphataemia prevalence after intravenous iron infusion is 45-75% (3,4,5). Significant hypocalcaemia after iron infusion has rarely been reported (3,6,7,8) and the frequency of hypocalcaemia following intravenous iron in the inpatient setting has not been characterised.

In this single-centre retrospective study, we sought to characterise changes in plasma calcium and phosphate following intravenous iron in inpatients. We hypothesized that hypocalcaemia occurred more frequently following iron infusion, and was more likely to occur with concurrent vitamin D deficiency.

All inpatients who received intravenous iron polymaltose or ferric carboxymaltose at the Royal Brisbane and Women's Hospital from January 2020 to September 2021, who had a general chemistry blood test within ten days prior to and following infusion, were included. We extracted all available results for corrected calcium (CCa) and phosphate (PO4) for 21 days before and after iron infusion. Seven-hundred-and-eighty-seven patients with 9168 blood samples were included.

The results showed a mean pre-infusion PO4 of 1.17mmol/L (range 0.47-1.91mmol/L) and post-infusion PO4 of 1.00mmol/L (range 0.33-1.85mmol/L), confirming a reduction in phosphate after iron infusion ($p<0.0001$). A significant increase in hypophosphataemia frequency after infusion was also demonstrated (7% vs 33%; $p<0.0001$). The temporal course of hypophosphataemia is shown in Figure 1. There was no significant difference between mean pre-infusion and post-infusion CCa, or in hypocalcaemia frequency. However, the temporal change in CCa showed an early post-infusion increase before a late decrease (Figure 2), suggesting an inverse relationship to PO4. Vitamin D levels were only available for 307 patients, however, deficiency was not associated with hypocalcaemia or hypophosphataemia occurring after iron infusion. In conclusion, our results confirmed significant hypophosphataemia following intravenous iron but did not demonstrate significant hypocalcaemia.





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Assessment of the clinical utility of plasma C-telopeptide (CTX) versus N-telopeptide (NTX) of type 1 collagen in metabolic bone disease

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Background: Bone turnover markers are used in the management of metabolic bone diseases. The commonly available bone resorption markers are C- and N-terminal telopeptide of type 1 collagen (CTX and NTX respectively) in monitoring patients with osteoporosis.

Aim: To assess the clinical utility of plasma CTX (ng/L) versus urine NTX (nmol BCE/mmol creatinine).

Methods: Fasting Metabolic Bone Study is a set of biochemistry tests (based on venous blood and urine samples after an overnight fast) requested for investigation and management of patients with metabolic bone diseases. From May to July 2022, PathWest laboratory dual reported CTX and NTX in all FMBS (NTX was the bone resorption marker since October 2004). Clinical details were identified from pathology request forms and available electronic medical records. To date, 232 out of 453 studies have been reviewed.

Results: We excluded patients with renal impairment (n=20) and age <30 (n=21). Given the sex specific reference intervals, our initial analysis was based on 151 female patients (NTX <50 nmol BCE/mmol creatine and CTX <800 ug/L). In our laboratory, bone resorption is well suppressed when NTX <20 nmol BCE/mmol creatinine and CTX <280 ng/L.

There was no correlation between NTX and CTX. The correlation coefficient was 0.45 when NTX 20-50 nmol BCE/mmol creatinine was compared to CTX, and 0.52 when CTX 280-800 mg/L was compared to NTX. There were 33 patients with suppressed NTX <20 but 7/33 (21%) had CTX >280 ug/L. Of the 19 patients on denosumab, 10 patients had suppressed NTX with corresponding CTX <90 ng/L. Further analysis will be performed in patients on bisphosphonate and menopausal hormone therapy.

Conclusion: Preliminary data identifies no clear correlation between NTX and CTX. If bone turnover markers are used in clinical management of patients, it is important to monitor with one specific marker.

Relationship between volume and type of alcohol consumption and bone mineral density in men

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Background: There is conflicting data relating to the effect of volume and type of alcohol consumed on bone mineral density (BMD).

Aim: Determine the cross sectional and longitudinal associations between amount and type of alcohol consumption and BMD in a community dwelling cohort of middle-aged to older men. We hypothesised that both volume and type of alcohol consumed impact on BMD

Methods: Participants (n=693) were derived and representative of the Florey Adelaide Male Aging Study (FAMAS) cohort (n=1195). Bone density was determined by dual energy X-ray absorptiometry at commencement and 5-year follow-up. Alcohol consumption was determined through Cancer Council Victoria Diet Questionnaire for Epidemiological Studies Version 3.1, and was classified into beer (high/low concentration), red wine, white wine and port/sherry/spirits. Multivariable regression was performed to assess cross sectional and longitudinal associations between types of alcohol intake and BMD.

Results: Oestrogen concentration alone was different between the FAMAS cohort and analysis cohort (Wilcox sign-rank test $p = 0.015$). Cross-sectionally, whole-body BMD was not associated with type or volume of alcohol consumed. Spinal BMD was inversely associated with volume of low strength beer consumption β -0.021 g/cm^2 (95% CI -0.034, -0.008, p<0.001). Longitudinally, there was no association between volume or type of alcohol consumption and whole-body BMD (corrected for baseline). Change in spinal BMD was inversely associated with change in full strength beer consumption β -0.006 g/cm^2 (95% CI-0.012, -0.001, p = 0.030).

Conclusion: In middle-aged to elderly men, volume or type of alcohol consumption does not appear to be associated with whole body BMD in small volumes. There appears to be a relationship between beer consumption and changes in spinal BMD compared to other forms of alcohol. Further research is required to determine the underlying cause of beer associated changes in BMD.

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The association between asymptomatic vertebral fractures and mortality risk in older participants in CaMOS.

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Clinical vertebral fractures are the most common osteoporotic fracture and are associated with increased mortality risk. However, 2/3 of these are asymptomatic (AVFs) and do not come to medical attention. The role of AVF on mortality risk is unknown.

Aims: This study aimed to determine the association between AVFs and mortality risk in older participants.

Methods: Participants, aged 50+ from the Canadian Multicentre Osteoporosis Study, were followed for 17 years. AVFs were determined at baseline from spine X-rays by trained radiologists using the Genant Method of grades 1, 2 and 3 according to respective 20-25%, 25-40% and >40% loss of vertebral height. Mortality risk for maximum grade and number of AVFs were evaluated using gender-specific Cox-proportional hazards models, adjusted for age and multi-morbidities.

Results: The cohort included 1755 men and 4442 women, of which 254 (14.5%) and 611 (13.8%), respectively, had an AVF. There was 8.6%, 4.3% and 1.5% of men with a maximum grade of 1,2 and 3 respectively and 6.6%, 4.8% and 2.4% for respective groups in women. There were 450 deaths in men and 795 in women over 71703 person-years. Kaplan-Meier curves demonstrated increased mortality risk only for grades ≥2. Therefore, grade 1 AVFs were included with no fracture in the Cox-model.

Maximum grade 2 was associated with increased mortality risk by 1.14 (0.76-1.72) and 1.08 (0.81-1.42) and maximum grade 3 with 1.46 (0.85-2.54) and 1.61 (1.17-2.23) for men and women respectively.

Mortality risk was further increased by increasing number of AVFs; by 1.31 (0.69-2.48) and 1.45 (1.05-1.50) for 2+ AVFs in men and women respectively.

Conclusion**:** Moderate to severe AVFs were associated with poorer outcomes in patients and thus should be considered for treatment in the clinical setting. Further research is required to determine whether the excess mortality is mediated by subsequent increased fracture risk.

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The Psychological Impact of Trauma

Loretta White[1]

1. Starship Hospital, New Zealand

Loretta will present on the Psychological Impact of Trauma. She will first start by discussing what trauma is and the different types of trauma a person might experience, including acute, complex, and developmental trauma and criteria for post-traumatic stress disorder (PTSD). She will discuss the ACE's study and model of how adverse childhood experiences influence health and wellbeing throughout the lifespan. She will then discuss how trauma affects a person's development, brain, relationships and functioning but highlight that there can be resilience following trauma. Loretta will consider the trauma faced by families with children with paediatric health conditions and discuss how this might present in the families that we work with.

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Trauma-informed family assessment and interventions

Anne Sinclair[1]

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Trauma is recognised as the psychological and emotional damage from a life threatening event. Trauma informed assessment is important in healthcare to understand the history of trauma for children and families and how this has impacted upon their psychosocial functioning and resilience. An understanding of trauma can problem more appropriate support, therapeutic interventions and referrals for each member of the family. Trauma informed care then involves core principles of establishing safety, trust, choice, collaboration, empowerment and respect for diversity.

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Providing trauma-informed care to children or teenagers with diabetes and their family members

Gilli Lewis[1]

1. Wellington Regional Hospital, Wellington, WELLINGTON, New Zealand

In this session I will explore how education and service delivery needs to be amended for children and caregivers who have experienced trauma prior to or at diagnosis of type 1 diabetes. I will do this by providing a couple of case examples illustrating how assessment of trauma experienced can help inform care strategies with children, teenagers and their

caregivers. I will describe some of different ways in which education and care can be delivered to help children and their caregivers heal from the trauma they have experienced.

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(Ullrich)-Turner syndrome almost a century after the initial descriptions: insights that Otto and Henry could not have imagined

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Advances in endocrine care of girls with Turner syndrome (TS) have been incremental, rather than fundamental. Over the past 30 years growth hormone regimens have been massaged to optimize height outcomes, with recent evidence supporting treatment initiation in the preschool years to prevent growth failure, rather than waiting for the inexorable development of short stature. Similarly, sex hormone replacement has evolved from the intentionally delayed initiation of puberty in the mid-teens, using large doses of conjugated oral estrogens extracted from the urine of pregnant mares, to more physiologically-timed administration of transdermal low-dose synthetic estradiol.

However, the critical insights into TS pathophysiology have come from the field of genetics. After Ford's 1959 discovery of the underlying '*sex chromosome anomaly*' progress was slow, with almost 40 years elapsing until identification of the first gene unequivocally implicated in the TS phenotype: the *short stature homeobox-containing* gene (*SHOX*). Recent advances have been driven by sophisticated genetic technologies focusing on 3 groups of candidates: pseudoautosomal genes, X-Y gene pairs and genes that escape X-inactivation. These approaches have identified dosage-sensitive genes associated with conditions that contribute to the morbidity and early mortality of TS, including urinary tract anomalies, autoimmune disorders, sensorineural hearing loss, premature ovarian failure and aortopathy.

One of the most striking findings has come from epigenetic analyses that have revealed generalized hypomethylation of the 45,X genome, affecting the autosomes as well as the X chromosome. In addition to disturbing the regulation of downstream gene networks, this fundamental genetic malfunction may predispose to meiotic non-disjunction, resulting in the sex chromosome aneuploidy underlying TS. Complementing the basic molecular/genetic analyses, studies of clinical cohorts provide insights into neurodevelopmental variations associated with the cognitive and behavioral profile of TS. For example, high-resolution structural MRI scans have demonstrated region-specific reduction in brain grey matter growth during the teenage years in girls with TS, with differential effects associated with parent-of-origin of the retained X chromosome. The brain volume deficit was exacerbated by estrogen deficiency, raising further concerns regarding delayed estrogen replacement in these hypogonadal girls. In another study, reduced grey matter volume was detectable as early as one year of age, indicating likely prenatal onset of the TS neuroanatomic phenotype.

Despite these significant advances, diagnosis remains delayed for many affected girls and women, undermining the potential value of early intervention strategies. Non-invasive prenatal testing has so far shown limited predictive value for TS. However, an intriguing new bioinformatic approach to aid early diagnosis uses a highly accurate computer-based tool for pattern recognition of TS facial features. Unraveling the genetic and biological basis for TS pathophysiology, accompanied by earlier identification of those who escape perinatal diagnosis, offers the opportunity to mitigate some of the multisystem problems of TS.

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Endocrine abnormalities in Prader Willi syndrome

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Prader Willi syndrome is a complex neurodevelopmental disorder. Many of the hallmark clinical features appear to result from hypothalamic dysfunction. Endocrine disorders associated with this condition include obesity, type 2 diabetes, growth hormone deficiency and hypogonadism. Central adrenal insufficiency and TSH deficiency have also been reported. This presentation will review these endocrine disorders from a developmental perspective and discuss current therapies.

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Klinefelter syndrome - from birth and beyond

Penny Hunt[1]

1. Christchurch Hospital, Christchurch, New Zealand

Klinefelter's syndrome (KS;47XXY) is the most common sex chromosomal anomaly affecting 1:650 newborn males. The classic phenotype of tall stature, gynaecomastia and small testes occurs in adolescence / adulthood, with specific difficulties in learning and socialization frequently noted in childhood. Despite huge advances in medical knowledge over the years, the majority of affected males remain undiagnosed. The spectrum of disease in Klinefelter's syndrome through the ages will be reviewed, together with treatment strategies including the current role of assisted reproductive technology.

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Effect of the Polynesian-specific CREBRF gene variant on lactogenic activity during mouse pregnancy

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1. University of Otago, Dunedin

The recent discovery of a Polynesian-specific gene variant with links to metabolic function shows promise as a potential drug target for treating metabolic disorders. The missense variant, termed R458Q, of the CREBRF (CREB3 regulatory factor) gene, has been associated with an increase in body mass index and yet, almost paradoxically, protection against type-2 diabetes. This gene variant has also been shown to be protective against the development of gestational diabetes mellitus (GDM) althought the mechanisms underlying this effect are yet to be elucidated. It has been shown in rodents that the hormones prolactin and its homologue, placental lactogen, play a key role in driving maternal adaptations of glucose regulation, and dysfunction of these adaptations can contribute to the development of GDM. Here, we aimed to use a mouse model with a 'knock in' of the R458Q gene variant of CREBRF to investigate if this gene variant leads to increased secretion of these pregnancy hormones conferring a 'gain-of-function' protective mechanism. First, we characterised prolactin secretion patterns in early pregnancy in control and R457Q CREBRF gene knocked-in (KI) mice fed either a standard diet or a high fat diet. Tail tip blood samples were collected at 8 time points over a 24 hour period in early pregnancy and prolactin was measured using a highly sensitive enzyme-linked immunosorbent assay (ELISA). A terminal blood sample was collected in late pregnancy (day 16) to measure total lactogenic activity using a live cell bioassay. In early pregnancy, KI mice have significantly higher prolactin levels compared to wildtype mice, in both the HFD-fed and control diet fed groups (2-way ANOVA, Effect of genotype P=0.0178, n=5-7 per group). These results support the hypothesis that enhanced prolactin secretion during pregnancy could contribute to the protective effect of this CREBRF gene variant in the development of gestational diabetes.

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Functional genomics for premature ovarian insufficiency: gene discoveries and patient diagnoses with clinical impact

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The aim of this research program is to use genomic sequence analysis to identify the genetic cause of a major form of female infertility, premature ovarian insufficiency (POI). POI is characterised by amenorrhea and elevated follicle stimulating hormone before the age of 40. It can be an isolated condition or syndromic and associated with various co-morbidities such as cancer predisposition, hearing loss, neurodegeneration or muscle weakness.

We have studied a diverse cohort of over 100 young women with POI using whole exome sequencing, followed by gene-centric and variant-centric filtration to identify likely causative genes and variants. The role of candidate variants in POI pathology was validated using various approaches such as the study of patient cell lines, modelling in *Drosophila* or zebrafish and *in-vitro* functional assays.

This approach has led to multiple novel POI gene discoveries, such as *TP63*, *TFAM*, *MRPL50, HROB, REC8,* and *GGPS1*. These discoveries have highlighted the important role of mitochondria and meiosis for ovarian functioning. For example, we have established *TFAM* and *MRPL50* as novel syndromic POI genes. We demonstrated disrupted mitochondria in patient cell lines, and abnormal ovarian development and function in zebrafish or *Drosophila* with disruption of the orthologous genes. We have also shown that genomic sequencing can alter and improve patient management and outcomes. For example, in several cases exome sequencing identified syndromic POI before its full clinical manifestation. This enabled surveillance and early intervention for associated co-morbidities, such as hearing loss, cancer or neurodegeneration.

Although not yet incorporated into clinical guidelines for the management of women with POI, our study demonstrates that genomic sequencing has the potential to enable new diagnoses and to change outcomes for women with this relatively common form of infertility, while also providing new insights into ovarian biology and pathology.

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Genome wide association study meta-analysis finds *DENND1A*, *C8orf49 and XBP1* associated with lean PCOS.

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Lean and obese women with Polycystic Ovary Syndrome (PCOS) display different clinical characteristics, implying a different pathophysiology depending on body mass index (BMI). PCOS phenotypes, stratified by BMI, are potentially genetically distinct.

This study aimed to detect genotype differences between lean and overweight/obese PCOS-affected women and explore distinctions in genetic architecture based on BMI.

Case-control genome-wide association study (GWAS) data from Caucasian PCOS-affected women and controls were pooled from six international PCOS research groups, and separated according to three BMI stratifications (lean BMI<25kg/m^2, overweight BMI >25-<30kg/m^2 and obese BMI >30kg/m^2). A meta-analysis of GWAS data (Meta-GWAS) from each BMI tier was performed.

The population comprised 257,155 women (6,273 cases and 250,822 controls) from Australian, American, Netherlands, Estonian and Finnish cohorts. Almost half of the population (47.1%, n=120,983) were of lean BMI. Two genetic loci meeting genome-wide significance ($p<5x10^{-8}$) for lean PCOS were identified - rs12000707, within *DENND1A* (9q33.3; $p=1.55x10^{-12}$) and rs2228260 within *XBP1* (22q12.1; $p=3.68x10^{-8}$). *DENND1A* is a well-recognised genetic risk locus for PCOS and is associated with hyperandrogenism and ovulatory dysfunction. *XBP1* is involved in glucose and lipid metabolism, suggesting a plausible biological link with PCOS. The signal at *XBP1* is part of a large linkage disequilibrium (LD) block containing other genes, including *CHEK2*, with putative PCOS involvement.

Gene-based association testing identified *C8orf49* as significantly associated with lean PCOS. *C8orf49* is located in close proximity to two other genes previously implicated in PCOS, *GATA4* and *NEIL2*, and may be part of a PCOS-susceptibility gene cluster on chromosome 8. The association between these genetic loci and lean PCOS is a novel finding.

The SNPs and genes identified in this study provide further evidence of distinct genetic architecture underlying lean and overweight/obese PCOS. The identification of loci associated specifically with lean PCOS is of significant interest.

Sex dependent disruptions to the dopaminergic, GABAergic and glutamatergic systems throughout development of preterm born guinea pigs

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Preterm birth is associated with a significantly increased risk of neurobehavioral disorders, in particular attention deficit hyperactivity disorder (ADHD) and anxiety. These disorders are associated with disruptions to key neurotransmitter systems, including the dopaminergic system, and the inhibitory GABAergic and excitatory glutamatergic balance. These neurotransmitters play critical roles that control cognition and behaviour, however, are sensitive to damage. Preterm birth

results in the premature exposure to the *ex-utero* environment, as well as removal from the placentally derived inhibitory neurosteroids. We propose that these damages disrupt key neurotransmitter systems which leads to the development of neurobehavioral disorders.

Dunkin Hartley time-mated guinea pig dams were allocated to fetal collection (preterm fetal; GA62, or term fetal; GA68), preterm induction of labour (preterm neonate; corrected postnatal day 1, or preterm juvenile; corrected postnatal day 40), or spontaneous term labour (term neonate; 24hrs old, or term juvenile; postnatal day 40). Relative mRNA expression of key neurotransmitter receptors in the frontal cortex were quantified by RT-PCR.

Dopamine receptor 1 (*DRD1*) mRNA expression was reduced in preterm male fetuses, neonates and juveniles compared to term born (p=0.01, p=0.02, and p=0.01 respectively). $GABA_A$ receptor α6 subunit (*GABRA6*) expression was increased in preterm male and female neonates compared to term born (p=0.02, p=0.03 respectively). $GABA_A$ receptor α4 subunit (*GABRA4*) expression was reduced in preterm male and female juveniles compared to term born (p=0.02, p=0.03 respectively). Male and female preterm neonates had increased glutamate NMDA receptor subunit 3A (*GRIN3A)* expression compared to term born (p=0.01 and p=0.03 respectively).

This study showed that preterm birth resulted in altered expression of receptors in the dopaminergic, GABAergic, and glutamatergic pathways that persisted throughout infancy and into childhood. These long-term changes may contribute to increased incidences of neurobehavioral disorders following preterm birth. Further studies are required to determine the mechanisms underpinning these changes

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Preconception weight loss, rather than weight loss in pregnancy, improves renal outcomes in a mouse model of obesity

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Maternal obesity increases risk of chronic kidney disease in the mother. In this study, we aimed to determine if preconception weight loss with diet modification improves kidney outcomes in late gestation, over diet change in pregnancy. Methods:C57BL/6 female mice were fed either a high-fat-diet (HFD) or chow diet for 8 weeks. To induce pre-pregnancy weight loss, 8 HFD-fed dams were switched to chow diet pre-pregnancy 4 weeks prior to matting. This was compared to a group of 8 HFD-fed dams who underwent 'diet switched' to chow diet after conception. 8 dams continued on chow as controls and HFD for comparison. Maternal body weight and glucose tolerance were observed prior to collection of blood and kidney samples, either prior to pregnancy or during late gestation. Serum creatinine, urinary creatinine and albumin were measured. Gene expression within kidney tissue was measured using real-time PCR, and protein expression measured by immunohistochemistry. Results: HFD-fed dams had increased renal expression of Insulin receptor and Fatty acid synthase (both $P<0.05$) pre-pregnancy and higher Urine Albumin: creatinine ratios (UACR) compared to control ($P<0.01$). In the group with 'diet switch' after conception, though gestational weight gain was lower than the other 3 groups ($P<0.05$) and body weight similar to the control group ($P<0.01$), glucose tolerance was impaired compared to control ($P<0.05$), and kidney tissue had increased expression of metabolic and oxidative stress markers, (8-OHDG $P<0.0001$, FAS, $P<0.05$) compared to mice who underwent preconception weight loss. Preconception renal fibrosis markers (Collagen IV, Fibronectin) and UACR were significantly reduced in mice who achieved preconception weight loss compared to HFD fed mice ($P<0.05$). These effects were sustained in pregnancy. Conclusion: Preconception weight loss has benefits on renal health in the preconception period and in pregnancy overweight optimisation in pregnancy alone.

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STAT3-independent actions of leptin on puberty onset and reproductive activity

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The adipose-derived hormone leptin plays an integral role in normal reproductive function. The canonical Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) is the most extensively studied leptin receptor (LepR) pathway. It's well known that neural STAT3 deletion results in hyperphagia and obesity, but its reproductive role is less clear.

Previous data suggests STAT3-signalling, while critical for leptin's effects on body-weight, may be unnecessary for reproduction [1]. Since reproductive capacity of C57BL/6 mice is unaffected by metabolic challenges [2], this surprising finding warrants re-evaluation on a more suitable strain. Hence, the aim of this experiment was to determine whether STAT3 knockout (KO) in a DBAJ/2 background, a strain susceptible to reproductive impairments and metabolic challenges, would reveal the requirement for STAT3 in reproductive function.

Transgenic mice with LepR-specific-deletion of STAT3 were generated using Cre-loxP. Reproductive and metabolic effects were explored in two background strains: C57BL/6 and DBAJ/2 (n=6-11 per group). Puberty onset was measured post-weaning by daily visual examination of the genitalia. Reproductive cyclicity (females) and reproductive organ weight (both

sexes) were assessed as adults. Metabolic effects were assessed via body and abdominal-fat weight and fasting glucose levels. Additionally, brain tissue was used to assess cellular leptin-responsiveness of STAT3.

Analysis of body weight revealed STAT3 KO females had significantly increased body weight (by 11 weeks [p=0.0093]) compared to controls (unpaired t-test). STAT3 KO males had normal bodyweight regulation. All STAT3 KO mice exhibited unchanged puberty onset, estrous cyclicity, and reproductive organ weight compared to control mice.

These data support the conclusion that leptin's actions on puberty timing and reproductive function are independent of STAT3. Another possibility we're investigating is that LepR-Cre is not powerful enough to drive complete STAT3 excision. Nevertheless, the role of different LepR signalling-pathways has become particularly relevant to the multiple functions of leptin.

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2. 2. Tortoriello, D.V., J. McMinn, and S.C. Chua, Dietary-Induced Obesity and Hypothalamic Infertility in Female DBA/2J Mice. Endocrinology, 2004. 145(3): p. 1238-1247.

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Two-sided involvement of a placenta-specific enzyme in pregnancy health

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Preeclampsia (PE) is a life-threatening complication of human pregnancy. Research in the field has identified many factors that are associated with PE, and in general these factors are regarded as villains in pregnancy health. We have been focusing on a protease that is not well expressed in any tissues except the placenta; it is also detected in the maternal circulation from early stages of pregnancy to term because of placental secretion. In PE, especially in the early-onset subtype, this enzyme is significantly elevated in the placenta as well as in the maternal circulation. Our studies strongly suggest that high levels of this protease circulating in the maternal blood but derived from the placenta may disturb maternal vascular homeostasis and contribute to the development of PE. To understand the dilemma as to why the placenta needs to make such a seemingly "destructive" factor that is not produced/wanted by any other organs in the body, we investigated its role in placental development using a number of approaches including derivation and differentiation of placental stem cells. Our results to date suggest that this villain enzyme which is so closely associated with PE is actually critical for human trophoblast differentiation and function. In this talk I will discuss our recent studies in this area, and I will also share our research on the potential utility of this enzyme in early detection of PE including the late-onset subtype which occurs far more frequently than the early-onset cases but are more difficult to detect pre-symptomatically.

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Imaging and computational tools to assess the placenta in fetal growth restriction

Alys Clark[1], **Toby Jackson**[1], **Sophie Couper**[1], **Mary Spring**[1], **Devanshi Jani**[1], **Seyed Ali Mirjalili**[1], **Peter Stone**[1], **Jo James**[1]

1. University of Auckland, Auckland, New Zealand

Fetal growth restriction is a complex multi-factorial condition that can be difficult to detect clinically. There are several known anatomical and functional differences in placentae from fetal growth restricted pregnancies compared with normal pregnancies. These differences are exhibited across a range of spatial scales from the size of the placenta to a sparser blood vessel and capillary network within the placenta. These differences impact the placentas ability to deliver oxygenated blood to the fetus, and hence fetal growth. Traditionally ultrasound imaging has been used to infer the function of the placenta by Doppler assessments in the major utero- and feto-placental arteries. However, recent years have seen a rise in new techniques to investigate the placenta both in vivo (magnetic resonance imaging) and ex vivo (high resolution 3D imaging such as microCT). Here I will present computational modelling as a tool to interpret imaging of the placenta and explain its current and potential future roles in interpreting magnetic resonance imaging, and in linking imaging data from in vivo and ex vivo modalities. Finally, I will discuss steps toward interpreting fetal and uterine Doppler imaging, guided by computational modelling, that may in the future provide a means to better identify at risk pregnancies.

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In vitro 3D models of Developing Placenta

Georgia Kafer[1]

1. University of the Sunshine Coast, Petrie, QLD, Australia

The human placenta is a transient organ built largely from specialised epithelial cells known as trophoblasts. Aberrations in trophoblast development and function are associated with many placental abnormalities. Our understanding of how different trophoblast aberrations cause placental dysfunction has historically been hampered by a lack of suitable systems to model

early human trophoblast development. Twenty years ago, researchers demonstrated that trophoblast cells could be differentiated from human embryonic stem cells (hESCs). Initially, the generation of trophoblast from hESCs attracted considerable debate owing to the tightly held notion that extra-embryonic cells (trophoblast) could not be generated from embryonic cells. Two decades on, work contributed by numerous independent research teams across the world has collectively established that pluripotent stem cells (embryonic and induced (iPSCs)) can be differentiated into trophoblast through manipulation of BMP4, FGF and TGF-β signalling. Pluripotent cells can generate major trophoblast cell types including cytotrophoblast (CTB), syncytiotrophoblast (STB) and extra-villous trophoblast (EVT). In this presentation I will outline the benefits of using a pluripotent cell base for modelling trophoblast biology. I will discuss how trophoblast biology varies when cells are cultured in 2-dimensional vs 3-dimensional (3D) systems. I will further provide an overview of how the 3D culture of trophoblasts into “organoids” can model dynamic features of placental villi including CTB-STB fusion, release of STB aggregates and migration of EVTs. I will finally discuss how placental organoid structures (placentoids) can be used to answer previously untestable placental research questions and some anticipated future directions of placental organoid – or “placentoid” research.

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Circular RNAs in the placenta

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Interest in the field of circular RNAs (circRNAs) is rapidly growing. Not only are circRNAs found throughout the healthy body, but they are also dysregulated in pathological states (the most researched of these being cancer). While research into the function and abundance of circRNAs has progressed, our overall understanding of these molecules remains primitive. Importantly, recent studies are elucidating new roles for circRNAs in pregnancy, particularly in the placenta. Given our knowledge that placental development shares commonalities with a “controlled cancer”, and that many of the genes responsible for circRNA production in cancer are also highly expressed in the placenta, it is likely that the same genes act in the production of circRNAs in the placenta. Furthermore, placental development shares many key signalling pathways and hallmarks with tumour growth and metastasis.

In this study we have isolated and purified circRNAs from placental tissue at multiple gestational ages classified as "term" (n=4 for 37, 38, 39, 40, 41 and 41+ weeks' gestation). These circRNAs have been sequenced and undergone bioinformatic analyses to determine their relative expression as gestation advances. As circRNAs are known to accumulate in tissue, due to their highly robust structure making them immune to exonucleases, we determined whether circRNAs play a role in ageing of the placenta.

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Endocrine sequelae of Cystic Fibrosis

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The outlook for children, adolescents and young adults living with cystic fibrosis (CF) has changed enormously in the past two decades due to significant changes in clinical practice over this period. It is important for Endocrinologists, finding themselves in this new landscape, to understand the implications of novel therapeutics and altered clinical trajectories on the management of Endocrine disorders. This session will explore some of the changes in CF care including CFTR modulator therapies on bone health, and glycaemia.

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Gut microbiome treatment

Wayne Cutfield[1]

1. University of Auckland, Auckland, New Zealand

Available soon

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Normal Puberty or Polycystic ovary Syndrome (PCOS)?

Stella Milsom[1]

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Polycystic ovary syndrome (PCOS) is the most common cause of infertility in adult women, and often manifests in adolescence. The cardinal features of PCOS are hyperandrogenism, ovulatory dysfunction, polycystic ovaries, and insulin resistance, although not all will be present in an individual female. PCOS is associated with potential long term sequalae such metabolic syndrome, type 2 diabetes, poorer psychological health and possibly endometrial carcinoma and cardiovascular disease. The aetiology of PCOS remains poorly delineated.

Current guidelines caution against early diagnosis in adolescence, given that polycystic ovary morphology (PCOM) and ovulatory dysfunction are common in this age group and alternative diagnoses such as energy deficiency causing acquired hypogonadotropic hypogonadism may mimic PCOS. However, early recognition of PCOS potentially provides opportunities to encourage and embed lifestyle habits likely to minimise the sequalae of PCOS.

PCOS is diagnosed in adult women if at least two of the following criteria are met: ovulatory dysfunction, hyperandrogenism, and polycystic ovary morphology (PCOM) and assuming other endocrinopathies have been excluded. In contrast, in adolescent girls, PCOM is nonspecific and a diagnosis of PCOS or probable PCOS should be based on the presence of both ovulatory dysfunction and hyperandrogenism.

Lifestyle modification in all but lean PCOS underpins clinical management with potential gains in menstrual regularity, and reduction in metabolic risk. Pharmacological options to reduce the symptoms of androgen excess and ovulatory dysfunction vary between countries and there is sparse data with which to guide clinical management decisions in younger patients. Moreover, there is a lack of longitudinal data regarding the natural history of untreated PCOS and whether intervention has the potential to mitigate longer term sequalae and protect future fertility.

This brief presentation will focus on recognition and implications of a potential PCOS diagnosis in adolescence and suggest management strategies.

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The challenges of managing type 1 diabetes in emerging adults in Aotearoa New Zealand

Ryan Paul[1]

1. Waikato District Health Board, Hamilton, New Zealand

Available soon

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The hope, reality and challenges of conserving amphibians and reptiles through genome storage and assisted reproduction

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Amphibians and reptiles are among the most threatened vertebrate taxa globally. Storing gametes and other live cells in genome resource banks and using assisted reproductive technologies (ARTs) to retrieve live animals from the stored material are two powerful, complementary tools for arresting and reversing biodiversity decline. However, the degree of development of ARTs and cryopreservation technologies in amphibians and reptiles differ markedly. These differences are explained in part by different perceptions of the taxa, but also to the very different reproductive anatomy and biology associated with the evolutionary transition from the aquatic to the terrestrial environment, and external to internal fertilisation and development. Artificial fertilisation with cryopreserved sperm is becoming a more widely developed and utilised technology for amphibians: > 30 species have had live, motile cells recovered post-freeze/thaw and several have produced reproductively competent progeny following artificial fertilisation. However, in contrast, artificial insemination leading to production of live young has been reported in few reptiles, and while sperm have been successfully cryopreserved, the production of live offspring generated from cryopreserved sperm has not been reported. In both amphibians and reptiles, a focus on sperm cryopreservation and artificial fertilisation or artificial insemination has been at the expense of the more challenging development of cryopreserving oocytes and embryos, which has not been achieved for either taxon. However, encouraging break throughs in fish oocytes and whole embryos using vitrification and laser warming hold great promise for amphibians. Alternative technologies such as the use of sophisticated stem cell/primordial germ cell cryopreservation and transplantation approaches also hold promise for the recovery of diploid and female genomes in cases where oocytes/embryos cannot be cryopreserved. I will discuss our group's work on the development of cryopreservation technologies and ARTs for the conservation of amphibians and reptiles with a focus on what we can achieve now, ongoing challenges and where we are heading next.

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Breeding system and conservation management of kiwi species

Isabel Castro[1], **Malin Undin**[1], **Caitlin McLeod**[1], **Eliana Ramos Pallares**[1], **Peter Lockhart**[1], **Barbara Durrant**[2], **Simon Hills**[1], **Wei-Hang Chua**[1]

1. Massey University, Palmerston North, MANAWATU-WANGANUI, New Zealand

2. Reproductive Sciences, San Diego Wildlife Alliance, San Diego, USA

Kiwi are a remnant of a group of ancient birds belonging to the group palaeognathae. There are five species one in the North Island (Brown Kiwi) and four in the South Island (Rowi, Tokoeka, Pukupuku and Roroa). The mating systems of these species vary from monogamy to cooperative breeding. We found that Brown Kiwi shows a diversity of mating systems from monogamy to polyandry and polygynandry. Interestingly Kiwi females are larger than males (sexual dimorphism) and in the Pukupuku and Brown Kiwi the male incubates alone (sex role reversal). This sort of behaviour is common in polyandrous species living in extreme environments where the females and males' reproductive success depends on rearing as many offspring as possible, during a short and often hazardous breeding season. This does not seem to be the case for brown kiwi where females lay up to two clutches of two eggs per season and the life span is long (≥50 years). So, we have been fascinated by this unusual species and are studying several aspects of their breeding ecology. An intriguing aspect of a breeding system where males incubate is the hormonal environment of the males. We measured the levels of prolactin on male and female kiwi. Female prolactin concentrations increased at the time of oviposition, whereas male prolactin concentrations rose gradually between the pre-breeding and incubation periods. Male testosterone decreased during incubation. We are currently looking at whether females take male roles (sex role reversal) to match incubation of males. We will be measuring hormones that are involved in masculinisation in other species (androstenedione and testosterone). In addition, we are looking at breeding behaviours that could be mediated by such hormones. Finally, we have found that members of breeding units are less related to each other than expected suggesting that Brown Kiwi can detect kin. We are investigating whether kiwi can smell differences in individuals that allow them to select partners to avoid inbreeding. The variable mating system of kiwi as well as their potential ability to discriminate between relatives can provide benefits to management by decreasing inbreeding and maximising reproductive success.

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Targeting female fertility in invasive vertebrate pests using gene drives

Stephen Frankenberg[1]

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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 invasive 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 propagation 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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The hybridised genome of New Zealand brushtail possum reveals novel marsupial imprinting and germline epigenetic reprogramming

Donna Bond[1], **Oscar Ortega-Recalde**[1], **Melanie K Laird**[1], **Kyle S Richardson**[1], **Finlay Reese**[1], **Bruce C Robertson**[2], **Yolanda van Heezik**[2], **Amy L Adams**[2], **Takashi Hayakawa**[3], **Wei-Shan Chang**[4], **Erich D Jarvis**[5], **Neil J Gemmell**[1], **Alana Alexander**[1], **Timothy A Hore**[1]

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3. Faculty of Environmental Earth Science, Hokkaido University, Hokkaido, Japan

4. Faculty of Science, The University of Sydney, Sydney, Australia

5. Vertebrate Genome Laboratory and HHMI, The Rockefeller University, New York, USA

The brushtail possum is a protected and treasured species in its native range of Australia, but also a devastating folivore and predator introduced into New Zealand. Like other marsupials, possums give birth to altricial young which undergo most of their development suckling on the teat. Intensive study from a pest-control perspective has meant possums are a model marsupial, yet many aspects of their reproductive biology are unknown.

Here we report the first chromosome-level assembly of the possum genome, and using large cohorts of RNA-sequencing in pouch-young and adults, identified metabolic signatures recording the transition from a milk diet to herbage at weaning.

Nuclear and mitochondrial analysis showed high levels of nucleotide diversity in the New Zealand possum is due to hybridisation between Tasmanian and mainland source populations. This diversity, along with phasing of long-read methylation sensitive sequencing, allowed us to distinguish expressed parental alleles throughout the genome and identify allele-specific methylation. Through this process, we uncovered several possum genes with imprinted, parent-specific expression not yet seen in other species. This result challenges the expectation that marsupial imprinting is restricted to orthologues of imprinted genes in humans and mice.

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Establishing positive relationships with food in toddlers and pre-school aged children

Lesley S Youde[1]

1. Paediatric Diabetes and Endocrinology, Royal North Shore Hospital, St Leonards, New South Wales, Australia

Optimizing glycaemic control for toddlers and preschool children with type 1 diabetes (T1D) is crucial to minimise acute and long-term diabetes complications and support neuro-cognition, development and health-related quality of life [1]. International guidelines promote the establishment of early, positive lifestyle behaviours such as the provision of nutritious family-centred meals as a cornerstone for achieving optimal glycaemic control in pre-school children.

A diagnosis of T1D in early childhood is known to increase the risk of developing poor feeding relationships and suboptimal nutritional intakes [2]. The development of a positive feeding relationship between parent and child can be challenged by the additional demands of daily diabetes care. Parents have additional mealtime tasks to attend to such as blood glucose monitoring, carbohydrate counting and pre-prandial insulin administration [3]. Parental concern, combined with perceived problematic child eating behaviours, may lead to the adoption and persistence of a variety of non-responsive feeding practices.

The aim of this presentation is examine the research in this area, review guidelines and current clinical practice and to explore options for supportive nutritional interventions.

The conclusion of the presentation will outline early interventions to establish responsive feeding relationships, encourage more healthy core foods, raise parent's awareness of the effect of 'treat' food and snacking and offer advice on alternative approaches for mealtime management.

1. Sundberg F, Barnard K, Cato A, de Beaufort C, DiMeglio LA, Dooley G, et al. ISPAD Guidelines. Managing diabetes in preschool children. Pediatr Diabetes. 2017; 18(7):499-517.
2. Streisand R, Monaghan M. Young children with type 1 diabetes: challenges, research, and future directions. Curr Diab Rep. 2014; 14(9):520.
3. Cunningham NR, Vesco AT, Dolan LM, Hood KK. From caregiver psychological distress to adolescent glycaemic control: the mediating role of perceived burden around diabetes management. J Pediatr Psychol. 2011; 36(2):196-205.

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Body language: Promoting positive body image and relationships with food in young people with diabetes

Lotte Williams[1]

1. Auckland District Health Board, Grafton, AUCKLAND, New Zealand

A practical perspective on developing a positive relationship with food and body acceptance in young people with diabetes.

Diet culture, weight stigma and the Health at Every Size Approach will be discussed.

This will include an overview of body image and practical strategies for working with young people with diabetes.

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Detecting unhealthy relationships with food in young people with diabetes

Carmel Smart[1]

1. John Hunter Childrens Hospital, Lambton Newcastle, NSW, Australia

Type 1 Diabetes (T1D) is associated with increased risk of eating disorders. International guidelines exist around screening for disordered eating in paediatric clinics; however research shows few clinics in Australia and New Zealand meet this recommendation. Key barriers include lack of the diabetes team confidence around use of tools and ongoing disordered eating management. This talk will share experiences around screening tools, including our preliminary development of a tool that incorporates diabetes technologies; and potential strategies diabetes teams can employ to support disordered eating management.

The following will be discussed:

- Our research reporting screening practices for disordered eating in paediatric Type 1 Diabetes (T1D) clinics in Australasia.
- Results of our ongoing internationally collaborative study that aims to develop a tool with clinician assessed (including from T1D technologies), parent, and adolescent items that is brief enough for consistent use, highly sensitive and minimally suggestive.
- Taking the next step: Potential strategies diabetes teams can use when disordered eating behaviours are detected in an adolescent.
- Working with the Eating disorder team: Nutritional approaches that support disordered eating management at home
- Practical aspects and key learnings from inpatient management of adolescents with T1D and ED at the John Hunter Children's Hospital, Newcastle, Australia

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Nutrition strategies to support re-creating a healthy relationship with food – a case study of an inpatient encounter

Sarah Beer[1]
1. Starship Child Health, Greenlane, AUCKLAND, New Zealand
Available soon

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Medical management of eating disorders in adolescents and young people with diabetes

Juliet Berkeley[1]
1. Canterbury District Health Board, Christchurch, New Zealand
Available soon

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Mineralocorticoid receptors in cardiovascular disease

Morag Young[1]
1. Prince Henry's Institute of Medical Research, Clayton, vic, Australia
Available Soon

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Ghrelin – cardiovascular control in health and disease

Daryl DOS Schwenke[1]
1. University of Otago, Dunedin, OTAGO, New Zealand
Ever since ghrelin was first discovered in 1999 as the endogenous ligand for the growth hormone secretagogue receptor, this gut-derived hormone has been implicated in a diverse range of physiological functions. Of particular interest, ghrelin has emerged as a potent modulator of the cardiovascular system, with therapeutic actions in a plethora of cardiovascular morbidities. Here, I present a series of investigations that employ a variety of experimental approaches to illuminate the pleiotropic benefits of *exogenous* ghrelin in treating pulmonary hypertension, acute myocardial infarction and peripheral artery disease, and highlight key mechanistic pathways that ghrelin targets to elicit its physiological effects. I will also discuss how reduced circulating levels of *endogenous* ghrelin may present as a unique and significant risk factor for the onset and severity of cardiovascular disease. Finally, looking forward, I consider the idea that down-regulation of the ghrelin pathway may be preferentially inherent in specific populations that are known to have a greater pre-disposition to CVD in New Zealand.

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Circulating cardiac biomarkers linking molecular physiology and clinical outcomes

Chris J Pemberton[1]
1. University of Otago, Christchurch, CANTERBURY, New Zealand
Breadcrumbs of evidence suggesting the heart has an endocrine function aside from its mechanical circulatory role had been hinted at in the mid-20th Century, but it wasn't until 1981 when the first clear evidence that the heart had true endocrine function (bioactive hormone and receptor) was documented. This initial report identified the first member of the natriuretic peptide (NP) family - known as ANP - and pioneered a new research field known as cardio-endocrinology. Within 30 years, the field of cardio-endocrinology had expanded to acknowledgement of the fact that proteins/DNA/RNA/lipids/metabolites from just about every organ in the body can affect cardiac function and stability, either directly or indirectly. Thus, whilst the heart contains and releases NPs (ANP and BNP) to promote vascular relaxation and renal diuresis, protein factors from the immune system (suPAR), bone marrow/normoblasts (ERFE), nervous system (urocortins), kidney (EPO), stomach (Ghrelin), vasculature (CNP/adrenomedullin) and multi-organ present stress markers (GDF-15) provide physiologic and clinical information regarding cardiovascular status. Alongside these proteins, circulating

DNA/RNA (of many varieties), lipids and peptide/protein metabolites also provide molecular signals and information that reflect nuclear/cytosolic transcription & translation, proteolytic degradation and energy expenditure in nascent and fulminant cardiovascular disease states, including coronary artery disease, myocardial infarction and acute heart failure.

This talk will provide a snapshot of some of these factors and their biomarker capability, as well as efforts directed towards their pharmacological use and/or modulation of cardiovascular diseases to reduce the burden on patients and health systems

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Updates in lipid management

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Cardiometabolic medicine is gaining momentum as a subset of both endocrinology and cardiology specialties with combined cardiometabolic services forming to address the significant burden of cardiometabolic disease in various centres. There have been multiple recent advances in the diagnosis and treatment of lipid disorders with significant improvements in both lipid concentrations and cardiovascular events demonstrated in multiple clinical trials. Awareness of inherited lipid disorders is growing, particular for familial hypercholesterolaemia (FH), which is the most prevalent autosomal co-dominant disorder of low-density lipoprotein catabolism that is present in ~1:300 people in the population. However, further work is required as currently less than 10% of people with FH are current identified and treated. I will outline the benefits of availability of genetic testing for FH and recent guidelines. I will also describe emerging specific lipid / lipoprotein lowering therapies including monoclonal antibodies and antisense oligonucleotides.

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ACR TI-RADS has limited utility in a real-world setting: correlation of results and patient outcomes in a cohort presenting for thyroid ultrasonography

Tom Wilkinson[1], **Thomas Cawood**[1], **Anthony Lim**[1], **Penny Hunt**[1], **David Roche**[2]

1. Canterbury District Health Board, Christchurch, NZ, New Zealand

2. Canterbury Southern Community Laboratories, Christchurch, New Zealand

Aims: The American College of Radiology Thyroid Image Reporting and Data System (ACR TI-RADS) was developed to predict malignancy risk in thyroid nodules using ultrasound features, to identify patients who would most benefit from investigation with fine needle aspiration (FNA)(1). TI-RADS was derived from a pre-existing database of FNA results, raising uncertainty about applicability to unselected patients presenting with a thyroid nodule in whom the risk of malignancy may be lower. We assessed malignancy rates in a cohort of patients presenting for ultrasound and the impact of routine reporting of TI-RADS on patient outcomes.

Methods: Records for all patients presenting for thyroid ultrasonography in Canterbury, New Zealand were retrospectively reviewed across two intervals: 1/1/2017-30/6/2018 (prior to implementation of TI-RADS reporting) and 1/1/2020-30/6/2021 (after implementation of TI-RADS reporting). Malignancy rates were calculated for nodules >10mm with a definitive FNA or histology result.

Results: 1210 nodules were identified in 582 patients prior to implementation of TI-RADS. 1253 nodules were identified in 625 patients after implementation of TI-RADS. After implementation of TI-RADS, fewer patients proceeded to FNA (49% vs. 60%, $p<0.01$) or surgery (14% vs. 18%, $p<0.05$), with no difference in cancer diagnoses (3% vs. 4%, NS), however FNA procedures would not have decreased if ACR TI-RADS recommendations were strictly followed. TI-RADS category was associated with malignancy rate (0% in TR1 and TR2, 3% in TR3, 5% in TR4, and 12% in TR5). Notably, 63% of nodules were graded TR3 or TR4, where rate of malignancy did not meaningfully differ from baseline.

Conclusion: TI-RADS category is associated with malignancy rate in patients presenting for thyroid ultrasound, however is non-discriminatory in the majority of nodules. Nodules classified as “highly suspicious” have only a modestly increased malignancy rate. Routine implementation of ACR TI-RADS appears to alter clinical decision making for only a minority of patients.

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A Clinical Decision Making Support Tool for Active Surveillance of Low Risk Papillary Thyroid Cancers

Eleanor White[1, 2], **Bridget Abbott**[1, 3], **Geoffrey Schembri**[1, 4, 3], **Roderick Clifton-Bligh**[1, 2, 4], **Matti Gild**[1, 2, 4]

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4. University of Sydney, Sydney, NSW, Australia

Active surveillance (AS) is an alternative to surgery in select patients with very low risk papillary thyroid cancer (PTC). Many clinicians feel ill-equipped in selecting appropriate patients. Our aims were: (1) develop a web-based decision support tool to assist clinicians in identifying patients appropriate for AS; and (2) evaluate the prevalence of patients suitable for AS in a tertiary centre.

A web based clinical support tool was developed utilising evidence based characteristics for AS suitability. A retrospective database was interrogated for patients who underwent hemithyroidectomy between 2012 - 2021 with final histopathology demonstrating PTC.

Between 2012 - 2021, 763 patients underwent hemithyroidectomy with final histopathology PTC. 316 patients were excluded (incomplete data, histopathology other than PTC, incidental PTC or hyperparathyroidism). We applied the tool to the remaining 447 patients. 352/447 (79%) were unsuitable due to the thyroid nodule ≥2cm. 95/447 (21%) were potentially suitable for AS. We restricted our final analysis to the cohort with pre-operative FNA demonstrating a Bethesda V or VI result 60/95 (63%).

51/60 patients were female and 9/60 male, age range between 19 – 77, median age 36.5 years. 10 patients had completion hemithyroidectomy, 4/10 demonstrating malignancy in contralateral lobe. All 4/10 had I-131 and 4/10 patients without malignancy in contralateral lobe had 1-131. No patients had evidence of disease recurrence.

Subgroup analysis of 22 patients were analysed to ascertain if AS features were easily identifiable on pre-operative ultrasound. Overall, 18/22 (82%) patients would have been suitable utilising this tool, without incorporating 2mm to capsule. 4/22 were excluded due to presence of extrathyroidal extension on ultrasound.

Clinical support tools are an accessible way of empowering clinicians to decide suitability for active surveillance. In a cohort of patients who had hemithyroidectomy for PTC, 60/447 patients (13%) may have been suitable for AS. Further validation studies are required.

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ctDNA in medullary thyroid cancer

Ayanthi Wijewardene[1, 2], Martyn Bullock[2], Bin Wang[3, 4], Matti Gild[1, 2], Catherine Luxford[2], Roderick Clifton-Bligh[1, 2]

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2. Cancer Genetics Laboratory, Kolling Institute, The University of Sydney, Sydney, NSW, Australia

3. Precision Medicine, Precision Medicine, Syd Path, St Vincent's Hospital, Sydney, NSW, Australia

4. St Vincents Clinical School, University of New South Wales, , Sydney, NSW, Australia

Background- Liquid biopsies are a minimally invasive approach to obtain biomarkers such as circulating tumor DNA (ctDNA) in peripheral blood. ctDNA is validated as a powerful tool in lung and breast cancer[1-3]. To date, there is limited and conflicting studies evaluating its role in thyroid cancer[4]. Our study aimed to evaluate the utility of ctDNA in advanced thyroid cancers.

Methods: Eight patients >18 yo with metastatic medullary thyroid cancer (MTC) had 10 mL of blood collected in Streck cell-free DNA blood tubes. Plasma was isolated by two rounds of centrifugation at 2,000 g for 10 mins. The ctDNA was extracted using Qiagen's QIAamp Circulating Nucleic Acid Kit. Purified ctDNA was quantified using a Qubit fluorometer, and 3.2-114ng ng analysed using Oncomine Pan-Cancer Cell-Free Assay run on an Ion Torrent Genexus system. Genetic testing of germline and tumoral DNA had been performed as per standard care; patients with germline mutations were excluded. Clinical data was obtained from medical records.

Results: Tumor DNA sequencing found 7 patients had a RET p.M918T and 1 a RET p.E632_L633del mutation. Detectable circulating levels of these mutations were found in 4/8 patients. Of the 4 patients negative by ctDNA sequencing, all samples were collected post-commencement of Selpercatinib treatment, except for one patient with a low (2 mL) plasma volume. In all patients positive by ctDNA, samples were collected prior to Selpercatinib treatment; in these patients sequential samples, 3 showed greatly reduced or undetectable levels following Selpercatinib. In contrast, the patient with a RET p.E632_L633del mutation showed increasing plasma concentrations of the mutation in the setting of progressive disease. Positive correlation was seen between mutant copies/ml and calcitonin (r=0.25) and CEA (r=0.64).

Conclusion: ctDNA is a novel biomarker, with potential to monitor disease progression in patients with driver mutations and advanced thyroid cancers.

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Mammary-specific ablation of Cyp24a1 reduces mammary density and inhibits tumour development in MMTV-PyMT breast cancer mice

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Active vitamin D (1,25(OH)2D) has been shown to regulate numerous cell processes in mammary cells. Degradation of $1,25(OH)_2D$ is initiated by the mitochondrial enzyme 25-hydroxyvitamin D 24-hydroxylase (CYP24A1), and provides local control of $1,25(OH)_2D$ bioactivity. CYP24A1 is coamplified with a known oncogene in human breast cancer tissue, and thus CYP24A1 may be an important player in contributing to the dysregulation of cell growth through lowering local cellular $1,25(OH)_2D$ production. To assess the role of CYP24A1 activity in normal mammary development, we used mammary epithelial-specific Cyp24a1 knockout mice to demonstrate reduced terminal end bud number, ductal outgrowth and branching during puberty and alveologenesis during early pregnancy [1]. These mice were then used to create mammary epithelial-specific Cyp24a1 knockout in the PyMT mouse breast cancer model (KO) to examine its role in tumour progression. Specific CYP24A1 ablation was confirmed by histology and qPCR analysis. Breast tumour initiation was significantly delayed with palpable tumours occurring at 10 weeks in KO animals (mean=0.140 cm3, SD=0.061 N=17) compared to 7 weeks (mean= 0.385 cm3, SD=0014, N=17) in control animals (CYP24A1 flox/flox Cre- and CYP24A1wt/wt Cre+). At 13 weeks, tumour volume in KO mice was 75% smaller than in control mice ($p < 0.0001$). Furthermore, tumour size at endpoint in KO (6 cm^3) was observed at age 18 weeks (mean= 5.6 cm^3 SD=0.42 N=19) compared to 13 weeks in control animals (mean= 6.5 cm^3 SD=0.22 N=17). Breast tumour number in KO mice (4.6 ± 0.5 N=20) was significantly decreased at death (17-18 weeks) compared to 13-14 weeks control mice (7.9 ± 0.3 N=20). Finally, CYP24A1 knockout animals showed a dramatic reduction in lung metastasis development compared to control animals (2/10 Vs 9/10, $P<0.001$ N=10). These data suggest inhibiting CYP24A1 is a potential approach to activating the vitamin D pathway in breast cancer prevention and therapy.

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PMR-116, a novel inhibitor of ribosome biogenesis with antitumor activity in preclinical models of prostate cancer

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Advanced prostate cancer is characterised by mutations and amplifications of genes involved in regulating protein synthesis. PTEN-loss stimulates activity of the mTOR pathway, while amplification of MYC leads to increased ribosome biogenesis and elevated mRNA translation rate. Our previous work has demonstrated the efficacy of co-targeting ribosome biogenesis, via inhibition of RNA Pol I activity, and 4E-BP1 phosphorylation to suppress prostate cancer growth *in vivo* in GEMM of PCa and in patient-derived xenografts(1,2).

In a collaboration with Pimera Inc., we investigated the efficacy of their new lead RNA Pol I inhibitor PMR-116 in models of prostate cancer. PMR-116 is well tolerated *in vivo* in mice and can be given at 300mg/kg weekly. Using the Hi-MYC mouse model of PCa we show that dosing 6-month-old mice once weekly for 4 weeks can decrease the incidence of invasive lesions by up to 85% compared to vehicle control while reverting glands to patterns of low grade intraepithelial neoplasia. PMR-116 rapidly inhibits proliferation in the Hi-MYC model with a 50% decrease in Ki67 observed 12 hours after oral administration. Conversely, PMR-116 showed minimal anti-tumour efficacy in the PTEN-null model of PCa suggesting that elevated MYC signalling may be required for optimal response.

To further validate our promising GEMM results in more clinically relevant human-derived models, we used patient-derived xenografts lines we established from multidrug-resistant, metastatic PCa(3). PMR-116 treatment decreased tumour volume in all PDX tested including complete response in a line in which tumour volume decreased by ~90% compared to baseline.

We believe this new RNA Pol I inhibitor shows promising results in a wide range of preclinical models of androgen receptor dependent and independent PCa and may exert higher efficacy in tumours expressing high levels of MYC. PMR-116 is currently in Phase I dose escalation trial in patient with solid tumours (ACTRN12620001146987).

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Bipolar Androgen Therapy (BAT) inhibits the growth of patient-derived models of advanced prostate cancer.

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Publish consent withheld

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A mediation analysis of the effect of testosterone treatment in the T4DM trial

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Background: In the T4DM study of males at high risk of, or with newly diagnosed type 2 diabetes (T2D), testosterone (T) treatment reduced T2D at 2 years by 40% beyond lifestyle change alone [1].

Aim: To test whether the T treatment effect on glycaemia is mediated through changes in fat mass, skeletal muscle mass and strength, and oestradiol (E2).

Methods: A randomised placebo-controlled trial enrolling 1007 men, 50-74 years, waist circumference ≥ 95cm, serum T ≤ 14nmol/L (chemiluminescent RIA) and either IGT or newly diagnosed T2D on an OGTT. Participants were enrolled in a lifestyle program and randomised 1:1 to 3 monthly IM injections of 1gm testosterone undecanoate or placebo. Mediation analyses used standard methodology with natural effects models, for the 2 primary outcomes of T2D at 2 years - OGTT ≥ 11.1mmol/L and 2-hour glucose change from baseline, modeled by logistic regression and linear regression respectively. Potential mediators of interest were fat mass, % abdominal fat, lean mass (DXA) and non-dominant hand-grip strength (handgrip dynamometry) and Oestradiol (LCMS) (with and without adjustment for SHBG). Mediation was adjusted for baseline covariates (risk factors and baseline mediators). Direct and indirect effects were estimated.

Results: There were complete data for 709 participants (70%). The unadjusted OR for T2D at 2 years was 0.53 (95% CI: 0.35-0.79), and 0.48 (95% CI: 0.30-0.76) adjusting for baseline covariates. Addition of the potential mediators attenuated the treatment effect, OR 0.75 (95% CI: 0.42-1.36). In mediation analyses 65% of the total effect for 2-hour glucose ≥ 11.1mmol/L, and 66% of change in 2-hour glucose from baseline were mediated. Only fat mass remained prognostic in the full model (OR: 1.23; 95% CI: 1.09-1.39; p<0.001).

Conclusion: Fat mass mediated only part of the T-treatment effect on glucose metabolism; further work on the causal pathways is warranted.

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Serum testosterone is inversely associated with leucocyte telomere length in men.

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Objective

Older men on average have lower testosterone concentrations compared with younger men, and more age-related comorbidities. Whether lower testosterone concentrations contribute to biological ageing remains unclear. Shorter telomeres are a marker for advanced biological age. We tested the hypothesis that testosterone concentrations are associated with leucocyte telomere length (LTL), in middle to older aged men.

Design

Cross-sectional analysis of the UK Biobank study, involving community-dwelling men aged 40-69 years.

Methods

Serum testosterone and sex hormone-binding globulin (SHBG) were assayed. Free testosterone was calculated (cFT). Leucocyte telomere length (LTL) was measured using Polymerase Chain Reaction. Linear mixed models were fitted to LTLs.

Results

In 167,706 men, median age 58 years, standardised LTL was inversely associated with total testosterone after adjusting for sociodemographic, lifestyle and medical factors (P<0.001; effect size 0.09, 95% confidence interval [CI], 0.08-0.10 longer LTL for men with total testosterone at median of lowest quintile [Q1] vs highest [Q5]). This relationship was attenuated after additional adjustment for SHBG (P=0.003; effect size 0.03, CI=0.02-0.05). The association between cFT and LTL was similar in direction but lower in magnitude to that found with total testosterone. After adjusting for sociodemographic, lifestyle and medical factors, SHBG was inversely associated with LTL (P<0.001; effect size 0.12, 95% CI 0.10-0.13 longer LTL for SHBG at median Q1 vs Q5). These results were largely unchanged after additional adjustment for testosterone (P<0.001; effect size 0.10, CI=0.08-0.12).

Conclusions

Total testosterone and SHBG were independently and inversely associated with LTL. Men with higher testosterone or higher SHBG had shorter telomeres, which would be consistent with more advanced biological age. These findings argue against a role for testosterone to slow biological ageing in men.

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Gonadal germ cell cancer risk in DSD

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Individuals who have a Difference of Sex Development and Y chromosomal material in their (gonadal) karyotype are at increased risk for developing a malignant gonadal germ cell cancer (GCC). These GCC can be either seminomas (dysgerminomas) or non-seminomas and are always preceded by an *in situ* neoplastic lesion: germ cell neoplasia *in situ* in a (dysgenetic) testicular context or gonadoblastoma in a context of undifferentiated gonadal tissue. Therefore, prophylactic gonadectomy in childhood has long been a standard procedure.

As it becomes increasingly clear that the risk for GCC development varies widely across diagnoses and that often the gonads produce hormones that are beneficial for patients, gonadectomy is now often postponed until adulthood, or replaced by a watchful surveillance program for gonads *in situ*.

In this session, the pathobiology of gonadal GCC is explained from a developmental perspective, and risk profiles per group of conditions presented. Unfortunately, tools for the surveillance of abdominal gonads are limited and none of the available techniques can reliably detect an early *in situ* neoplastic lesion. The available tools are briefly outlined and practical recommendations are given that can be readily applied in a clinical context.

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Syndromic Forms of Intersex Conditions

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Intersex conditions (also known as Differences of Sex Development) are congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. Commonly, intersex conditions cause gonadal dysgenesis due to mutations in genes required during fetal life for gonadal development. These genes often play a role in other organ systems. Such extra-gonadal effects of these mutations lead to co-morbidities or syndromic forms of intersex conditions. In this talk,

several examples of Syndromic DSD in 46,XY patients will be discussed. Firstly, variants in the FGF9 ligand or in its receptor, FGFR2 occur in 46,XY intersex patients, some with synostoses. FGF signalling variants affect Sertoli cell differentiation or proliferation. Secondly, a form of alpha thalassemia occurs in patients with ATRX syndrome leading to under virilisation or feminisation of XY individuals. In an ATRX mouse knockout model, the formation of a 'giant' PML nuclear body in Sertoli cells leads to testis degeneration. Thirdly, a number of intersex cases of unknown genetic etiology are under study and undergoing whole exome and whole genome analyses. These patients have rare co-morbidities, such as intellectual disability, heart and kidney defects, tall stature, and advanced bone age.

See posters by Uyen Le et al., Brittany Croft et al., and Nayla Leon Carlos et al.

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SRB The Robinson Research Institute Award

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Available soon

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SRB Mercy Perinatal Mid-Career Medal Session

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Prof Tu'uhevaha Kaitu'u-Lino is a NHMRC RD Wright (CDF2) and Dame Kate Campbell Fellow in the Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne. She leads the Diagnostics Discovery and Reverse Translation in Pregnancy team under the wider Translational Obstetrics Group, and currently has two major research focuses; 1) Identifying new diagnostics to identify preeclampsia and fetal growth restriction (to reduce the incidence of stillbirth) 2) Understanding the pathogenesis of these diseases to assist in identifying new therapeutic targets.

A mid-career scientist, Prof Kaitu'u-Lino has published >125 peer-reviewed publications in leading specialist journals and prestigious interdisciplinary journals. She is currently CI on a NHMRC Project Grant, a NHMRC Ideas Grant, an ARC Discovery Project, a NHMRC Fellowship and a NHMRC Synergy grant. Since being awarded her PhD in 2008 (and with 35 months of career interruption since) she has been CI on over $13.5M in research funding.

Prof Kaitu'u-Lino is currently Deputy Head of the Department of Obstetrics and Gynaecology at the Uni of Melbourne and a Program Organising Committee co-chair for the Society for Reproductive Biology. She regularly acts as an ad hoc reviewer for international journals and sits on the Editorial Board for the journals Placenta and Scientific Reports. She is passionate about mentoring the next generation of scientists and clinician scientists and is currently supervising 5 PhD and 3 honours students. A mum of 4 and step-mum of 2, Prof Kaitu'u-Lino is a strong advocate for women in STEM.

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Multidimensional diagnostics with artificial intelligence for adrenal tumours and endocrine hypertension

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Mass spectrometry provides possibilities to simultaneously measure multiple endocrine-related analytes to more accurately diagnose hormonal disorders than possible using any single analyte alone. The same multi-analyte panel may also be applied to different disorders. Thus, measurements of metanephrines and methoxytyramine can be used for diagnosis of phaeochromocytoma or paraganglioma (PPGL) and neuroblastoma, whereas a single mass spectrometry-based steroidomics panel can be employed for primary aldosteronism (PA), adrenocortical carcinoma, Cushing syndrome and other disorders of steroidogenesis. Moreover, patterns in test results can be used to assess post-test probabilities of disease and subtype disorders according to tumour location, disease-causing mutations or even malignant risk. The problem remains how to best interpret such multidimensional data. With today's advances in computational power, this can be achieved using machine learning (ML) classification-based algorithms. Sufficiently large numbers of patients are crucial for training and internal validation, which should be followed by external validation. For training, correct classifications are essential, though problematic for some disorders. We have specifically developed ML models for PPGL and PA. For PPGL we have employed datasets of over 700 patients with and over 3200 without tumours to train and validate ML models for determination of post-test disease probabilities and assessments of metastatic risk. Predictions of ML-models out-perform those of specialists in the field. For PA we have developed ML models using a retrospective cohort of 462 tested patients, including 139 with unilateral disease with 58 due to *KCNJ5* mutations. Those ML models are now undergoing external validation in a prospective study that has to date enrolled 542 eligible patients. Although use of our models for screening was compromised by incorrect classifications due to immunoassay inaccuracy, one model nevertheless performs similarly to aldosterone:renin ratios. Significantly improved performance was achieved by incorporation of plasma potassium and renin

with that steroidomics-based model. Among the patients in the trial, 186 have received follow-up, including 38 with unilateral disease confirmed by post-surgical biochemical cure. Genotyping has identified *KCNJ5* mutations in 13 patients, all correctly predicted by ML to have PA with *KCNJ5* mutations. Ten had their adrenals removed without AVS evidence of lateralisation. Such patients with an identified adrenal mass may thus proceed directly to surgery without need for confirmation studies or AVS. Although applications of multidimensional diagnostics with artificial intelligence show promise for diagnostic stratification of endocrine disorders, it remains important to establish that outcomes for patients are improved compared to traditional procedures.

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Primary aldosteronism: lessons from cells, clinics and consumers

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Available soon

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Placental endocrine *Igf2* regulates maternal metabolism with impacts on fetal growth

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Objectives: Hormones released from the placenta are thought to adapt maternal metabolism so that nutrients are optimally partitioned to the fetus for growth. Likely central to this process are imprinted genes, such as *Igf2* (insulin-like growth factor 2) that is highly expressed by the conceptus and vital for promoting fetal growth. Here, we explore how deletion of *Igf2* in the placental endocrine zone impacts on maternal metabolism and fetal nutrient supply and growth.

Methods: *TpbpaCre* females were mated with male *Igf2*-floxed mice to produce whole litters with selective *Igf2* under-expression in the placental endocrine cells (*Jz-Igf2*^{UE}). On day 16 of gestation, dams underwent either a glucose/insulin tolerance test, or were anesthetised and received a non-metabolisable analogue of glucose (^{3}H-methyl-D glucose) to determine maternal-fetal glucose transfer. Maternal pancreas was harvested for beta cell mass quantification and maternal blood and placentas were collected for the proteomic identification of placental hormones.

Results: Compared to pregnant control dams, *Jz-Igf2*^{UE} dams showed greater glucose tolerance and insulin sensitivity. Moreover, *Jz-Igf2*^{UE} dams showed reduced pancreatic beta cell mass, but greater glucose-stimulated insulin secretion (measured during the glucose tolerance test). Materno-fetal glucose transfer and fetal growth were decreased in *Jz-Igf2*^{UE} dams. Proteomic analysis of maternal plasma identified reduced prolactin levels in *Igf2*^{UE} dams, while placental analysis identified 310 proteins altered between control and *Jz-Igf2*^{UE} dams, such as pentraxin, leptin receptor and TNRF1.

Conclusions: *Igf2* influences placental hormone production, with important implications for maternal metabolic changes that promote fetal nutrient supply and growth.

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Polar lipidomics to predict pregnancies at risk of fetal growth restriction and preeclampsia

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Lipids serve as multifunctional metabolites that have important implications for the pregnant mother and developing fetus. Abnormalities in lipid metabolites have emerged as potential risk factors for pregnancy diseases, such as preeclampsia, and fetal growth restriction. The aim of this study was to assess the biomarker potential of lipid metabolites for early detection of pregnancy complications.

Plasma samples from the Biomarker and Ultrasound Measures for Preventable Stillbirth (BUMPS) cohort were collected at 36 weeks' gestation, prior to diagnosis of fetal growth restriction (<5th birthweight centile, n=49), preeclampsia (n=17), both fetal growth restriction and preeclampsia (n=6), and gestation-matched controls (n=72). Analysis of maternal plasma was conducted by Metabolomics Australia and polar lipidomics was performed on the Agilent 6490 LC-QQQ mass spectrometer to quantify 468 lipids. MetaboAnalystR 3.2 was used for data pre-processing and MATLAB R2020b for statistical analysis.

Cholesterol esters (CE15:0, CE16:1, CE17:1, CE22:4, CE24:6) were significantly increased in maternal circulation of women who delivered fetal growth restriction babies at term (P<0.002, n=55 vs n=72 controls). Further analysis revealed cholesterol ester 17:1 provided the best predictive power for fetal growth restriction pregnancies (AUC=0.71). Phosphatidylinositol (P=0.0002), Phosphatidylcholine (P=0.0003), Diacylglycerol (P=0.0007) and Triacylglycerol (P=0.0008, n=23 vs n=72 controls) were the top dysregulated lipids in the maternal circulation of patients who later developed preeclampsia. Phosphatidylinositol was the best lipid to predict patients who developed preeclampsia later in pregnancy (AUC=0.81).

This study successfully quantified 468 lipids in maternal plasma collected from patients at 36 weeks' gestation, who were later diagnosed with fetal growth restriction or preeclampsia later in pregnancy. The predictive capacity of generating lipid profiles for gestational disorders holds significant potential as a non-invasive assessment of maternal and fetal health. More studies are required to further investigate lipid metabolism in pregnancy and determine if lipid analysis provides a therapeutic window to treat gestational disorders.

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Maternal C1q is required for haemodynamic adaptations to pregnancy, decidual vascular remodelling, and healthy fetal growth

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Preeclampsia is a pregnancy complication associated with impaired spiral artery remodeling. Serum concentrations of C1q are decreased in some women with preeclampsia. C1q is produced by trophoblast cells and macrophages that, along with uterine natural killer (uNK) cells, facilitate spiral artery remodeling. Regulatory T (Treg) cells are anti-inflammatory cells which maintain immune homeostasis during these processes. The significance of maternal C1q deficiency on pregnancy outcomes has not been investigated. We hypothesized that maternal C1q deficiency would impair spiral artery remodeling and fetal growth which may be associated with a reduced Treg cell abundance. Female mice with a null mutation in *C1qa (C1qa-/-*) were mated to BALB/c male mice. Treg cell proportions were assessed using flow cytometry. Blood pressure was measured before and during pregnancy using tail cuff plethysmography. Ultrasound bio-microscopy assessed uterine artery function. Wild-type C57BL/6 female mice mated to BALB/c males were controls, and significant differences (P<0.05) were assessed. *C1qa*-/- dams had a 25% reduction in Treg cell abundance in mesenteric lymph nodes on day 3.5 post coitum. At mid-gestation, decidual artery lumen area was reduced by 41%, indicative of impaired spiral artery remodeling, and uNK cell abundance was reduced by 11%. *C1qa*-/- dams demonstrated uterine artery dysfunction with a ≥16% increase in artery resistance and pulsatility indices at mid-gestation. Mean arterial pressure (MAP) was not different between *C1qa*-/- and wild-type dams before or at mid-gestation. However, in late-gestation, MAP decreased in wild-type mice, but not in *C1qa*-/- mice, indicative of failed haemodynamic adaptations to pregnancy. In late-gestation, fetal weight and fetal: placental weight ratios were reduced in *C1qa*-/- dams by ≥10% compared to controls. In conclusion, maternal C1q deficiency has adverse consequences for maternal vascular adaptations to pregnancy and the underlying mechanism likely involves Treg cells. These data suggest a key role for C1q in uterine vascular remodeling.

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Regulation and function of SPINT1 in human placental trophoblasts

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Serine Peptidase Inhibitor Type 1 (SPINT1) is expressed by placental trophoblasts and its ablation in mice leads to compromised placental development and consequently embryonic lethality1,2. In humans, placental and circulating SPINT1 levels are reduced in fetal growth restriction (FGR), a complication mediated by placental insufficiency3.

The aim of this work was to assess the regulation and function of SPINT1 in human placental cells using a first-trimester human trophoblast stem cell (hTSC) line. Placental insufficiency and FGR is associated with placental hypoxia, which we have previously found to cause reduced SPINT1 in isolated term cytotrophoblasts. Here, we tested whether this was also the case in hTSCs. Indeed, hypoxia (1% Oxygen) significantly reduced SPINT1 mRNA expression by 40% (p<0.01) and secretion by 50% (p<0.01) relative to normoxia (8% Oxygen).

We next assessed whether SPINT1 is released from the surface of placental cells by matrix metalloproteinases (MMPs). We treated hTSCs with batimastat, an MMP inhibitor and found SPINT1 secretion into culture media was significantly reduced at a dose of 10uM batimastat by 28%, relative to control (p=0.0165). This suggests that while MMPs may contribute to SPINT1 release, there may also be alternate proteases that contribute.

SPINT1 inhibits the degradative activity of several cell surface proteases, including matriptase and prostasin. We next assessed whether silencing SPINT1 (siRNA) in hTSCs would significantly alter protease activity by utilising a substrate

which fluoresces when cleaved by matriptase or prostasin. We observed a non-significant increase in matriptase activity accompanies loss of SPINT1.

We've shown placental SPINT1 is reduced by hypoxia and MMPs may contribute to its release from the placenta. We also confirm a likely role for SPINT1 in impairing matriptase/prostasin activity in human placental cells. Further studies are currently underway to ascertain the molecular regulators of SPINT1 and its potential role in cellular proliferation and differentiation.

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Reduced placental (P)RR inhibits trophoblast growth and placental development

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Introduction

The renin-angiotensin system (RAS) is vital for appropriate placental development. The (pro)renin receptor ((P)RR) plays a crucial role in activating the RAS. We have shown that (P)RR promotes trophoblast proliferation, migration, and invasion *in vitro*. In this study, we assess the role (P)RR plays in placental development *in vivo*.

Methods

GFP-expressing lentiviral packaged gene-constructs were used to specifically knockdown (P)RR expression in the trophectoderm of mouse blastocysts. Zygotes were collected from super-ovulated C57/BL6/CBA-F1 mice and cultured until the blastocyst stage. Following zona pellucida removal, blastocysts were incubated for 6h with either vehicle (no-virus), control virus (vehicle shRNA and GFP;$1x10^8$VP/ml), or (P)RR-knockdown virus ((P)RR shRNA and GFP;$1x10^8$VP/ml) before being transferred into recipient pseudo-pregnant Swiss CD1 female mice. Fetal and placental tissues were collected and assessed at embryonic days 10 and 18.

(P)RR levels were assessed in the labyrinth zone of day 18 placentae via qPCR and immunoblot. Additionally, stereological merz grid analysis was performed on placental tissue to assess the volumetric distribution of trophoblasts, fetal capillaries, and the maternal blood space.

Results

Embryo viability and the fetal-placental weight ratio were decreased at term in the (P)RR-knockdown group compared with the control groups (P=0.034 and P=0.04, respectively). (P)RR-knockdown successfully reduced (P)RR mRNA and protein levels in the placental labyrinth zone (P=0.002 and P=0.034, respectively).

Trophoblast volume and surface density were decreased in (P)RR-knockdown placentae when compared with the control groups (both, P<0.0001). In contrast, maternal blood space was increased in (P)RR-knockdown placentae (both, P<0.0001). Furthermore, syncytiotrophoblast barrier thickness was reduced in the (P)RR-knockdown placentae (P=0.026).

Discussion

Reduced placental labyrinth (P)RR expression decreases placental trophoblast number and total surface area for exchange, thus decreasing trophoblast barrier thickness. Decreasing (P)RR expression also decreases fetal-placental weight. Taken together these data suggest that placental (P)RR is important for placental development and functional capacity.

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C-type natriuretic peptide relaxes omental arteries in a new ex vivo model of preeclampsia.

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Preeclampsia is a serious pregnancy complication, associated with widespread maternal vascular dysfunction. C-type natriuretic peptide (CNP) contributes to vascular homeostasis, acting through NPR-B and NPR-C receptors; however the role of CNP in preeclampsia is unknown. This study characterises the expression of CNP during pregnancy and investigates whether CNP can dilate maternal arteries in several *ex vivo* models of preeclampsia.

CNP, NPR-B and NPR-C mRNA expression was assessed in placental tissue (preterm n=86; term n=49) and omental arteries (n=17) from preeclamptic and normotensive pregnancies via qPCR. Human primary cytotrophoblasts (n=3) and placental explants (n=5) were cultured under normoxic (8% O_2) or hypoxic (1% O_2) conditions and mRNA expression was assessed. Using wire myography, we investigated the effects of CNP on dilation of omental arteries dissected from fat biopsies, collected during caesarean section of healthy pregnancies (n=22). Arteries were pre-constricted with serum from preeclamptic patients, or recombinant endothelin-1 (vasoconstrictor high in preeclampsia) to model vasoconstriction associated with preeclampsia. Pre-constricted arteries were then treated with synthetic CNP (0.001-100uM), or vehicle, and vascular relaxation assessed. In further studies, arteries were pre-incubated with NPR-B or NPR-C antagonists prior to serum-induced constriction, investigating the mechanistic signalling within the model.

The mRNA expression of CNP, NPR-B and NPR-C was not altered in placenta or omental arteries from preeclamptic pregnancies compared to normotensive controls. Moreover, NPR-C was undetected in cytotrophoblasts, and hypoxia did not alter placental expression of CNP. Furthermore, CNP directly stimulated maternal artery vasorelaxation in an innovative model of preeclampsia (constriction driven by serum). However, its actions were not attributed to NPR-C receptor signalling, nor endothelin-1 driven constriction pathways.

This study demonstrated that CNP expression is not altered in the placenta or maternal vasculature in preeclampsia. However, collectively the data suggest that enhancing CNP could offer a therapeutic strategy for reducing systemic vascular constriction in preeclampsia.

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Bioprinting a placental model to study the effects of current and emerging treatments of preeclampsia

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Preeclampsia is a cardiovascular disorder of pregnancy without a cure, in part due to the lack of reliable model systems for the investigation of human pregnancy. In our recent work we developed and utilised a three-dimensional (3D), bioprinted, trophoblast organoid model for studying aspects of placental development and function during pregnancy. We then aimed to employ this model to study the effects of current and emerging treatments of preeclampsia; namely aspirin, metformin and mesenchymal stem/stromal cell-derived extracellular vesicles (MSC-EVs), on trophoblast function.

First trimester trophoblast cells, ACH-3P (1), were used to generate 3D organoids using a RASTRUM bioprinter (Inventia Life Science) and normal culture medium for up to 12 days. After 5 days, select wells were treated with TNF-α (10ng/ml), a cytokine increased in preeclampsia (2,3), to induce an inflammatory environment. On Day 8, aspirin (0.5mM), metformin (0.5mM) or MSC-EVs (10µg/well) were added to wells +/- TNF-α. Organoid growth and cell viability was captured using an IncuCyte. Harvested and fixed organoids were immunolabelled for trophoblast subtype-specific markers E-cadherin, human leukocyte antigen (HLA-G) and β human chorionic gonadotropin (β-hCG).

Cells encapsulated within a polyethylene glycol (PEG)-based hydrogel self-formed organoids over 12 days, demonstrating invasive capabilities within the matrix. There was no significant difference in the organoid area between treatment groups ($p>0.05$). Similarly, there was no significant difference in the viability of cells treated with each condition ($p>0.05$), confirming that the treatments were not toxic to the trophoblast cells. Villous cytotrophoblast, extravillous trophoblast and syncytiotrophoblast populations were confirmed by fluorescent confocal microscopy.

Our novel 3D trophoblast organoid model recapitulated key trophoblast subtypes of early placental tissue. Preliminary results showed no significant influence of aspirin, metformin or MSC-EVs on the viability or proliferation of trophoblasts was noted. We intend to further study their effects on trophoblast differentiation and invasion relevant to placental development.

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Meier-Gorlin syndrome and primordial dwarfism

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Primordial dwarfism is an umbrella term for a number of genetic conditions - all defined by extreme growth restriction, where both height and head size are reduced, normally in proportion. The genetics of these conditions continues to be unravelled. They are generally recessive and impact several essential functions in the cell required for growth and proliferation, including the centrosome and mitosis, DNA damage and repair, splicing, and DNA replication. My research has identified the majority of genes linked to Meier-Gorlin syndrome, a primordial dwarfism where affected individuals also have defining characteristics of microtia and patellae a/hypoplasia. The clinical and genetics of these conditions will be discussed, along with our latest research in harnessing this genetic power to study more common conditions.

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Emerging new therapies for skeletal dysplasia: changing the rules of the game

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Skeletal dysplasia are a group of heritable conditions caused by abnormalities in the development, growth, and maintenance of the human skeleton. Many of these conditions have had their molecular basis revealed over the past 20 years and, recently, several potential precision therapies have emerged for these conditions.

This talk will overview the new emerging therapies for skeletal dysplasia, highlighting Achondroplasia and Schmid metaphyseal dysplasia as examples of this new paradigm, and discuss the pioneering clinical trials that are evaluating the safety and effectiveness of these new treatments.

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Anti-Müllerian hormone (AMH)-mediated preantral follicle atresia is a key determinant of antral follicle counts in mice.

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Aims: AMH is deemed to inhibit the activation of the primordial follicle to begin follicle development. However, recent studies suggest that AMH has a stronger effect on inhibiting the progression of primary follicles to the secondary stage[1,2]. This study aimed to (1) determine if primary follicle survival was higher in *Amh*$^{-/-}$ mice and (2) examine whether AMH over-expressing mice exhibit higher rates of primary follicle atresia.

Methods: Primordial, transitioning, primary, secondary and antral follicle counts were counducted on histological ovary sections from *Amh*$^{+/+}$ and *Amh*$^{-/-}$ mice. Active Casapase-3 immunohistochemistry was conducted on wild-type and AMH-overexpressing (*Thy1.2-AMH*$^{Tg/0}$) mouse ovaries to search for evidence of increased primary follicle atresia.

Results: AMH deficiency in 100-120 day-old mice caused a lower primordial follicle number but a higher activation rate. Meanwhile, a significant increase in primary, secondary, small antral, and medium antral follicle counts was found in *Amh*$^{-/-}$ mice compared with wild-type animals, indicating differing rates of developing follicle atresia between genotypes. The caspase-3 staining revealed high rates of atresia in late primary/early secondary follicles of *Thy1.2-AMH*$^{Tg/0}$ mice.

Conclusion: Results below show that AMH mediates preantral follicle loss and is a predominant influence on small antral follicle numbers, in addition to inhibiting primordial follicle activation during folliculogenesis. This new finding suggests that the role of AMH is not to conserve the ovarian reserve to prolong fertility but instead to prevent the antral follicle pool from becoming too large.

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Iodine excess and thyroid dysfunction in women undergoing oil-soluble contrast hysterosalpingography

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Hysterosalpingography with oil-soluble contrast medium (OSCM) improves pregnancy rates[1]. However, OSCM has high iodine content and long half-life, leading to potential iodine excess[2,3]. The aim was to determine the pattern of iodine excess after OSCM hysterosalpingography and the impact on thyroid function.
For this, a prospective cohort study was conducted on 196 consecutive consenting eligible women who underwent OSCM hysterosalpingography in Auckland, NewZealand (2019-2021)[4]. Participants underwent hysterosalpingography with OSCM followed by serial monitoring of thyroid stimulating hormone (TSH), free thyroxine (FT4) and urine iodine concentration (UIC) for 24 weeks (compliance>95%). A delayed pelvic X-ray was taken 45 minutes after hysterosalpingography and OSCM retention was graded by the radiologist. The primary outcome was development of subclinical hypothyroidism (SCH), defined as a non-pregnant TSH >4 mIU/L with normal FT4 (11–22 pmol/L), in those with normal baseline thyroid function.
Iodine excess (UIC ≥300 µg/L) was almost universal (98%) among the participants, with UIC peaking usually by four weeks. There was marked iodine excess, with 90% and 17% of participants having UIC ≥1000 µg/L and >10,000 µg/L, respectively. Iodine excess was prolonged with 67% having a UIC ≥1000 µg/L for at least three months. OSCM retention grading was associated with the magnitude of iodine excess (p<0.001). SCH developed in 38%; the majority (96%) were mild (TSH 4–10 mIU/L) and most developed SCH by week 4 (75%). Three participants met the current treatment guidelines (TSH >10 mIU/L). Thyroxine treatment of mild SCH tended to improve pregnancy success [p=0.046]. Hyperthyroidism (TSH <0.3 mIU/L) occurred in 9 participants (5%). A higher baseline TSH or lower baseline iodine levels were associated with an increased risk of developing SCH.
OSCM hysterosalpingography resulted in marked and prolonged iodine excess. SCH occurred frequently with late-onset hyperthyroidism occasionally. Regular thyroid function tests are required for 6 months following this procedure.

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Colocalisation of prolactin receptors on warm-sensitive neurons in the preoptic area.

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PACAP expressing neurons are found in discrete areas within the hypothalamus of the brain and have been implicated in a wide range of physiological functions such as food intake, fertility, anxiety, and thermoregulation. During pregnancy and lactation, the maternal brain undergoes many adaptations to support optimal outcomes, such as increases in food intake, infertility, suppression of anxiety and changes in body temperature. Many of these maternal physiological changes are hypothesized to be induced by the hormonal milieu of pregnancy, particularly the increasing level of prolactin and its homologue placental lactogen. Here, we examine the colocalization of prolactin receptor (Prlr) with PACAP in the mouse hypothalamus with the aim of determining if PACAP neurons could be regulated by changing prolactin/placental lactogen during pregnancy and lactation. First, to investigate the degree of PACAP neurons that express Prlr we used a transgenic mouse line ($PACAP^{Cre}/Prlr^{lox/lox}$) in which cells that express both Prlr and PACAP produce green fluorescence protein (GFP). We observed GFP in various hypothalamic nuclei, including the preoptic area (POA), ventromedial nucleus, paraventricular nucleus and the retrochiasmatic area, indicating that these nuclei have populations of neurons that co-express Prlr and PACAP. Within the POA, where high levels of Prlr and PACAP colocalization was observed, PACAP is expressed in warm-sensitive neurons (WSNs) which are involved in the regulation of body temperature. To assess Prlr expression in WSNs during pregnancy and lactation, when core temperature is more tightly regulated, we performed RNAscope *in situ* hybridisation for Prlr and PACAP in the POA of virgin, pregnant and lactating C57BL/6 mice. Overall, our data indicates colocation of PACAP and Prlr in a variety of neuronal populations, and hence PACAP neurons may be a key target for prolactin-induced adaptations in the maternal brain.

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17 alpha estradiol signalling in metabolism is not exclusively dependant on estrogen receptor alpha expression in gaba- or glutamatergic neurons in male mice.

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17 alpha estradiol (17αE2), a non-feminising stereoisomer of 17 beta-estradiol, has been shown to prolong lifespan and improve health in a sex-specific manner in male, but not in female mice. Recent studies have demonstrated the pivotal role of estrogen receptor alpha (ERα), one the main estrogen receptors, in mediating the effects of 17αE2 on metabolic health. However, the specific tissue or neuronal signaling pathway that 17αE2 acts through remains to be elucidated. ERα expression in glutamatergic (GLUT) and GABAergic (GABA) neurons (principal excitatory and inhibitory neurons in the brain respectively) in the hypothalamus are essential for estradiol signalling. Therefore, we hypothesised that knocking out ERα

from one/both of these neuronal populations would completely attenuate the beneficial metabolic effects of 17αE2 in males. Using an established brain specific ERα KO model in VGAT and VGLUT neurons (Vgat/Vglut2-Cre^{+}Esr), KO and WT mice were placed either on a high fat diet (HFD) inducing metabolic dysfunction, or on a HFD containing 17αE2. Over 12 weeks body weight, reproductive organ weight and glucose tolerance was recorded and at the end of the experiment hypothalamic brain and liver tissues were assessed to test whether 17αE2 effects on metabolic dysfunction were inhibited in either model. Our results show that neither genotype completely blocked the effects of 17αE2 on metabolism, suggesting that other neuronal populations or tissues may be involved in 17αE2 signalling.

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Prolactin-induced switch in neurohormone secretion in maternal brain

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Transitioning into motherhood involves complex alterations in the mother's physiology to allow successful nursing of the offspring. These adaptations rely on the brain responses to hormones secreted during pregnancy and lactation. The mammalian brain is highly adaptive, and studies have shown that the source of the adaptation lies within individual brain cells, the neurons. In the rodent models, we have identified a pronounced switch in the function of a group of hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons that control release of maternal hormone, prolactin. These neurons are normally identified as dopaminergic, which is induced by prolactin to release dopamine that in turn inhibit further prolactin secretion. It is widely assumed that this identity remains fixed throughout their lifetime. We recently overturned this long-held dogma by showing that these neurons switch their neurochemical type during pregnancy and lactation, essentially becoming another type of neuron for the duration of these conditions1. This is a critical adaptation for lactation, which requires elevated prolactin. The physiological consequences of this remarkable transition are unknown, but we believe that it may functionally reverse the action of these cells, changing them from inhibiting to enhancing the secretion of prolactin. Using various transgenic rodent crosses and cell-type specific Cre-expression coupled with Cre-inducible viral vectors, we have shown that during lactation, these neurons 1) required prolactin to switch to lactation phenotype1, 2) remain responsive to prolactin2, 3) anatomically contacting each other and undergo morphological alteration that suggest increased synaptic inputs3 and 4) change in neuronal activity pattern indicative of possible release of new neurochemical content. In addition, we have also revealed the possible role of the newly synthesised neurochemical from the TIDA neurons in promoting prolactin secretion. Collectively, these findings provide an original insight into the physiological significance of the adaption performed by the TIDA neurons to sustain lactation

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Potential interplay between the immune system, steroid hormones and reproductive tissue during very early pregnancy in sheep

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Several genes linked to immune function are differentially expressed in reproductive tissues and immune cells in models of greater and lesser fertility. As a model of poor fertility, we studied peripubertal animals, where embryo loss is approximately twice that observed in adult animals. A significant proportion of this loss occurs very early in pregnancy, by day 4, therefore, we focused on the isthmus of the oviduct, comparing gene expression in peripubertal animals to adult animals. Potential differential expression for proteins involved in steroid hormone signalling, growth factors and proteins important for immune function were observed. Several genes, including growth factors and receptors as well as interleukins and their receptors, were correlated to concentrations of progesterone and oestradiol. To better understand the potential importance of the immune system during very early pregnancy, and how the local immune system may change in response to an embryo, we examined how the local and peripheral immune system differed between non-pregnant and pregnant ewes. Principal component analysis revealed separation of function of local and peripheral lymph nodes, providing evidence of specialisation of function of the immune system around the reproductive tissues to facilitate establishment of pregnancy. Analysis revealed an effect of pregnancy on gene expression in both lymph nodes and reproductive tissue. Analysis of gene categories revealed that expression of genes of the T cell receptor pathway in reproductive tract tissues was associated with pregnancy status. Thus, differential gene expression observed between pregnant and non-pregnant animals is consistent with specialisation of immune function of the reproductive tract to facilitate establishment of pregnancy. The importance of the T-cells for early pregnancy was highlighted through pathway analysis.

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Transitioning gender affirming hormone therapy (GAHT) prescribing into primary care.

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Aim

In Aotearoa New Zealand (NZ), GAHT is commonly started in secondary care settings. However, it is increasingly being initiated in primary care due to increasing demand and greater recognition of the barriers that transgender people face when accessing secondary care services. The use of routine psychological assessments prior to GAHT initiation can be experienced by patients as pathologising, and can result in feeling the need to say the 'right thing' to access treatment.[1] It can be challenging to find a mental health professional to conduct this assessment at all. An alternative approach (sometimes referred to as the "informed consent model") is one where the primary care team works in collaboration with patients, provides education about GAHT, and helps to support patients' understanding of the risks and benefits of GAHT so they can make well-informed decisions about their health. A need was identified for guidelines to assist primary care providers in this work.

Methods

Health professionals from primary and secondary care, were brought together to collaborate on the development of prescribing guidelines. Authors represent a range of professions including general practice, endocrinology, sexual health, adolescent health, psychology and peer support. Feedback was sought from members of RNZCGP and PATHA before release.

Results

The "Aotearoa New Zealand guidelines for commencing gender affirming hormone therapy (GAHT) for adults in primary care" was developed based on international guidelines, but localised to the NZ context, to support GPs to follow a staged process to initiate GAHT in primary care. Common concerns and questions were addressed, and practical tools such as checklists and patient information sheets included.

Conclusion

Supporting primary care clinicians who wish to initiate GAHT, by increasing their knowledge and confidence, and providing them with the tools to do so, has the potential to remove barriers for trans and non-binary people and contribute to improved healthcare experiences and wellbeing.

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Diabetes through the lifespan: The paediatric perspective

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Available soon

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Youth and young adults with Type 1 diabetes and very unhealthy glucose control – what does advanced technology have to offer?

Ben Wheeler[1]

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Youth and young adults with type 1 diabetes are well recognised as being at additional risk for the development of very unhealthy glucose control. Compounding this, in those struggling with diabetes, traditional management options often fail to reduce disease burden and restore glucose levels to within target ranges. However, in recent years diabetes technology has rapidly advanced. Advanced diabetes technology now offers the promise of a paradigm shift in how those struggling with the burdens of diabetes management can be treated. This talk discusses the evidence for advanced hybrid closed loop at this complex life stage, and proposes that those experiencing the most burden may often have the most to gain from closed loop therapy.

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Pathophysiology of hyperinsulinemia in obesity-infertility through hypothalamus-pituitary-ovary axis in over-eating obese mouse model

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Infertility occurs more frequently in overweight and obese women with disruption of regular menstrual cycles and reproductive hormone profiles. Although the clinical impact of obesity on female reproduction has been investigated with improvement by reducing weight, the pathological molecular mechanism of such infertility is still not clear. A simple overeating obese mouse line (MC4R KO) is characterized by hyperphagia, obesity, hyperinsulinemia, progressive insulin resistance, NAFLD, and development of infertility. We demonstrated a remarkable development of female reproductive hormone disturbance, with dysregulated rodent oestrous cycles, and significantly reduced formation of corpus luteum (CL). At the same time, hyperinsulinemia occurred before any significant difference in fasting blood glucose levels. Dapagliflozin is an SGLT2 inhibitor used clinically in the treatment of obese diabetes with the demonstrated improvement of obesity in this mouse model[2]. In this experiment, dapagliflozin treatment (1 mg/kg/day for 14 weeks from 14-week-old) of MC4R KO

female mice improved glucose tolerance, restored partially the pulsatile profiles of growth hormone (GH) and luteinizing hormone (LH), including the amount of pulsatile secretion, mean pulse mass, and pulsatile secretion, and changed approximate entropy and secretion mode. The oestrous cycle was partially but significantly normalized, and the number of CL was markedly increased in female MC4R KO mice by the dapagliflozin treatment. The expression of genes related to reproductive regulatory factors and hormones in the hypothalamic and pituitary was significantly elevated by the dapagliflozin treatment. Based on the above data, it may be concluded that dapagliflozin recovers reproductive function in an obese mouse model through recovering reproductive endocrine profiles. Dapagliflozin treatment may potentially be useful in the treatment of infertility in clinic obese patients.

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THE METABOLIC EFFECTS OF A *CREBRF* GENE VARIANT IN NEW ZEALAND WOMEN

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An Arg457Gln missense variant in the *CREBRF* gene (rs373863828) is prevalent in Polynesian (Aotearoa New Zealand Māori and Pacific) populations but rare in other ethnic groups. The A (minor) allele of this variant is associated with increased BMI, but paradoxically reduced risk of Type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM). rs373863828-A is associated with increased glucose-stimulated insulin release in response to a meal in NZ Māori and Pacific men, however, a similar effect has not been investigated in women. This presentation will discuss what we know about the rs373863828-A variant so far and presents preliminary data from our recent work on the metabolic effects of rs373863828-A in NZ women.

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IGF-1 assay – is it fit for purpose?

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Insulin-like growth factor-1 (IGF-1) plays a role in the diagnosis and treatment of growth hormone access and deficiency. As the interpretation of IGF-1 much depends on age- and gender-specific reference limits, the standard deviation score (SDS) is proposed as an indirect measure of GH status. Various IGF-1 immunoassays are used by laboratories which often provide non-comparable results due to the inherently high biological variation of IGF-1, lack of assay standardisation, as well as analytical variation and shifts in test results caused by lot changes of IGF-1 reagents, and variations in locally adapted reference limits.

To assess the clinical impact of IGF-1 test results, we designed clinical case-based vignettes and surveyed adult and paediatric endocrinologists in Australia and New Zealand to gain insight into their clinical decision making and action based on IGF-1 results. The case scenarios were designed to assess the degree of variation in IGF-1 results around age and gender-specific reference limits that would potentially affect clinical decisions. This lecture will present the outcomes of this survey and highlight clinician's opinion about the clinical use and utility of IGF-1 test results. Endocrinologists' feedback assists laboratories in deriving clinically relevant acceptance criteria for the analytical performance of IGF-1 methods and provides valuable information to laboratories and the diagnostics industry on the needs for improved analytical and clinical performance of IGF-1 assays.

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Endocrine Dynamic Testing – harmonisation across Australia and New Zealand

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The Endocrine Society of Australia (ESA), Australasian Association of Clinical Biochemists (AACB) and Royal College of Pathologists of Australasia (RCPA) formed a joint working group to address the harmonisation of dynamic endocrine test protocols (HEDT) within Australia and New Zealand which has been recently endorsed by the New Zealand Society of Endocrinology (NZSE). The final protocols are freely accessible from the society websites and adaptable for local use. The working group aims to provide evidence based, updated protocols which remove unnecessary differences, provide common cut-off and testing procedure, as well as information on assay differences.

This presentation provides an opportunity to clarify and discuss controversies surrounding endocrine dynamic tests using illustrative case studies.

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Congenital Adrenal Hyperplasia (CAH) in infants, children, adolescents and adults. What is different in diagnosis and management ?

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The presentation will discuss 21 hydroxylase deficiency, comprising 95% of all CAH.

Presentation: Infants born with severe deficiency of 21 hydroxylase are virilised. Affected females are detected by obvious virilisation but absent gonads in the early hours of life.Boys are frequently missed, due to lack of recognition of penile enlargement or hyperpigmentation of genitalia. Presentation is often late, with adrenal crisis at 10 – 14 days but can be delayed up to 6 weeks post-partum. Less severe enzyme deficiency manifests later in both sexes, either with virilization but no salt loss or with tall stature and accelerating linear growth beyond that expected for the family. The less severe the enzyme deficiency, the later in childhood diagnosis will be made. Primary or secondary amenorrhoea with childhood acne or early hirsutism should prompt consideration for non-classical CAH (NCCAH). PCOS may be the first time at which mild NCCAH is detected. The mildest forms may not be detected until adulthood when reduced male or female fertility or oligomenorrhoea may precipitate investigation leading to diagnosis.

Diagnosis: At all ages 17 hydroxyprogesterone (17OHP) is the primary diagnostic parameter but if enzyme deficiency is less and suspicion is high, a short synacthen test may be required in older children. Deranged electrolytes relate solely to Addisonian crisis . For children with CAH, at time of diagnosis bone age is extremely advanced compared to chronologic age, increasing suspicion, even if basal 17OHP hydroxyprogesterone is normal.

Reasons for diagnosis are prevention of morbidity and mortality, prevention of progressive virilisation with early overgrowth, reduction of the growth window, early epiphyseal fusion and adult short stature. Significantly raised androgens cause reduced breast growth at puberty in girls, testicular adrenal rest tissue in boys (TARTs), occcasionally seen in other organs. Management at all ages should focus on adequate suppression of 17 OHP and androgens, to prevent these changes.

Management Key at all ages requires sufficent glucocorticoid and mineralocorticoid to prevent hyperandrogenism, to regularise menstrual cycles in females and to prevent TARTs with consequent reduced fertility in males. Dexamethasone should usually be avoided due to pathologic weight gain. Pregnancy for women with CAH requires specialist advice. Dissatisfied and frustrated patients frequently default from followup in adulthood.

What is new? Recent introduction of newborn screening has reduced risk of missed early diagnosis and thus unecessary morbidity and mortality. Trials of new treatment modalities include modified glucocorticoid, mimicking normal diurnal variation. Research is ongoing into possible adrenal cell implantation and for genetic engineering.

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An approach to discordant thyroid function tests

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Most thyroid function tests (TFTs) are readily interpretable with thyroid stimulating hormone (TSH) and free T4 +/- free T3 results conforming to well recognised patterns. Occasionally, TFTs may be discordant with the clinical presentation. Biotin is a well-recognised cause of interference in some assays, where patients may be falsely attributed as having hyperthyroidism.

In addition, TFTs may be discordant with elevated free T4 +/- free T3 and non-suppressed TSH, which if not correctly interpreted may lead to inappropriate interventions and patient harm. The differential diagnosis includes biological causes such as TSH secreting pituitary adenoma and thyroid hormone resistance, where early resort to thyroid hormone receptor genotyping can often give a prompt diagnosis and avoid protracted other investigation.

In the first instance, however analytical artefacts should be excluded including heterophilic antibody interference which can be investigated by checking results on an alternative platform and using antibody blocking strategies. Albumin variants (familial dysalbuminaemic hyperthyroxinaemia [FDH]) and also pre-albumin variants can be investigated by mass spectrometry +/- genotyping. Macro-TSH is another cause of discordant TFTs, potentially confounding interpretation.

Close liaison between clinicians and the laboratory is essential for an optimal investigative approach, to avoid potentially inappropriate intervention and to ensure optimal patient outcomes.

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Exposure to an oestrogenic endocrine disruptor causes transgenerational effects on male reproductive development

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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 study aimed to determine the transgenerational effects of estrogenic EDCs on penis and testis development, and fertility in males. Gestating F0 female mice were exposed to 100ug/kg of diethylstilbestrol (DES), a clinically relevant EDC. The effects of DES were monitored in the F1-F4 male descendants. Reductions in pregnancy rate and mating index were observed up until the F4 generation in DES descendants. The anogenital distance (AGD) was also significantly reduced in DES descendants, confirming a decrease in androgen production. Hypospadias rates were significantly increased up to the F3 generation, and testis weights were significantly reduced up to the F4 generation. Sperm motility was also significantly impacted up to the F4. These results indicate a transgenerational effect of DES on multiple reproductive parameters including fertility, AGD and testis development. This study suggests that exposure of pregnant mothers to DES has significant implications for multiple generations of DES descendants and raises concerns about the effects of exposures to multiple estrogenic EDCs present in our environment.

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Both prophylactic and therapeutic vaccination protect against *Chlamydia muridarum* infection-induced impairment of spermatogenesis

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Chlamydia is the most common bacterial STI, with 131 million new infections annually, affecting both females and males equally. Many infections are asymptomatic and go undiagnosed and untreated. Our recent studies in mice (1-4) and men (5) suggest male chlamydial infection adversely impacts spermatogenesis. In mice, chlamydia is rapidly transported from the urethra to the testes in macrophages where Sertoli cells, Leydig cells and testicular macrophages are all infected. Infection causes decreased sperm motility, decreased zona-binding, global DNA hypomethylation and increased DNA damage, and adversely affects offspring development of infected sires. In infertile men we also detected *Chlamydia* infection in 45% of patients attending infertility clinics.

Using our male mouse model of *C. muridarum* infection we evaluated the effectiveness of intranasal vaccination with chlamydial major outer membrane protein, combined with ISCOMATRIX adjuvant to protect against sperm damage. Prophylactic vaccination protected against infection-induced impairment of sperm motility, morphology, oocyte-binding, and DNA damage and significantly reduced and, in some cases, cleared chlamydial burden from the testes, prostates and epididymides. Therapeutic vaccination, initiated after the establishment of infection also reduced the chlamydial burden in the testes, prostates and epididymides and provided partial protection against sperm DNA damage and abnormal sperm morphology.

Vaccine-mediated protection was not associated with classical interferon-secreting Th1 cells, which protect female mice against infection, but rather a combination of multifunctional CD4/8+ T cells in both the lower reproductive tract and testes, combined with local and systemic IgG and IgA production. These T cells included cells with a regulatory phenotype (Trem), suggesting that protective immune mechanisms must operate in concert with protection of the immune privilege in the testes to maintain successful spermatogenesis.

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Novel macrophage populations re-define the immunological environment of the mouse testis

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Resident macrophages in the rodent testis are heterogeneous in morphology, distribution and gene expression profiles. Recent studies have concentrated upon macrophages in the two main testicular compartments, the peritubular and interstitial macrophages. However, two other testicular compartments, specifically the rete testis (RT) and the subcapsular region (SC),have more direct contact with the external environment, and are implicated in initial responses to auto-immune challenges and ascending bacterial infections. Macrophage subsets were identified by immunofluorescence, using an antibody against F4/80 (all macrophages) in testis sections from adult mice expressing a GFP-transgene at the CX_3CR_1 (macrophage chemokine receptor) locus, $Cx_3cr_1^{gfp/+}$.Macrophage subsets were further defined by expression of CD206 (anti-inflammatory) and MHCII (antigen-presentation) and quantified by stereology.Macrophage volume density was 9-fold higher in the peri-epithelium and interstitium of the RT than in the rest of the parenchyma. Whereas parenchymal peritubular macrophages were $MHCII^+CD206^-$ and interstitial macrophages were $MHCII^-CD206^+$, most macrophages in the RT expressed both MHCII and CD206. Macrophages in the SC also expressed MHCII more frequently than macrophages in the parenchymal interstitium. A multiplex RNAseq analysis established that highly-purified macrophage preparations from mouse testes, comprising macrophages from all compartments, express genes encoding proteins involved in antigen-presentation (MHCII, *Cd86*, *Ciita*) and inhibition of inflammatory responses (*Il10*, *Socs1*, *Nfkbiz*, *Stat6*, *Gata3, Inpp5d, Shpk*).However, these cells displayed relatively low expression of genes involved in bacterial and viral responses, and were unresponsive to bacterial lipopolysaccharide. This study has identified a novel,large population of resident macrophages in the RT that is both antigen-presenting and anti-inflammatory,but deficient in anti-microbial responses. We hypothesize that these macrophages play the major role in inducing immune tolerance to spermatozoa emerging from the seminiferous tubules. Alterations to the protective function of these macrophages during inflammation and infection may lead to sperm autoimmunity or more severe inflammatory damage.

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A novel role for Interferon-epsilon in protecting the male reproductive tract against Zika virus infection

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Viruses, including HIV, mumps, Zika and SARS, frequently infect the testis and disrupt fertility. This has been attributed to an inability of spermatogenic cells to produce interferons (IFN) or IFN-induced proteins, required for viral resistance. Challenging this dogma, we discovered that interferon-epsilon (IFNe), a type-I IFN first identified in female reproductive epithelia, is constitutively expressed by meiotic and post-meiotic spermatogenic cells and testicular macrophages in mice and humans. We aimed to investigate the anti-viral role of IFNe in the testis, using an established mouse model of Zika virus infection and a human Sertoli cell-line (HSerc, ScienCell^{TM}). Adult wildtype mice (WT), $Ifne^{-/-}$ mice lacking IFNe, and $Ifnar1^{-/-}$ mice lacking the IFNAR1 receptor subunit required for IFN-signalling, received a single intraperitoneal injection of Zika virus (PRVABC59, $5x10^5$ pfu in saline). Controls received saline only. Reproductive organs were collected 7 days post-infection (peak illness). Infected WT mice lacked histological evidence of orchitis or epididymitis, but infected $Ifne^{-/-}$ and $Ifnar1^{-/-}$ mice displayed testicular hyperaemia, oedema, and immune cell infiltrates. The epididymis of infected $Ifne^{-/-}$ and $Ifnar1^{-/-}$ mice displayed immune cell infiltrates, epithelial damage, luminal obstruction and fibrosis. Expression of critical Leydig cell (*Cyp11a1*, *Cyp17a1*) and spermatid genes (*Tnp1*) was also reduced in infected $Ifne^{-/-}$ and $Ifnar1^{-/-}$ mice. The HSerc cell-line was infected with 5 or 10 MOI Zika virus, and treated with 100IU recombinant human IFNe either 12h before or 1h after infection. qPCR for viral RNA and plaque assays for infectious virus performed 24h post-infection showed that IFNe pre-treatment reduced the viral load by ~98%. Post-infection IFNe treatment reduced viral RNA by ~70% and infectious virus by 97%. These data indicate that IFNe plays a key role in protecting the testis against Zika virus, shifting the

existing paradigm of testicular anti-viral defences, and identifying IFNe as a potential therapeutic for testicular viral infections.

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Oxidative stress elicits a sequence of complex stress response tactics at different stages of germ cell development

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Publish consent withheld

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Erectile Dysfunction on the Rise: Is Exposure to Endocrine-Disrupting Chemicals a Risk Factor?

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Erectile dysfunction is an extremely prevalent condition globally and is estimated to have doubled since 1995. Although several risk factors including ageing, genetic mutation, and environmental factors (such as smoking) play a role, they do not fully account for this rapid increase. Thus, we must identify novel risk factors for erectile dysfunction. The corpus cavernosum (CC) is a critical vascular structure which mediates erection via relaxation of the smooth muscle, which in turn engorges the penis with blood. Given that vascular physiology and developmental patterning of the CC are exceptionally sensitive to endocrine signaling, endocrine-disrupting chemicals (EDCs) may alter these pathways to increase the risk of erectile dysfunction.

We exposed isolated adult mouse CC samples to the potent estrogenic-EDC diethylstilbestrol (DES) and the phytoestrogen genistein. In addition, male mice were subjected to systemic DES exposure via drinking water at environmentally relevant levels during sexual development. Using our optimized ex vivo wire myography protocol, we examined contractility and relaxation of treated CC samples and mesenteric arteries.

Isolated and systemic EDC exposure significantly altered CC relaxation and constriction, respectively. Systemic DES exposure also significantly altered constriction of the mesenteric arteries. These results suggest that EDC exposure is a likely risk factor for erectile dysfunction and that dietary EDC exposure impacts not only erectile function but the cardiovascular system. Indeed, erectile dysfunction is a well-established symptom of cardiovascular disease, a leading cause of death globally. Our study is one of few addressing these links, thus it is critical to continue these experiments to further elucidate the impacts of EDCs on sexual and cardiovascular health.

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Temperature dependent phosphorylation of SRSF10 as a mediator of heat stress induced infertility

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The most common presentation of male infertility are men that produce low numbers of spermatozoa, that are poor in motility and morphology (also known as oligoasthenoteratozoospermia, or OAT). From an aetiology perspective, there is evidence to suggest that testicular hyperthermia is a leading cause of OAT. However, it is unknown why the process of spermatogenesis must remain 3-4^{O}C below core body temperature.

To understand this further, the testes of mice were placed in a water bath and exposed to either control or elevated temperatures. A phospho-proteomic analysis of testicular cells that were exposed to these conditions showed changes in the phosphorylation level of Serine Rich Splicing Factor 10 (SRSF10). SRSFs are known to play a major role in the regulation of mRNA alternative splicing, particularly controlling the inclusion of exons during RNA maturation. To understand the kinetics of phosphorylation further, isolated round spermatid cells were placed at different temperatures. We found

SRSF10 was highly phosphorylated when round cells were incubated below 35^{O}C suggesting kinase involvement. However, above 35^{O}C, SRSF10 was dephosphorylated suggesting phosphatase involvement.

Using siRNA, we have begun to identify the family of thermo-sensitive kinases that directly regulate the phosphorylation of SRSF10. It appears that CLK-like kinase 2 (CDK2) phosphorylates SRSF10 in a temperature dependent manner. Work is now continuing to identify the phosphatase(s) that are responsible for the removal of phosphorylation.

The finding that SRSF10 is regulated by temperature, suggests testicular hyperthermia results in aberrant alternative splicing, leading to both a loss of meiotic cells and poor sperm quality. The data suggests that a key reason for lower testicular temperature is the regulation of alternate mRNA transcripts.

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Ovarian cancer therapeutics – where are we now and where are we headed?

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Ovarian cancer (OC) is the deadliest form of gynaecological malignancy and, in particular the epithelial histologic subtype (EOC), are often diagnosed at an advanced stage3,4. More than 90% of primary OC are EOC: high-grade serous, low-grade serous (LGSC), mucinous, endometrioid, and clear cell carcinomas5. The remainder are very rare variants, including Sex Cord Stromal tumours (granulosa cell tumours, Sertoli-Leydig tumours), which can be endocrine responsive6. The national WEHI-Stafford Fox Rare Cancer Program (SFRCP) streamlines rare cancer research, including that of rare ovarian cancer variants.

Of 609 SFRCP accruals to date, 217 have OC. We perform NGS testing to guide therapeutic outcomes: ~Whole Genome Sequencing (WGS) on fresh tumour samples and Whole Exome Sequencing (WES) on FFPE samples, depending on tumor purity (TP). H&E sections undergo anatomical pathology review to confirm diagnosis and to estimate TP.

Choice of therapeutics relevant for OC depends on an accurate OC subtype diagnosis. PARP inhibitor (PARPi) therapy has been transformative, particularly for women with EOC or carcinosarcoma with a *BRCA1/2* mutation in their germline and/or OC. We are exploring mechanisms of PARPi resistance and the use of novel DNA repair inhibitors. Other novel therapeutics in development will be discussed, including less toxic antibody drug conjugates; the concept of targeting specific molecular aberrations (eg for mucinous OC or LGSC); and our experience with stromal tumours.

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The highs and lows of clinical research in infertility

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One in fifty births in New Zealand are now the result of fertility treatments. Yet in spite of the high profile of fertility treatments such as in vitro fertilisation most of the interventions have been poorly researched. Many treatments have been introduced without evaluation by randomised controlled trials. This is particularly so in the case of add-ons for in vitro fertilisation. In response to these concerns a body of work has been undertaken to improve the reporting and conduct of clinical trials as well as highlight the gaps in the knowledge base for fertility treatments. This presentation will showcase the highs and lows of infertility research.

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Science-medicine partnerships: making a difference for infertile and cancer patients

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IVF is one of the greatest scientific-medical success stories of our times, starting as a revolutionary and controversial technique in the 1970s, to now being a mainstream and sometimes publicly-funded procedure that has produced >8 million babies. Since the early days of Nobel Laurate Bob Edwards' pioneering scientific contributions to IVF, scientists have always had a disproportionately large role in IVF, compared to other medical practices. However, this contribution is arguing diminishing with time as IVF has developed into a mature and highly corporatized medical practice. Despite this, overall, IVF has a low success rate of just 18%/cycle initiated and is completely ineffective in certain patient groups, e.g. women >43 years. Hence there is still room for significant improvement, especially given the inevitable March of Progress of science, bringing regular new insights into basic mechanisms regulating mammalian fecundity. One such area of major scientific advancement over the past decade is the molecular and cellular mechanisms regulating oocyte development, maturation and ovulation. These advances are important as oocyte quantity and quality are fundamentally rate-limiting to the success of IVF. This new knowledge has implications for the efficacy of oocyte in vitro maturation (IVM), which is a reproductive technology that makes use of oocytes from patients that have received minimal or no gonadotrophin stimulation. Whilst IVM

brings many advantages to patients, particularly those suffering from polycystic ovarian syndrome and also young cancer patients requiring urgent fertility preservation, IVM is less successful than IVF. Capitalising on the new scientific advances to improve the efficiency and clinical uptake of IVM has been challenging. My group has made a concerted effort to partner with bold and innovative clinician researchers who are prepared to conduct pre-clinical and clinical trials, grounded in solid science. This science-medicine partnership is making a difference for infertile and cancer patients.

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Transforming Fertility Treatment

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Infertility affects 10-15% of couples worldwide. Depending on the cause of infertility, treatment ranges from medications to stimulate ovulation through to more complex assisted reproductive technologies (ARTs), such as *in vitro* fertilisation (IVF). These fertility treatments have been reasonably successful; however, they all rely on decades-old medications that promote or suppress hormone activity (e.g., Clomid, Decapeptyl and Gonal-F). While the hormones of the hypothalamic-pituitary-gonadal axis are certainly critical in controlling female fertility, they are not the only factors involved. Ovarian follicle growth and development also depends upon the activity of transforming growth factor-ß (TGF-ß) proteins, including Anti-Müllerian Hormone (AMH), growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), inhibin A and inhibin B. We have modified each of these proteins with the aim to provide new opportunities to regulate female fertility.

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Polycomb-dependent epigenetic programming in the oocyte impacts neurological outcomes in the next generation

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Epigenetic programming in the developing germline can be altered by environmental influences, such as diet and drugs, and affect health in offspring. Embryonic Ectoderm Development (EED) is an essential component of Polycomb Repressive Complex 2 (PRC2), a highly conserved epigenetic regulator required in oocytes for offspring growth and development. PRC2 catalyses histone 3, lysine 27 tri-methylation (H3K27me3), thereby repressing developmental genes in multiple tissues.

To determine whether PRC2 programming inherited impacts on brain development and behaviour, we deleted *Eed* only in growing mouse oocytes and analysed outcomes in offspring. Histological and immunofluorescent analyses of neurological markers in cortex were conducted in fetal and adult offspring, and extensive behavioural testing was conducted in adulthood.

Consistent with a role for EED-dependent maternal programming, oocytes lacking EED had severely depleted H3K27me3, and an extensive range of neurodevelopmental genes were de-repressed. Post-implantation offspring from *Eed*-null oocytes demonstrated a significant neurological developmental delay and altered cortical patterning, including increased numbers of neural progenitors and disrupted neuronal patterning. Reduced brain weights were also observed at birth compared to genetically identical controls ($n=22-28$, $p<0.005$). Remarkably, altered cortical patterning was sustained in adult offspring from *Eed*-null oocytes with reduced BRN2 positive late-born neurons in the cortical plate ($n=5-7$, $p<0.001$). Extensive behavioural testing revealed that offspring from oocytes lacking EED had object recognition memory impairment and a severely blunted response to the challenge drug methamphetamine ($n=27-29$, $p<0.001$).

These data provide novel evidence that EED-dependent epigenetic programming in oocytes plays an essential role in regulating non-genetically inherited impacts on brain development, including sustained effects on cortical patterning and behaviour in adult offspring. Moreover, as this work reveals a sustained role for EED in mediating non-genetic impacts on offspring neurogenesis, determining whether therapeutic EED-inhibiting drugs may also alter oocyte programming, and affect offspring neurodevelopment, cognition and/or behaviour should also be assessed.

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PUMA blockade protects oocytes from chemotherapy-induced damage

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Irreversible ovarian damage and depletion of oocytes are devastating side effects of many cancer treatments; 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 in young girls and women receiving cancer treatment. PUMA, an apoptotic protein, triggers oocyte death following exposure to DNA-damaging insults, like chemotherapy. In fact, genetic loss of PUMA preserves fertility post-chemotherapy without compromising offspring health. Excitingly, a small molecule PUMA inhibitor (PUMAi) has recently become available, making PUMA blockade for fertility preservation a real therapeutic possibility for the first time.

To assess whether PUMA blockade can prevent oocyte apoptosis post-chemotherapy, postnatal day 5 C57BL6/J ovaries were cultured *ex vivo* for 5 days in media containing the cyclophosphamide metabolite 4-HC (2μM) ± PUMAi (200μM). Whilst 4-HC alone significantly depleted primordial follicles by 85% ($p<0.01$), primordial follicle numbers in ovaries from the 4-HC+PUMAi group were not significantly different from controls. Next, adult mice were administered 10mg/kg PUMAi 2 hours before and 20 hours after 150mg/kg cyclophosphamide. This regimen was based on a previous study in which PUMAi prevented intestinal stem cell apoptosis post-chemotherapy. Remarkably, primordial follicle numbers were approximately doubled in the cyclophosphamide + PUMAi group versus cyclophosphamide alone (858±122 vs. 386±106, $p<0.05$). This is extremely promising, as genetic knockout models of PUMA prove that partial protection of just 12% of follicles sustains female fertility.

Collectively, these data demonstrate that PUMA blockade is a promising avenue for fertility preservation prior to chemotherapy treatment. Further studies are already underway to optimise the PUMAi regimen for maximal ovarian protection; ensure that PUMAi does not impact the anti-tumour efficacy of chemotherapy treatment; and to determine whether PUMAi can prevent primordial follicle apoptosis *in vitro* in human ovarian tissue.

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Trace-level exposure of Per- and Poly-fluoroalkyl Substances (PFAS) via drinking water is associated with poor oocyte quality and embryo development in mice.

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Fertility is considered a 'sixth vital sign' because it can forewarn other health issues, including exposure to environmental toxicants. Coincident with an animal facility upgrade, our female mice exhibited profound loss of embryos during pre-implantation development. Extensive investigations uncovered that drinking water was the cause and water composition analysis was undertaken to identify the responsible toxicant(s). Trace elements were within recommended levels and phthalates undetectable; however, Per- and Poly-fluoroalkyl Substances (PFAS), a family of persistent organic pollutants and endocrine disrupting chemicals, were detected at 4.3ng/L. Compared to controls, mice exposed to the PFAS-contaminated water exhibited oocytes that were shrunken or had small polar bodies, and failed to form embryos. To investigate further, mice were given three local sources of drinking water contaminated with PFAS at 0.6ng/L, 2.8ng/L, or 4.4ng/L for 9 weeks (n=5-6/group). Mice consuming PFAS-contaminated water had decreased oocyte quality ($p<0.04$), 2-cell ($p<0.02$), and blastocyst development rates ($p<0.004$) compared to mice consuming PFAS-free (MilliQ) water. PFAS concentration was negatively correlated with oocyte viability ($r=-0.89$, $p<0.0001$). Alarmingly, these PFAS levels are representative of those in Australian drinking levels and well within current "safe level" guidelines. To directly test the effects of trace-level PFAS, a relevant mixture of PFOA, PFOS, and PFHxS at 5ng/L or 50ng/L was given to female mice for up to 6 months. Water for human consumption (drinking fountain) was included for comparison. Systemic effects were monitored and reproductive parameters analysed. PFAS exposure, even at these trace levels, was associated with extensive hair loss (n=29-47/group), altered ovarian folliculogenesis, decreased embryo mitochondrial function ($n\geq11$, $p<0.03$), decreased blastocyst cell numbers (n=13-21, $p=0.008$), reduced fetal weight and size (n=12-27 fetuses from 2-3 pregnancies, $p<0.01$), and increased neonatal mortality. This data that water deemed suitable for human consumption has detrimental effects on mammalian embryo development has important implications for water quality policies.

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Spatial real-time RNA asymmetries differentiate translation capacity of inner and outer cells in the preimplantation mouse embryo

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RNA localisation has indispensable roles for establishing asymmetries and coordinating cell fate decisions during early embryogenesis across many non-mammalian species[1-4]. To direct the spatiotemporal distribution of RNA within the cells of an embryo[5], the microtubule-cytoskeleton provides highly sophisticated trafficking pathways[6-7]. Yet, it remains unknown whether subcellular heterogeneities exist during mammalian preimplantation development and how they contribute to cell fate.

Here, using advanced live imaging we visualise global RNA transcripts at high spatiotemporal resolution from fertilisation to the blastocyst stage in the living preimplantation mouse embryo. For the first time, we discover apicobasal RNA asymmetries specific to outer cells of the 16-cell stage embryo, which coincides with cell fate decisions and the emergence of the pluripotent inner cell mass. Highly clustered RNAs accumulated proximal to the basal membrane, while more dispersed RNA foci were identified apically as the embryo reaches the late 16-cell stage. The targeted distribution of membrane-less RNA molecules is facilitated by the microtubule-cytoskeleton, associated with lysosomes which serve as RNA transport vehicles. Furthermore, real-time tracking of RNA revealed distinct RNA subpopulations located in apical and basal regions of outer 16-cell stage blastomeres. Apically located RNA foci were more dynamic and accompanied an enrichment of translation components. Intriguingly, our discoveries of spatiotemporal RNA heterogeneities determining differential translation capacity are unevenly inherited by outer and inner daughter cells during subsequent cell divisions.

Here we provide novel insights into a subcellular mechanism driving asymmetric RNA localisation and compartmentalised translational regulation in outer cells of the 16-cell stage embryo, which may contribute to cell fate decisions during mammalian embryogenesis by serving as a fine-tuned mechanism for the control of gene expression. We envision that deciphering the spatiotemporal processes underpinning early embryogenesis may facilitate the development of techniques to target subcellular structures with precision for applications in regenerative and assisted reproductive medicine.

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Regulatory T cells modulate uterine natural killer cell activation to promote spiral artery remodelling in mice

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Early-onset preeclampsia is preceded by impaired remodelling of the decidual spiral arteries during early placentation and results in fetal growth restriction in 20-25% of cases. A specialised subset of anti-inflammatory T cells called regulatory T (Treg) cells are deficient in many women with preeclampsia. Using Foxp3-DTR mice that enable transient Treg depletion, we have shown that Treg cells are required for robust spiral artery remodelling. Uterine natural killer (uNK) cells are key drivers of spiral artery remodelling, but whether Treg cells interact with uNK cells is unknown. Here, we hypothesised that depletion of Treg cells would reduce anti-inflammatory cytokines within decidual tissue and uNK cell numbers or function would be altered. Foxp3-DTR mice were mated and given diphtheria toxin (DT) on gestational day (gd) 3.5 and 5.5 to deplete Treg cells, with vehicle-treated mice as controls. On gd 10.5, decidua were collected for cytokine gene expression analysis by qPCR and immuno-staining with Dolichos Biflorus Agglutinin (DBA) lectin to detect uNK cells. Whole uterus was analysed by flow cytometry to quantify three distinct subsets of uNK cells – tissue-resident (trNKs), conventional (cNKs), and group 1 innate lymphoid cells (ILC1s) – each of which have unique roles in angiogenesis and tissue remodelling. qPCR showed that Treg cell depletion decreased *Il10* by 47% ($P<0.05$), *Tgfb* by 60% ($P<0.001$), and *Il35* by 58% ($P<0.01$). DBA staining showed Treg cell depletion led to a 20% reduction in total uNK cells ($P<0.001$). Flow cytometry showed Treg cell depletion reduced all three uNK cell subsets, with trNKs($NK1.1^+Nkp46^+EOMES^+CD49a^+$) reduced by 62%, cNKs($NK1.1^+Nkp46^+EOMES^+CD49a^-$) by 43%, and ILC1s($NK1.1^+Nkp46^+EOMES^-CD49a^+$) by 57% (all $P<0.05$). Together, these data show that Treg cell deficiency alters decidual cytokines and impairs uNK maturation, constraining uNK cell ability to facilitate spiral artery remodelling. It is therefore important to investigate Treg-uNK cell interactions in women with preeclampsia.

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Taking the EasyPhos route: a phosphoproteomic approach to investigating capacitation driven protein phosphorylation in human spermatozoa

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Publish consent withheld

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Prolactin modulation of thermoregulatory circuits provides resilience to thermal challenge of pregnancy and lactation

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Pregnancy and lactation represent significant challenges to thermal homeostasis due to the progressive generation of metabolic heat from fetal growth and development and then milk production. Since increases in body temperature can be teratogenic and hence detrimental to fetal development, this rising body temperature must be offset by counter-regulatory mechanisms in the mother to prevent hyperthermia. Active adaptation of maternal thermoregulation is most apparent by the decrease in body temperature observed in the last trimester of pregnancy in many species including humans, cows, and rodents, despite peak metabolic heat production from fetal development at this time. Our aim was to investigate the role of the pregnancy hormone prolactin in driving these maternal adaptations in body temperature regulation. First, using chemogenetic and immunohistochemical approaches we demonstrate that within the preoptic area of the hypothalamus (POA) warm-sensitive neurons (WSN), which detect elevations in body temperature and engage downstream effector pathways to lower body temperature, express prolactin receptors (Prlr). While acute prolactin administration did not influence body temperature in virgin male or female mice, the same treatment in lactating mice significantly lowered body temperature, suggesting in certain conditions prolactin action can influence thermoregulation. Deletion of Prlr from the POA lead to significantly elevated body temperature during pregnancy, but not in the pre-pregnancy state, suggesting that prolactin action on WSNs is important for maintaining appropriate body temperature specifically during pregnancy. Finally, while control mice could cope with pregnancy under a mild thermal challenge (30°C), mice lacking Prlr in glutamatergic neurons, which includes many WSNs, had poor pregnancy indicating that prolactin action in glutamatergic neurons provides resilience to thermal challenges during pregnancy. This work highlights a key role for prolactin to regulate the thermoregulatory circuits and provide optimal conditions for successful pregnancies.

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Prolactin as a key regulator of maternal care-giving behaviour in mothers

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Mammals give birth to dependent offspring which requires significant investment by a mother to care for and protect new-born young. To facilitate the display of essential maternal behaviours, elevated hormones during pregnancy act on a maternal neural network to drive behavioural change. Our work has focused on the pituitary hormone, prolactin and the placental analogue, placental lactogen. Previously we have shown that these hormones acting through the prolactin receptor (Prlr), have a critical role in the medial preoptic area of the hypothalamus (MPOA) to promote the onset of maternal nursing behaviour. Subsequently, we identified that in addition to the MPOA, many brain regions that regulate different aspects of maternal behaviour, also express the Prlr or receive prolactin-sensitive projections. Recently, we have found that prolactin has roles in modulating maternal protective behaviour and in ensuring interactions with offspring are rewarding for a mother. Rather than directing one aspect of maternal behaviour, prolactin appears to act throughout the maternal neural network to regulate a broad range of behaviours to ensure optimal care of offspring.

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Do hormonal contraceptives and cold mimetics modulate energy expenditure and metabolic function in young adults?

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Brown adipose tissue (BAT) is known to contribute to daily energy expenditure via the process of adaptive thermogenesis. The primary physiological regulator of BAT is exposure to ambient cold. In addition to its effects on energy expenditure, activation of brown adipose tissue has been associated with increased glucose and triglyceride clearance as well as improved insulin sensitivity. Despite this, few studies have addressed the mechanisms by which BAT activity is regulated in humans. Numerous animal studies have demonstrated that estrogen acts within the brain to regulate both reproductive and metabolic functions, in particular estrogen increases thermogenesis via activation of the sympathetic nervous system. Furthermore, in young healthy participants we have previously demonstrated that the degree of BAT activation in response to physiological stimuli is correlated with plasma concentrations of 17β-estradiol. However, it remains unknown as to whether exogenous ovarian steroids in the form of hormonal contraceptives (combined oral contraceptives and progestin only based contraceptives) can influence BAT activity and metabolic function. It also remains unknown as to whether menthol, a cold mimetic, can be used to increase BAT activity. We have used infrared thermography to measure changes in supraclavicular temperature (index of BAT activity). This presentation will describe the effects of hormonal contraceptives in women as well as sub-chronic menthol treatment in men on BAT heat production and glucose metabolism.

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Diabetes and Cognitive Function

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Diabetes mellitus (one of the most common endocrine disorders) and dementia are two of the most prevalent conditions affecting ageing individuals. The fact that type 2 diabetes is a risk factor for dementia became evident around 15 years ago, and now we also know that type 1 diabetes is associated with brain dysfunction. Questions still remain whether diabetes is a causal risk factor for dementia, or is merely a coexisting condition. If the former were true, it raises the interesting hypothesis that treating diabetes, its features, or its complications may reduce the risk of future dementia. In my presentation, I will present data from previous and ongoing observational studies in people at different stages of ageing, to provide insights into the complex relationship between diabetes and dementia and contextualise their findings to other work in the field.

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Doctors Won't Treat Patients for Conditions They Don't Have – Why the Definition of Osteoporosis Needs to Change

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For 30 years osteoporosis has been defined as a bone mineral density more than 2.5 standard deviations below the mean value in the young normal population. However, multiple studies have shown that 80% of older adults suffering fractures do not meet this definition. As a result, treatment is often denied those who at high fracture risk and greatly in need of intervention. We need to move away from a rigid diagnostic definition based on only a single risk factor, to a broader set of thresholds which allow for cost-effective fracture prevention strategies. Without such a change in approach, the ageing of our community will result in ever increasing numbers of fractures, with the morbidity and mortality resulting from that.

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Cardiovascular risk markers in adults with adrenal adenoma and possible autonomous cortisol secretion

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Adrenal adenomas are identified in 5% of abdominal computer tomography. While surgical treatment of overt hypercortisolaemia is recommended, many adenomas have possible autonomous cortisol secretion (PACS), defined as

morning cortisol 50–138 nmol/L after 1 mg dexamethasone suppression test (DST). While PACS is associated with increased cardiovascular mortality, it is not clear whether cortisol secretion underlies this association. We investigated mechanisms that may link PACS and cardiovascular mortality in adults with adrenal adenoma.

In this cross-sectional study, we characterised 18 adults with adrenal adenoma and PACS (64±11 years, 6 males) and 18 controls with non-functioning adrenal adenoma (66±10 years, 8 males). Reactive hyperaemia index (RHI) was measured by peripheral artery tonometry to quantify endothelial function and 24-hour ambulatory blood pressure (24h AMBP) monitoring was performed. Indices of insulin secretion and sensitivity were estimated by measuring glucose and insulin fasting and 30-minutely for two hours following a mixed meal (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat). Whole body composition was assessed by dual-energy x-ray absorptiometry.

Participants with PACS had significantly higher cortisol after 1 mg DST and 24-hour urinary free cortisol (24h UFC) and lower dehydroepiandrosterone sulphate than controls. Fasting glucose and glucose area under the curve after the mixed meal were higher and insulin secretion index trended lower in participants with PACS. There were no significant differences in Matusda Index, RHI, 24h AMBP or total or central fat mass between the groups (Table). Cortisol after 1 mg DST was weakly, but positively, associated with fasting glucose (r^2=0.12, p=0.04).

Adults with adrenal adenoma and PACS demonstrated fasting and postprandial hyperglycaemia with a trend towards lower insulin secretion, but no difference in insulin sensitivity, endothelial function, hypertension or fat mass. Hyperglycaemia secondary to impaired insulin secretion not sensitivity may underlie the association between PACS and increased cardiovascular mortality.

Table

	PACS	NFA	p-value
Number (n)	18	18	
Age (years)	64 ± 11	66 ± 10	0.64
Sex (Males, n, %)	6 (33%)	8 (44%)	0.73
Cortisol after 1 mg DST (nmol/L)	73 ± 23	27 ± 10	< 0.0001
Dehydroepiandrosterone sulphate (umol/L)	1.4 ± 1.4	4.5 ± 3.5	0.002
24h Urinary free cortisol (nmol/24h)	109 ± 71	63 ± 25	0.03
Fasting glucose (mmol/L)	6.3 ± 1.6	5.4 ± 1.1	0.05
Glucose AUC (mmol/ml*min)	1064 ± 117	885 ± 79	<0.001
Matsuda Index	4.2 ± 3.6	4.0 ± 2.1	0.47
Insulin secretion index	20.1 ± 9.5	40.2 ± 41.2	0.09
RHI	2.1 ± 0.7	2.0 ± 0.5	0.75
Systolic 24-hour AMBP (mmHg)	129 ± 18	126 ± 11	0.53
Diastolic 24-hour AMBP (mmHg)	81 ± 14	78 ± 9	0.46
Total body fat mass (kg)	41 ± 18	39 ± 10	0.69
Central fat mass (kg)	3.5 ± 2.0	3.5 ± 1.3	0.95

PACS: Possible autonomous cortisol secretion, NFA: Non-functioning adenoma

Impaired bone formation and reduced strength in young adults with inflammatory bowel disease (IBD): pieces of the fracture-risk puzzle

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IBD affects >100,000 Australians, commonly affecting adults aged <30 years. Fracture risk is increased and adds to significant morbidity in these young adults with IBD (IBD-YAs). Assessment of areal bone mineral density (aBMD) by dual energy x-ray absorptiometry (DXA) does not accurately identify most individuals at risk of fracture. Our aim is to identify clinically useful imaging techniques to facilitate fracture risk-stratification in IBD-YAs. Given their young age, we

hypothesised that IBD-YAs have features of impaired bone formation and reduced strength, identifiable through high resolution peripheral quantitative tomography (HRpQCT).

A cross-sectional study of 29 IBD-YAs requiring biologic therapy, and 26 YAs without IBD (hYAs), was conducted. Participants underwent HRpQCT imaging (n=37) and biochemical testing (n=50). aBMD and hip structural analysis (HSA) data were measured by DXA (n=55).

Mean (± SD) age was 30.9±11.7 years in IBD-YAs and 33.6±5.9 years in hYAs. Median disease duration was seven years (range <1–37 years). Vitamin D deficiency was identified in six (24%) hYAs and 10 (40%) IBD-YAs; none had severe vitamin D deficiency (<25 nmol/L).

HRpQCT at the tibia showed reduced trabecular thickness (0.246±0.021; hYAs 0.275±0.025, p=0.001) with less inhomogeneity (0.256±0.043 vs 0.297±0.058, p=0.038). Strength was reduced with lower stiffness (203837±516230 vs 253879±60744, p=0.023) and failure load (-11127.7±2640.7 vs. -13567.3±3013.5 p=0.030) in IBD-YAs compared to hYAs. IBD-YAs had lower femoral neck aBMD (0.907±0.355g/cm^2 vs. 1.052±0.127g/cm^2, p=0.02). HSA identified reduced cross-sectional area (154.1±31.1 vs. 171.0±35.3, p=0.037) and shaft cortical width (4.517±1.401 vs. 5.437±1.785, p=0.027).

Consistent with reduced strength and both impaired bone formation and increased bone loss, IBD-YAs have lower failure load, reduced bone size and cortical width as well as reduced aBMD and trabecular changes, all indices previously associated with increased fracture risk.[1,2] Longitudinal studies of larger IBD cohorts are required to confirm these findings and identify the variable with most clinical utility.

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Testosterone therapy on gender dysphoria, depression, and suicidality in trans and gender diverse individuals seeking masculinisation: a randomised controlled trial

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Publish consent withheld

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Prospective use of an online clinical support tool to validate a predictive risk model in differentiated thyroid cancer

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Background: We developed a predictive risk model which improved the ATA modified initial risk stratification by including stimulated thyroglobulin, histological assessment of extrathyroidal extension, and tumour size. The model was validated on a retrospective dataset[1]. The aim of our study was to prospectively validate this model as a decision support tool to guide the use of radioactive iodine(RAI).

Methods: An online clinical support tool was developed to allow for easy application of our risk model. After input of variables, the tool produced a four-tiered outcome: very low, low, intermediate, and high risk. The model was extended to provide RAI recommendations; no RAI for very low risk disease, 1GBq for low risk, 4GBq for intermediate risk and 6GBq for high risk. Clinicians were surveyed whether they agreed with the treatment recommendation. Patients over ≥18 y with new diagnosis of DTC treated between August 2021-2022 were included.

Results: 132 patients were prospectively included. Median age of patients was 50y(IQR 39-66), and 38% were male(50/132). Majority of patients had papillary thyroid cancer 85/132(64%), followed by follicular thyroid cancer 18/132(15%) and follicular variant 16/132(12%). Remaining patients were Hurthle cell(9/132, 7%), diffuse sclerosing variant (2/132, 1%) and tall cell (2/132, 1%). Using the decision support tool, 19/132(14%) were assessed as high risk, 82/132 (62%) intermediate risk, 26/132(20%) low risk and 5/132(4%) very low risk. 90 clinicians completed the survey and most agreed with the dosing recommendation; 86% of high risk(12/14), 97% of intermediate risk(57/59) and 100% in low(15/15) and very low risk(2/2).

Conclusion: Our risk model can be readily translated into clinical practice via our online clinical support tool. Our tool had high utilisation and demonstrated its capacity to guide risk stratification and RAI activity. Longer term analysis will be required to determine the impact of our risk model and therapy recommendation on cancer outcomes.

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Preconception weight intervention improves fertility and metabolic outcomes in mothers and offspring in a mouse model of maternal obesity

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Women with obesity are advised to lose weight before pregnancy, but evidence is lacking about the effectiveness of weight loss strategies to aid improved perinatal outcomes. Strategies are urgently needed to mitigate the increased risk of long-term effects of maternal obesity on the offspring, including diabetes, obesity, cardiorenal disease and metabolic associated fatty liver disease. This prestigious ESA Ken Wynne Postdoctoral Research Award has facilitated my work investigating the impact of preconception weight loss, with either the GLP-1 receptor agonist liragluitde, or dietary intervention, versus dietary intervention in pregnancy, in a mouse model of maternal obesity.

Methods: Maternal obesity was modelled in C57BL/6 mice; with dams fed a high fat diet (HFD) versus chow diet for 8 weeks and compared to lean chow-fed controls. In obese dams, liraglutide or diet modification (switch to chow) was utilised to induce pre-conception weight loss. Fertility rates were observed after mating. A further group of pregnant dams were switched from HFD to chow diet in early pregnancy. Maternal anthropometric measures, glucose tolerance and metabolic markers were measured at late gestation. Pregnant dams were either allowed to deliver their offspring or sacrificed at gestational Day 18-20 and maternal blood, kidney and placenta were collected. The offspring were weaned onto a HFD and their anthropometric measures, glucose tolerance, metabolic markers, liver and kidney examined at postnatal week 12.

Results: Pre-pregnancy liraglutide was the most effect pre-pregnancy strategy for improving maternal metabolic health, alongside fertility and fecundity. Pre- or post-conception diet intervention was effective at reducing adverse maternal metabolic outcomes in late pregnancy. The offspring of obese mothers were protected from the adverse foetal programming effects of maternal obesity, with improved metabolic and liver health in male offspring observed.

Conclusion: This research strongly supports pre-pregnancy intervention as an effective strategy to improve maternal fertility, and reduce adverse metabolic health in the mothers and the offspring. The challenge of weight management is to sustain long-term weight loss, and pre-pregnancy pharmacotherapy together with dietary intervention maintained in pregnancy, may be a useful strategy for some women with obesity desiring pregnancy.

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The discovery of a novel contraceptive method for women

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The unintended pregnancy rate worldwide is approximately 48% of all pregnancies. This high unintended pregnancy has caused burdens not only to the mother and the child but also the overall socioeconomic infrastructure. One of the causes of unintended pregnancy is the high failure rates (13-29%) of over-the-counter methods. As such, the novel contraceptives that are easily assessable and effective methods, such as non-hormonal contraceptives, could mitigate this unintended pregnancy issue. Our laboratory showed that inhibition of semen liquefaction in the female reproductive tract significantly prevented sperm migration in mice. Semen liquefaction is a biochemical process governed by prostate-specific antigen (PSA; a serine protease enzyme). PSA cleaves gel-forming proteins (semenogelins; SEMGs) and changes the semen from gel-like to watery viscosity. Highly viscous semen and abnormal liquefaction reduce sperm motility and contribute to infertility. However, specific inhibition of PSA activity has not been evaluated as potential contraceptive method for blocking semen liquefaction. To determine whether specific inhibition of PSA activity can be developed as a novel contraceptive target, fresh ejaculates were treated with PSA antibody for 30 min for viscosity assay and 4 hours for sperm motility assay. We found that PSA antibody 1) effectively inhibited liquefaction by preventing degradation of SEMGs resulting in a higher semen viscosity compared to control and 2) significantly decreased sperm motility. Moreover, we found that there are small molecule PSA-specific inhibitors that could act similarly to prevent semen liquefaction in humans. In conclusion, inhibition of PSA activity using a neutralizing antibody can be developed for potential usage as novel, non-hormonal contraceptives for both men and women.

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An altered perspective and an opportunity to change the future.

Mani Mitchell[1]

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In March this year I had an accident and sustained serious physical injury, my slow, complications riddled recovery has provided me with two things, lots of time on my back away from the day to day demands of the office, a life rare opportunity to be slow, to reflecting/analyze. A new, up close and personal and detailed highly varied - interaction with our health system.

It has both changed and sharpened my perspective.

This year when the New Zealand Government published its budget, there was for the first time in our history funding, 2.5 million for 'intersex work', by the NZ Ministry of Health.

- **$2.516 million** over four years to support health practitioners to provide best practice health care to intersex children and young people, and to empower intersex children and young people and their whānau to make informed decisions about medical interventions, which will better protect the rights of intersex children and young people and prevent unnecessary medical interventions from occurring

This budget initiative is the culmination of over two decades of collaborative work. Informed by a decade of consultation with the New Zealand Human Rights Commission and associated 'Round Tables', a joint presentation to the United Nations Committee on the Rights of the Child and the conclusions of the CRG (Clinical Reference Group) - A multi sectoral National Clinical Network established through the Pediatric Society of New Zealand and funded by the Ministry of Health established in 2017.

A unitary Progressive Labour Government that had made a commitment to improving intersex (VSC) variations sex characteristics health outcomes prior to the last election and Associate Health Minister, the Hon Dr Ayesha Verrall, who has a background and experience in medicine as a specialist and was instrumental in informing, developing this policy initiative.

This initiative has created a real and unique opportunity to move beyond historic conflicts and impasse, to focus on a shared common goals.

To work collaboratively and draw on best practice research, reports and emergent standards including (Prism NZ HRC report, 2020, Denise Steers PhD 2020, the peer support program developed by Bonnie Hart in Australia, and recently published 2022 WPATH, guidelines)

Develop a model that knows and values the importance of diversity, is aware of and addresses the issue of culture, violence, stigma, shame and discrimination, values and encourages effective communication, education and holistic integrated health care.

A model that takes the lessons, wisdom, learnings from the past and makes the future **better and safer** for everyone.

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Human rights considerations in the Australian context

Ghassan Kassisieh[1]

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In the context of recent legal intersex reform proposals in the Australian states and territories, this presentation explores how human rights considerations, particularly those concerning the bodily integrity of intersex people, can inform legal reform proposals and why the call for transparency and better decision-making processes can benefit everyone involved in intersex healthcare, including intersex people, their families and health professionals.

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Clinical decisions for and with children and their families

Erin Sharwood[1]

1. Queensland Paediatric Endocrinology, QLD

coming soon

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Changes in European practice around treatment decision-making, including regulation of medical care

Martine Cools[1]

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In the last decade, several aspects of care for individuals who have differences of sex development have become highly controversial. In 2015, the Council of Europe (CoE) has published an "issue paper on Human Rights and Intersex People",

resulting in a resolution in 2019, urging all member states to secure the protection of human rights of intersex people. In Spring 2022, an international conference was held in preparation of an update on the topic.

Several European countries have responded differently to the CoE's resolution, with legislation already in place in some countries, and legislative initiatives undertaken in others.

In this session, we will review the current legal situation in specific European countries, and try to understand some of the existing tension. We will present the results of a recent international collaboration among stakeholders from various backgrounds that aimed to promote discussion, achieve base-level consensus, and – where possible – provide provisional recommendations that may guide medical and psychosocial care while awaiting a broader consensus, with the hope of ultimately improving the care and well-being of all children and adults who have a DSD.

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Aggressive Pituitary Tumours - identification and management

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An important debate is simmering within the pituitary field – a proposed name change from pituitary adenoma to pituitary neuroendocrine tumour (PitNET). The primary controversy relates to use of the term "tumour" and concern about invoking undue anxiety within patients given that the majority of "tumours" are slow-growing and do not metastasise. Pituitary carcinomas (PC) are exceedingly rare, accounting for 0.2% of all pituitary tumours. However, 5-10% do behave in an aggressive manner, causing significant morbidity and mortality rates similar to PC, such that terms like "tumours with malignant potential" or even "carcinoma in situ" have been proposed for aggressive pituitary tumours (APT). The diagnosis of APT is a clinical one and defined in the 2018 European Society of Endocrinology Clinical Practice Guidelines as an invasive tumour with an unusually rapid tumour growth rate or clinically relevant growth despite optimal standard therapies[1]. This talk will outline important clinical, radiological and pathological features of APT and PC. Over the last decade significant advances in the management of these tumours has emerged. Temozolomide remains the first-line chemotherapy with second line therapy options including immune checkpoint inhibitors, anti-VEGF and other targeted therapies as well as peptide receptor radionuclide therapy. Timing of radiotherapy with oncological therapies is increasingly important. Many challenges remain such as patient selection, duration of therapy and predicting response to therapeutic options. Where available, tumour molecular testing can help guide management. All patients with APT and PC should be managed within an experienced pituitary multidisciplinary team.

[1] Raverot G, Burman P, **McCormack A,** Heaney A, Petersenn S, Popovic V et al. *European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas.* Eur J Endocrinol. 2018;178(3):265-76.

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Evolving diagnostic evaluation and classification of pituitary tumours

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Pituitary tumours comprise a pathologically diverse group of neoplasms and exhibit a wide spectrum of clinical behaviour. Although generally benign, a subset demonstrates clinically "aggressive behaviour" (10-15%) and more rarely progress to develop distant metastatic disease (0.1-0.2%). Pathological classification has evolved over the past few decades, continuing to approximate improving understanding of tumour biology. The 2017 WHO heralded a paradigm shift, with tumours now classified according to cellular lineage, ascertained by transcription factor and hormonal immunohistochemistry. Prognostic value of Ki67 and mitotic count were retained, along with recognition of new "higher risk" histological types. However, the individual and additive prognostic value of these factors remained to be determined. The recent WHO 2022 classification, which controversially has rebranded pituitary adenomas as pituitary neuroendocrine tumours (PitNETs), incorporates further refinement of lineage-based classification, notably with identification of a new "plurihormonal" type, characterised by expression of multiple lineage transcription factors within a monomorphous population of cells. Streamlined processes are required for diagnostic reproducibility and economic feasibility, in order for recent research advances to be translated into clinically meaningful impacts.

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Prolactin-induced suppression of gonadotrophin secretion during lactation

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In mammals, lactation is associated with a period of infertility, in order for a mother's metabolic resources to be directed towards caring for her newborn, rather than supporting another pregnancy. This lactational infertility is characterized by the suppression of kisspeptin neurons leading to a reduction in gonadotrophin secretion and a consequent cessation of ovulation. Lactation is accompanied by chronically elevated levels of the anterior pituitary hormone prolactin. Despite elevated prolactin, *per se*, being a well-recognized cause of infertility, the specific role that prolactin plays in lactational infertility, as distinct from other suckling or metabolic cues, is unclear. The aim of the present study was to determine whether prolactin action specifically on arcuate kisspeptin neurons suppresses gonadotroph secretion during lactation in mice. To investigate this we conditionally deleted the prolactin receptor (Prlr) from arcuate kisspeptin neurons ($Prlr^{lox/lox}$/$Kiss1^{Cre}$) and examined estrous cyclicity, luteinizing hormone (LH) secretion and arcuate kisspeptin neuronal activity. Neuronal activity was monitored in real time using kisspeptin neuron-specific GCaMP6 fiber photometry. As reported previously[1], in the diestrous virgin state, periodic events of elevated intracellular calcium (indicative of synchronous activity of the arcuate kisspeptin population) precede the release of LH, occurring approximately once an hour. As expected, control animals showed lactation-induced infertility, and only resumed normal estrous cyclicity following weaning (>20 days of lactation). These animals show a corresponding complete suppression of synchronised Ca^{2+} events from kisspeptin neurons till day 14 of lactation. In contrast, $Prlr^{lox/lox}$/$Kiss1^{Cre}$ mice showed early resumption of estrous cyclicity within 4-17 days of lactation ($p<0.0001$) which was preceded by early reactivation of the arcuate kisspeptin population. These observations show dynamic variation in activity of arcuate kisspeptin neurons associated with the hormonal changes of lactation, and provide evidence that prolactin action on those neurons is necessary for the suppression of gonadotrophin secretion during lactation.

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Pituitary imaging for endocrinologists - diagnosis and follow-up

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Over 20% of people have a pituitary lesion on a magnetic resonance imaging (MRI) study. Most commonly these are small pituitary neuroendocrine tumours (PitNETs) or Rathke's cleft cysts. The prevalence of a clinically significant PitNET is 1 in 1000 people. Clinical decision making is optimised when undertaken in the setting of a multi-disciplinary team (MDT). Radiology is an integral component of an MDT, and an experienced neuroradiologist is essential for both diagnosis and follow-up of these lesions. However, the endocrinologist should ensure they are familiar with the neuroradiology techniques and develop their own expertise in the interpretation of the images, particular as some general radiology practices lack a dedicated neuroradiology specialist to report them. MRI is the mainstay of pituitary neuroimaging and was first used clinically in the 1980s. A standard set of sequences for a pituitary MRI scan include T1 non-contrast coronal and sagittal, T2 coronal, T1 post-contrast coronal and sagittal plus dynamic sequences when looking for functional microadenomas. The slice thickness should be ≤2mm. Features to be noted include: size/volume, suprasellar extension and relationship to the optic apparatus, tumour invasion, internal tumour changes and any characteristics which might distinguish a PitNET from other sellar/parasellar masses. Newer sequences such as the contrast-enhanced 3D-T2-weighted SPACE sequence show promise to improve detection of small corticotroph PitNETs. Functional positron emission tomography (PET) scans using tracers such as ^{11}C-methionine and ^{68}Ga-DOTA-CRH have improved the detection of secretory microadenomas. MRI is preferred for radiological follow-up of sellar and parasellar masses. There are no Endocrine Society guidelines on the frequency of post-operative radiological follow-up, though some other national societies have formulated these. Volumetric growth rate across the first 3 years has been shown to predict need for re-intervention for non-functioning PitNETs. Macroadenomas probably need lifelong follow-up, while stable microadenomas can be safely discharged.

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Immunotherapy: Where to from Here

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Available Soon

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Checkpoint inhibitor autoimmune diabetes

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Checkpoint inhibitor associated autoimmune diabetes (CIADM) is a new subtype of type 1 diabetes (T1DM) that arises as a rare complication of immune checkpoint inhibitor (ICI) therapy. Data regarding this condition to date is limited to case reports and small case series.

We systematically review published evidence to identify presentation characteristics and risk factors for CIADM.

From the literature, 220 patients were identified. Mean age was 63.7years (±12.4), mean BMI was 24.7±6.6kg/m2 and 60% of patients were male. Pre-existing type 2 diabetes was present in 8.6%, with 4.5% requiring oral agents. All but 1 patient (99.5%) had prior exposure to either anti-PD1 or anti-PDL1 therapy. One patient had only anti-CTLA4 exposure. Median time from ICI to CIADM onset was 12 weeks (IQR 6-24). At presentation DKA was present in 69.2% and C-peptide was 0.13±0.2nmol/L. Other immune adverse events occurred in 45.4%, with thyroiditis in 18.1%.

T1DM autoantibodies were present in 40.7% (73/179), with anti-GAD being the commonest in 37.4%. T1DM autoantibody positivity was associated with DKA (p=0.003) and faster time to CIADM onset (p=0.002). Of the 88 patients with HLA haplotyping performed, 63.6% had susceptibility haplotypes for T1DM, and 9.0% patients had protective haplotypes.

CIADM commonly presents in DKA and T1DM autoantibody positivity is associated with earlier and more severe presentations.

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Beta cell replacement for type 1 diabetes

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Type 1 diabetes (T1D) destroys the pancreatic beta cells and the body’s capacity to produce insulin. Insulin replacement has been the main treatment for 100 years but it is not a cure for diabetes and it does not address the underlying mechanisms of disease.

Islet transplantation has been a successful treatment for over 20 years in a limited number of patients. The main indication for islet transplants is for T1D complicated by hypoglycaemic unawareness with frequent severe hypoglycaemia events. Two reports – one a prospective multicentre trial in the US and the other a large series of 255 islet transplants from Canada - have shown that islet transplantation can be carried out safely in major centres and treats hypoglycemia very effectively even when insulin independence is not achieved. Patient reported outcome measures reflect the success of the procedure. However, shortage of suitable donor organs and the need for immunosuppression to prevent allo-rejection and/or recurrence of autoimmunity limit its broad application. Islet autotransplantation is not subject to these limitations and is useful when pancreatectomy is being carried out in chronic pancreatitis.

New approaches to beta cell replacement will be built on the foundations laid by islet transplantation and will include much less limited cell supply and a variety of approaches to overcoming and evading immune attack.

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CAR-T cell therapies for endocrine-related cancers

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Immunotherapy with genetically engineered chimeric-antigen receptor (CAR) T cells is a profound advance in cancer therapy. CAR T cells exemplify a new generation of therapies that function through immune activation and have revolutionised outcomes for haematological malignancies. However, CAR T cells are less effective against solid cancers, as the immunosuppressive tumour microenvironment (TME) precludes T cell infiltration, activation and cytotoxicity following antigen recognition. It is becoming increasingly evident that CAR T cells are ineffective as a single therapy for solid tumours, and that combining CAR T cells with agents to pre-condition the TME are essential. These barriers must be overcome to confidently proceed to clinical trials for patients with endocrine-related cancers, such as prostate cancer.

Recent studies have revealed that low-dose chemotherapy can alter the TME to maximise CAR T cell trafficking and efficacy in preclinical models of prostate cancer. Mechanistically, carboplatin remodels the TME to overcome immune-suppression, resulting in early and persistent infiltration of activated CAR T cells into the tumour. Chemotherapy pre-conditioning induces a cascade of changes to the TME, including the induction of an inflammatory cancer-associated fibroblast phenotype, enhanced extracellular matrix degradation and re-orienting M1 macrophage differentiation, thereby enhancing T cell trafficking and retention in the tumour mass. Collectively, these data suggest that chemotherapy pre-conditioning may increase the efficacy of CAR T cell therapy for solid tumours, and could achieve durable tumour regression in men with prostate cancer who have exhausted all treatment options.

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Effective treatments for men with male-factor infertility.

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Male infertility is a medical condition affecting one in 20 men in the western world[1,2] and accounts solely, or in a contributory way, for ~50% of couples attending assisted conception (AC)[3]. The common approach to overcome male-factor infertility is through Assisted conception, which treats the symptom, not the cause. Several efforts have been made to treat males with poor semen quality, with promising results. For example, in clinical trials, (many dating back to the 1980s) scrotal cooling is an effective proven alternative form of AC that improves semen quality and natural pregnancy rates. For example, trials performed in 25 infertile men showed that in 18 men (70%), semen parameters improved with scrotal cooling[18], and 6 (24%) went on to conceive a natural pregnancy during the 14-week scrotal cooling regime, despite the fact they had been "trying" to conceive for 3-8 years previously[19-21]. A second trial involving 64 men, showed improvements in semen parameters in 66% of cases and a pregnancy rate of 27%[23] within 16 weeks of scrotal cooling. Despite the success of these studies, scrotal cooling has not been taken up as a therapeutic option due to three major issues. These include **1)** some devices have been too bulky for practical day-to-day use, **2)** some devices have been based on cold damp cloths, which is not practical for being worn under clothes **3)** some devices need access to refrigerator/freezer throughout the day. Here we investigate how this can be overcome.

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The application of reproductive engineering to manage the breeding stallion

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The logistics around managing stallions during the relatively short horse breeding season presents horse producers with a unique set of limitations that threaten the economic, environmental, and ethical sustainability of this industry. We are attempting to address these limitations via the application of reproductive engineering developments arising from a solid foundation of basic discovery science. These developments include a stallion sperm storage medium that extends the fertility of liquid-stored spermatozoa from two days to two weeks; a device that can be used on-farm to predict the fertility of a semen sample based on metabolic activity; a simple feed supplement that can reverse oxidative DNA damage in the male germline; and management strategies to reduce the effects of heat-stress on the spermatozoa of susceptible stallions. These reproductive engineering developments have all been facilitated by a foundation of basic research discoveries, the most significant being the finding that the default metabolic pathway of ATP production by stallion spermatozoa is oxidative phosphorylation. Although this pathway is extremely efficient, it does result in the production of large amounts of reactive oxygen species, which, when combined with the high concentrations of polyunsaturated fatty acids in the stallion sperm plasma membrane, make these cells particularly susceptible to oxidative damage, leading to a 'live fast, die young' phenomenon. This contrasts markedly with most other species, which almost exclusively utilise glycolysis, and therefore have very different requirements during in vitro storage. Other fundamental discoveries that have led to translational outcomes include a comprehensive proteomic characterisation of ejaculated stallion spermatozoa, and the identification of a cohort of stallions with an intrinsic susceptibility to heat-induced germline DNA damage. These examples of translating basic science into high-impact applications for stakeholders illustrate the importance of continued support for discovery research.

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New Generation of Mesh Technology for Prolapse Repair

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Pelvic floor disorders such as pelvic organ prolapse (POP) affect 1 in 4 women. Until recently, polypropylene meshes were often used to augment native tissue repair pelvic reconstructive surgery as a treatment option for POP. However, they have been associated with serious complications such as inflammation, pain and erosion. As a result, such non-degradable meshes have been banned in many countries including Australia and New Zealand. At present, there is no optimal therapy for the treatment of chronic POP leaving millions of women in despair. Polypropylene meshes bear no resemblance to the native vaginal tissue and elicit undesirable tissue responses which have been the underlying cause of its failure in the long term. Our team has been developing a new generation of biocompatible and degradable meshes that have better tissue integration. Furthermore, we showed that the repair and regeneration of tissue can be further enhanced by boosting the meshes with SUSD2+ Mesenchymal stem/stromal cells from women's endometrium (eMSCs). This presentation outlines the development of novel surgical constructs for vaginal repair by merging parallel technologies of stem cell science, nanotechnology, 3D printing and cellular bioprinting using unique preclinical mice, rat and ovine models. Our research delves deep into the interplay between immune cells and mesh design to understand tissue integration and foreign body response. This presentation will also feature our pre-clinical and clinical research into the development of a new generation of therapeutics for advancing maternal urogynaecological health.

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Improved high-throughput assay to detect endocrine-disrupting chemicals

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While the negative impacts of endocrine-disrupting chemicals (EDCs) on reproductive health are well recognised, they can only be properly identified and their risks mitigated using accurate and biologically relevant assays. Furthermore, assays need to be high throughput and practicable to cope with the burgeoning number of chemicals with potential endocrine disrupting action. Our understanding of endocrine signalling and mechanisms of EDC action has evolved greatly in recent decades, however in the field of EDC testing the application of such knowledge is lacking.

We applied contemporary advances in endocrine signalling to develop a novel high-throughput EDC assay able to be used for a broad range of sample types. This assay assesses multiple endocrine signalling pathways and their interactions, simultaneously. As a test case, we assessed a common herbicide, atrazine, for EDC activity by assessing pathway-specific markers of endocrine activation at single-cell resolution *in vitro*. Previous studies have shown that atrazine acts via interference with hormone metabolism, however other evidence suggests direct actions of atrazine on estrogen receptor alpha (ERa) signalling. This latter activity is not detectable using current high-throughput technologies. Our improved assay clearly detected the ability of atrazine to rapidly activate ERa.

This study demonstrates that applying more biologically-relevant contexts and integrating a broader understanding of endocrine signalling can improve the specificity of EDC detection in a high-throughput assay. An improved EDC detection assay will benefit researchers and legislators in mitigating the negative health and ecological impacts of EDCs.

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Recombinant AAV-mediated liver-targeted dual adrenal enzyme gene delivery in a model for congenital adrenal hyperplasia

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Despite the development of steroid replacement therapy in the 1950s(1-3), excess morbidity and mortality remains for people with congenital adrenal hyperplasia (CAH)(4), and alternative treatment options are sought. A recombinant adeno-associated viral (rAAV) vector could deliver wild-type *CYP21A2*, however one caveat is that rAAV vector genomes are predominantly episomal, therefore not replicated during cell division(5). The adrenal cortex continuously renews itself from progenitor cells(6). As a result, episomal gene therapy used in the adrenal cortex will only have a transient effect(7). A potential alternative is to induce ectopic expression of 21-hydroxylase in a stable organ extra-adrenally. We have shown that ectopic expression of 21-hydroxylase in the murine liver can restore aldosterone production and improve corticosterone (major glucocorticoid in the mouse(8))(9). This study sought to further restore corticosterone through the delivery of both *CYP21A2* and *CYP11B1* to the murine liver in 21-hydroxylase deficient mice.

Two vectors were produced: rAAV8-CYP21A2 and rAAV8-CYP11B1, both with a liver-specific promoter. Mice were injected intravenously with either rAAV8-CYP21A2 alone or with a stoichiometric mix of both vectors and harvested four weeks later. Using liquid chromatography tandem mass spectrometry, steroid profiles were compared between wild-type, and untreated, single enzyme (CYP21A2) treated and dual enzyme (CYP21A2 and CYP11B1) treated *Cyp21a1*$^{-/-}$ mice.

The median serum corticosterone was higher in dual enzyme vector treated than single vector treated *Cyp21a1*$^{-/-}$ mice, with dual vector treated corticosterone levels for both sexes restored to that of wild-type males (Figure 1).

Ectopic expression of enzymes *CYP21A2* and *CYP11B1* in hepatocytes of 21-hydroxylase deficient mice increased production corticosterone further than ectopic *CYP21A2* expression alone. While further studies are necessary, ectopic 21-hydroxylase expression could be an alternative genetic therapeutic option that overcomes the limitations of episomal gene therapy in a rapidly turning over organ such as the adrenal cortex.

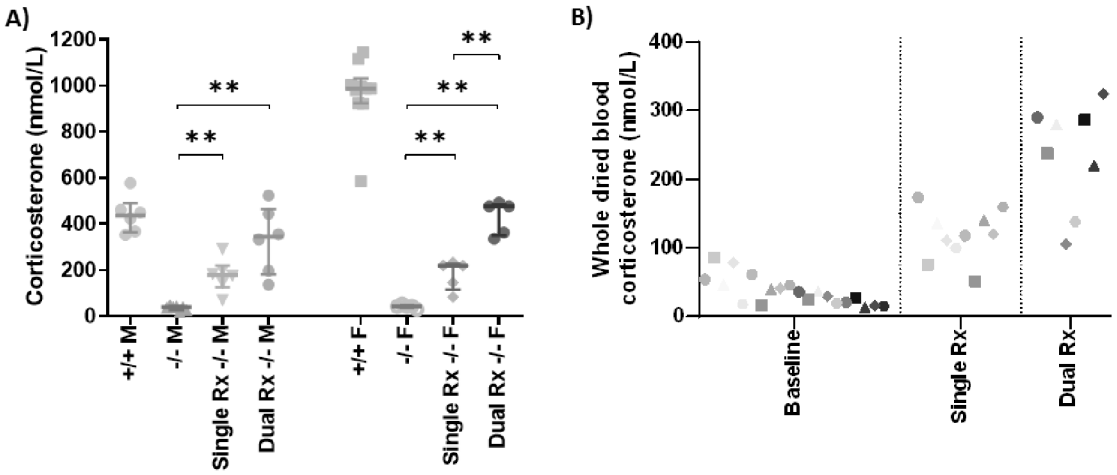

Figure 1. Corticosterone measured by liquid chromatography tandem mass spectrometry. Treated mice received either AAV8-CYP21A2 alone (Single Rx) or a stoichiometric mix of AAV8-CYP21A2 and AAV8-CYP11B1 (Dual Rx). **A)** Serum corticosterone collected by terminal cardiac puncture. Individual results are plotted with bars representing median and interquartile range for the treatment groups. The median corticosterone level was higher in dual enzyme treated mice than in single enzyme treated, in both sexes. Both treatments improved corticosterone from baseline. **B)** Whole dried blood spot corticosterone collected by non-lethal mandibular bleed at baseline and terminal cardiac puncture at 4 weeks post-treatment. Symbols represent the corticosterone level before and after treatment for individual mice. All mice except the single vector treated individual represented by the pale orange square had a higher post-treatment corticosterone level.
Genotype is represented as +/+ for wild-type *Cyp21a1*, -/- for homozygous mutant *Cyp21a1*. Male, M; Female, F.
** $p < .01$ with Mann-Whitney U test.

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Development of islet autoimmunity in Australian children at-risk of type 1 diabetes in the Environmental Determinants of Islet Autoimmunity (ENDIA) study

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The Environmental Determinants of Islet Autoimmunity (ENDIA) study follows Australian children with a first-degree relative with type 1 diabetes (T1D) in a unique pregnancy-birth cohort. Primary outcome is the development of persistent islet autoimmunity. Of children who develop multiple islet autoantibodies in the first 5 years , 85% are predicted to progress to clinical T1D before adulthood[1]. We aimed to characterise the pattern of islet autoantibody seroconversion in ENDIA children.

Participants were followed prospectively 3 monthly during pregnancy and the first 2 years, and 6 monthly thereafter. Autoantibodies to insulin (IAA), GAD, IA-2 and ZnT8 were measured by radio-binding and ELISA assays.

By August 2022, 92 of 1256 (7%) children aged median 4.9 (IQR 3.7, 6.3) years had developed one or more persistent islet autoantibodies or T1D (Table 1). First appearing autoantibody was most commonly IAA in 56/92 (62%), alone or in combination, followed by ZnT8A alone in 25% and GADA alone in 13%.

Probability of progression to persistent islet autoimmunity within the first 2 years was 2% for IAA alone-first. HLA DR4/DQ8 was predominant in these children (24/38). First appearance of other autoantibodies alone reached similar probabilities after 4 years. Relative risk of development of persistent islet autoimmunity using father with T1D as reference, was ~three-fold with multiple first-degree relatives with T1D (HR = 2.8), similar with a sibling proband (HR= 0.9), and lowest with a mother proband (HR = 0.7). Twenty children progressed to T1D – one with mild ketoacidosis. IAA-first, alone or in combination, was the usual sequence (18/20).

In conclusion, the predominant pattern in at-risk young children is IAA-first peaking early in association with high risk HLA type and progression to T1D. Other autoantibodies peak later. Early life patterns of islet autoimmunity may implicate different endotypes, with different environmental drivers from pregnancy and post-natal life.

Table: 1 Characteristics of ENDIA children developing islet autoimmunity and type 1 diabetes

	n	Age in years- median (IQR) at diagnosis or seroconversion	IAA first[1]/ GADA first[2]/ IAA & GADA first[3]/ ZnT8A first[4] (None)
Type 1 diabetes	20	2.5 (1.9, 4.8)	8/0/10/1(1)
Persistent islet autoimmunity (multiple islet autoantibodies)	59 (32)	3.1 (1.9, 4.1)	20/11/7/21
Persistent islet autoimmunity then later sero-revertor	13	1.0 (0.9, 1.3)	10/1/1/1
Single occasion islet autoantibody pending follow-up	21	3.5 (2.6, 5.1)	9/3/4/5
Transient islet autoimmunity on single occasion	67	1.8 (1.2, 2.9)	30/14/0 /23[5]
TOTAL	180	2.4 (1.2, 4.0)	77/29/22/51[5] (1)

[1]IAA first=IAA only or with IA-2A and/or ZnT8A, [2] GADA first= GAD only, [3]IAA and GADA first= IAA and GAD only or with IA-2A and/or ZnT8A, [4]ZnT8A first = ZnT8A only, [5]ZnT8 except for IA-2A in three cases.

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Precision medicine in intersex conditions through reprograming patient fibroblasts into testicular Sertoli-like cells

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Intersex are congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. After WGS, options for functional analysis of causative DNA variants identified in patients is limited to biochemical assays and animal models. Often, the functional consequences of causative variations cannot be elucidated using these methods as there is no cell model that authentically mimics fetal gonadal somatic cell types in which variants are expressed development. We have tried to mitigate this limitation by reprogramming readily available skin tissue derived dermal fibroblasts into Sertoli cells, which could then be used in functional assays. We employed a computational predictive algorithm for cell conversions called Mogrify™ to predict the transcription factors (TFs) required for direct reprogramming of human dermal fibroblasts into Sertoli cells. We established trans-differentiation culture conditions where stable expression of TFs was achieved in 46, XY adult dermal fibroblasts using lentiviral vectors. The resulting Sertoli-like cells (SLCs) were validated for Sertoli cells characteristics by several approaches. SLC shape and size resemble mature human Sertoli cells, as were cell adhesion and proliferation. RNAseq revealed Sertoli-cell specific genes such as *SOX9*, *PTGDS*, *BMP4*, or *DMRT1* were expressed as validated by IF. Conversely, markers of other testicular cell types and of the ovary were by and large not expressed. The trans-differentiation method was also applied to four fibroblast lines from 46, XY intersex patients. In a case of testicular dysgenesis of unknown (exome-negative) genetic etiology, the intersex SLCs express low levels of SOX9 consistent with the clinical diagnosis. This model will aid in variant interpretation following WGS towards improved diagnosis and clinical management of intersex.

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Chemokine receptor expression of CD4+ T cells responding to proinsulin predicts remission duration in Type 1 Diabetes

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Publish consent withheld

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National health care that does not fund continuous glucose monitoring drives inequity in Paediatric Diabetes: The New Zealand example.

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Aims: Continuous glucose monitoring (CGM) improves glycaemia for people affected by type 1 diabetes (T1D), but is not funded in Aotearoa/New Zealand. This study explores the impact of non-funded CGM on equity of access and associated glycaemic outcomes.

Methods: Cross-sectional population-based study collected socio-demographic (age, gender, prioritised ethnicity, socioeconomic status) and clinical data from all regional diabetes centres in New Zealand with children <15 years with T1D as of 1st October 2021. De-identified data were obtained from existing databases or chart review. Outcomes compared socio-demographic characteristics between those using all forms of CGM and self-monitoring of blood glucose (SMBG), and association with HbA1c.

Results: 1209 eligible children were evaluated: 70·2% European, 18·1% Māori, 7·1% Pacific, 4·6% Asian, with even distribution across socioeconomic quintiles. Median HbA1c was 64mmol/mol (8·0%), 40·0% utilised intermittently scanned (is)CGM, and 27% real-time (rt)CGM. CGM utilisation was lowest with Pacific ethnicity (37% lower than Māori) and the most deprived socioeconomic quintiles (quintile 5 vs. 1 adjusted RR 0·68; 95% CI, 0·56 to 0·83). CGM use was associated with regional diabetes centre ($P < 0·001$). The impact of CGM use on HbA1c differed by ethnicity: Māori children had the greatest difference in HbA1c between SMBG and rtCGM (adjusted difference -15.7mmol/mol; 95% CI, -21.9 to -9.5), with less pronounced differences seen with other ethnicities.

Conclusion: Inequities in CGM use exist based on prioritised ethnicity and socioeconomic status. Importantly, CGM was

independently associated with lower HbA1c, suggesting that lack of CGM funding contributes to health disparity in children affected by T1D.

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Age-dependent loss of cohesin protection in human eggs

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Aneuploidy in human eggs is a major cause of miscarriage, infertility and birth defects. While it is prevalent throughout the female reproductive lifespan, aneuploidies associated with loss of cohesion between sister chromatids are greatly increased as women age. Loss of sister chromatid cohesion is largely attributed to an age-dependent loss of the cohesin ring complex. However, the molecular causes of cohesin loss are largely unknown. By using super-resolution microscopy, we demonstrate that a localisation-specific loss of the cohesin protector protein, Shugoshin 2 (SGO2), contributes to an increase in aneuploidy that is associated with cohesion loss in eggs from older women. We show that SGO2 localises in two pools in human eggs; (1) surrounding the kinetochore (centromere) and (2) across the chromatid junction (pericentromere bridge). We observed that SGO2 is progressively diminished from the pericentromere bridge in eggs from older women. The loss of this specific pool of SGO2 is accompanied by an increase in inter-kinetochore distance and the number of single chromatids suggesting reduced sister chromatid cohesion. In contrast, SGO2 association with kinetochores is independent of age, inter-kinetochore distance or the incidence of single chromatids. Further, we show that SGO2 at the pericentromere bridge coincides with a pool of cohesin in human eggs. We demonstrate that the localisation of SGO2 to the pericentromere bridge is dependent on MPS1 and BUB1 kinase activity. Notably, depletion of SGO2 by MPS1 inhibition in human oocytes during meiosis I results in loss of cohesin protection leading to an increase in single chromatids at meiosis II. Overall, our data suggest that the age-dependent loss of SGO2 at the pericentromere bridge in human eggs contributes to the loss of cohesion integrity and the increased incidence of aneuploidy observed in human eggs with age.

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Connecting oocyte mitochondrial ultrastructure and function

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Poor oocyte quality is a leading cause of reduced fertility in humans and animals. However, the factors underpinning oocyte quality are poorly understood. Studies in several species have shown that during oocyte growth and maturation an increasing proportion of mitochondria become hooded, but the purpose of this structural change is unknown. We have also observed differences in the timing of this morphological change between good and poor quality oocytes in a sheep model [1]. The aim of our research is to understand how mitochondrial structure, function and oocyte quality are interconnected to identify novel ways of modifying oocyte quality. Adult sheep oocytes were treated with the mitochondrial inhibitors, FCCP, oligomycin and antimycin A for one hour at the start of in vitro maturation. Changes to the number of hooded mitochondria and mitochondrial membrane potential (MMP) were measured using transmission electron microscopy and confocal microscopy with TMRM staining. Oocytes treated with the electron transport chain uncoupler, FCCP, had decreased mean fluorescent intensity ($P = 0.0046$) and a trend towards an increased proportion of hooded mitochondria ($P = 0.0626$) compared to those treated with the vehicle control. In contrast, oligomycin (ATP synthase inhibitor) treatment decreased the percentage of hooded mitochondria ($P = 0.0287$) but had variable effects on oocyte MMP. Antimycin A had no effect on either the proportion of hooded mitochondria or MMP. These results support our hypothesis that the increased proportion of hooded mitochondria observed after oocyte maturation represents an increase in the uncoupling of the electron transport chain. Our continuing research will determine if altering mitochondrial structure and function can alter oocyte quality using in vitro embryo production techniques. This may lead to the development of modified in vitro maturation media that can improve oocyte quality.

1. Reader, K.L., et al., Mitochondria and vesicles differ between adult and prepubertal sheep oocytes during IVM. Reproduction, Fertility and Development, 2015. 27(3): p. 513-522.

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Dominant follicle selection in mice

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Mono-ovulatory dominant follicle selection is well-characterised through ultrasound studies tracking daily changes in follicle size during the ovarian cycle follicular phase. Poly-ovulatory dominant follicle selection is less clear due to difficulty tracking each antral from day-to-day with certainty. This study aimed to examine dominant follicle development in mouse ovaries to determine if the preovulatory follicles are recruited as a single, large cohort or if preovulatory follicles are recruited progressively across the estrous cycle. Mice were injected with of bromodeoxyuridine (BrdU) at estrus, metestrus and were euthanised 2 hours later. Incorporation of BrdU into the DNA of proliferating granulosa cells was detected by immunohistochemistry and mitotic indexes for 622 growing follicles were quantified using machine-learning image-analysis. Preantral follicles exhibited the lowest mitotic index but follicles underwent a growth spurt in the early antral stage, reaching their maximal rate of proliferation. Declining mitotic indexes were seen as follicles developed to the preovulatory stage. Few atretic follicles were observed at estrus but large numbers were present at estrus and diestrus. Interestingly, the atresia occurred in follicles beyond 170 µm indicating that this represents the onset of gonadotropin-dependent phase, which is substantially larger *in vitro* estimates. Preovulatory-sized follicles were rare at estrus but increasingly present at metestrus. Full preovulatory cohorts were established by diestrus, indicating that this phase is similar to follicle deviation (selection) in mono-ovulatory species. These data indicate that preovulatory cohorts accumulate progressively during the estrus cycle. Mathematical models of follicle growth established that there are two possible classes of follicles that can become preovulatory; (1) medium antral follicles that survived under low-FSH conditions at the end of the previous cycle and (2) small antral follicles with high mitotic indexes that can theoretically overtake slow-growing medium antral follicles. Future studies will examine how follicles can acquire the characteristics of future preovulatory follicles.

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The potential for transgenerational effects of endocrine disrupting chemicals on female ovarian development and reproductive health

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Differences in sexual development (DSD) are congenital conditions characterised by abnormal patterns of sexual development. DSDs are among the most common human birth defects and are drastically increasing in prevalence over past decades. Although there is a known affiliation between DSDs and epigenetic mutations, the increasing incidence of DSDs is too rapid to be caused by genetic factors alone. Thus, DSDs are thought to be consequential of chemicals within the exposome that accumulate as disease risk factors. Exposure to endocrine disruptor chemicals (EDCs), which mimic or disrupt normal hormonal signalling, has been directly linked with an increased prevalence of DSDs. The synthetic oestrogen Diethylstilbesterol (DES) is a key example of this. DES exposure has been shown to induce adverse reproductive health effects for exposed individuals and unexposed individuals past the F3 generation through multi and transgenerational inheritance respectively. In this study, we examined the ovarian phenotype in F1-F3 generation exposed females to determine whether DES exposure predisposed females to polycystic ovarian syndrome (PCOS) and primary ovarian insufficiency (POI). To define the transgenerational reproductive impacts in females following DES exposure, gestating F0 female mice were exposed to 2.5L/g of DES. Ovaries were analysed in offspring at D21pp and in mature adults for the number of follicles, follicle atresia, and the formation of ovarian cysts. Significant differences were seen between generations in follicle populations for both the D21pp and mature adult cohorts. By examining the morphological effects of DES exposure in young and aged female mice (up to the F3 generation) we can define the reproductive consequences of DES exposure. Such findings have broad implications for the millions of female DES descendants, while further deepening our understanding of the consequences of EDC exposure upon the prevalence of DSDs such as PCOS and POI.

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A pilot *in vivo* study: potential ovarian cancer therapeutic by placental extracellular vesicles

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The biological links between cancer and pregnancy are of interest due to parallel proliferative, immunosuppressive, and invasive mechanisms between tumour and placental cells. However, the proliferation and invasion of placental cells are strictly regulated. The understanding of this regulation is largely unknown. Placental extracellular vesicles (EVs) may play an important role in this regulation, as placental EVs are known to contribute to maternal adaptation, including the adaptation of the vascular and immune systems. We have previously reported that placental EVs significantly inhibited ovarian cancer cell proliferation by delaying the progression of the cell cycle. We, therefore, performed this pilot *in vivo* study to investigate whether placental EVs can also inhibit ovarian tumour growth in a SKOV3 human tumour xenograft model. A single intraperitoneal injection of placental EVs at 15 days post tumour implantation, significantly inhibited the growth of the tumours in our *in vivo* model. Signs of cellular necrosis were observed in the ovarian tumour tissues, but not in other organs collected from mice that had been treated with placental EVs. Expression of receptor-interacting kinase 1 (RIPK1) and mixed linkage kinase domain-like (MLKL), which are mediators of necroptosis were not observed in our xenografted tumors. However, extensive infiltration of $CD169^{+}$ macrophages and NK cells in ovarian tumour tissues collected from placental micro-EVs treated mice were observed. We demonstrate here that inhibition of ovarian tumour

growth in our xenograft model by placental EVs involves cellular necrosis and infiltration of $CD169^+$ macrophages and NK cells into the tumour tissues.

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Oocyte mitochondria as key regulators of follicle development

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Throughout the development of ovarian follicles, the oocyte increases 300-fold in volume and the surrounding granulosa cells undergo differentiation and proliferation. There is a constant cross-talk between the oocyte and the surrounding granulosa cells during folliculogenesis, however, how mitochondrial functions in oocyte is linked to follicle development remains poorly understood. During oocyte growth there is a dramatic increase in mitochondrial biogenesis resulting in an increase from around 1,000 to 200,000 mitochondria. Mitochondrial biogenesis requires mtDNA replication, largely driven by Transcription Factor A, Mitochondrial (TFAM) and mitochondrial fission, primarily driven by Dynamin-related Protein 1 (DRP1). Oocytes and their granulosa cells are in constant cross-talk via gap-junctions and it is essential for normal follicle and oocyte development. Mitochondrial function is largely regarded as cell autonomous, but it is unclear how compromised mitochondrial function in one cell type, the oocyte, may influence the development of the entire follicular compartment. We have addressed this question by examining follicular development in oocyte-specific knock-outs of *Drp1* and *Tfam*.

Targeted deletion of either *Drp1* or *Tfam* from primordial follicle oocytes does not significantly affect the folliculogenesis and production of fully-grown oocytes in prepubertal mice. However, around the time of puberty in both models, folliculogenesis is severely disrupted with a follicular arrest at primary and secondary stages. This mirrors other models in which oocyte-somatic cell communication is disrupted. Transcriptional analysis of TFAM and DRP1-deleted oocytes reveals that mitochondrial function in oocytes has key roles in regulating the levels of the regulators of the cellular inflammatory response. In conclusion, our results show that disrupting oocyte mitochondrial function has an extended phenotype beyond the oocyte that impacts the entire follicle at a specific stage of development. The precise mechanism of this effect requires further investigation but may involve disrupted oocyte-somatic cell communication accompanied by an upregulation of inflammatory signalling.

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Iodine and other factors associated with improved fertility following oil-soluble contrast medium hysterosalpingography

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Hysterosalpingography (HSG) with oil-soluble contrast medium (OSCM) improves pregnancy rates in infertile women by unclear mechanisms[1, 2]. OSCM has a high iodine content and long half-life with a potential to cause iodine excess post-HSG[3]. As a secondary outcome of a study examining the impact of this iodine load (SELFI study)[4], we determined factors associated with improved pregnancy rates following an OSCM HSG.

196 consecutive consenting infertile women undergoing OSCM HSG were recruited (Auckland, 2019-2021). Baseline levels and serial measurements of urine iodine concentration (UIC), thyroid function tests and anti-mullerian hormone (AMH) were performed for 6 months post-procedure. Pregnancy and treatment with levothyroxine during the study period were documented.

Median age of the cohort was 36 years (26-49 years). Pregnancy rates were high in women under 40 years with a steep decline in rates in women ≥ 40 years (51% in < 35 years, 47% in 35–39 years and 16% in ≥40 years). A higher AMH was associated with greater chance of pregnancy. 29% participants were iodine deficient at baseline, while 55% iodine sufficient, and 16% had iodine excess. A lower UIC at baseline was associated with a greater likelihood of pregnancy following the HSG. Women who became pregnant had baseline UIC 21% lower than those who did not become pregnant (95% CI -38%, -1%; p=0.042). Among participants with subclinical hypothyroidism(n=79) following HSG, there was a trend for those treated with levothyroxine to get pregnant compared to those not treated and this result was significant when only women under 40 years were considered (63% vs 37%; p=0.047).

In conclusion, OSCM HSG improved pregnancy rates, but was less useful in women aged ≥40 years. Iodine deficiency was relatively common in this cohort and increased iodine levels from OSCM exposure may contribute to the improved fertility observed with this procedure.

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Using single-cell RNA sequencing to determine granulosa cell mediated regulation of primordial follicle activation

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Female fertility is dictated by the number of oocyte-containing primordial follicles within the ovary. These follicles are established early in development and their selective activation represents the definitive first step towards oocyte maturation and ovulation. Although evidence supports crosstalk between the oocyte and its supportive somatic granulosa cells, the intrinsic molecular mechanisms that regulate ovarian follicle activation are largely uncharacterised.

In this study, we used single-cell RNA sequencing (scRNAseq) to examine the transcriptional profile of mouse embryonic and neonatal ovaries at three timepoints: embryonic day (E) 18.5, postnatal day (PND) 4 and PND7. These timepoints correspond with the major developmental events of primordial follicle formation and activation. In total, 24,810 cells were sequenced with high confidence, and we identified five distinct clusters of granulosa cells. Bioinformatic subcluster analysis revealed a distinct cluster of pregranulosa cells that appeared to be undergoing follicle activation, evident by the subtle change in known granulosa cell genes including *Cdkn1b* (p27kip1), *Amh* and *Inhbb*. Additionally, this cluster was uniquely differentiated by the expression of *Tnni3*, *Slc18a2*, *Fam13a* and *Klf2*, all of which are novel to follicle activation. To understand the role of *Cdkn1b*/$p27^{kip1}$ (a cell cycle inhibitor) as a potential regulator of these novel activation genes, we performed transcriptomic analysis on *Cdkn1b*$^{-/-}$ knockout mice. The scRNAseq gene expression signature of activating granulosa cells was observed precociously in *Cdkn1b*$^{-/-}$ ovaries, suggesting that $p27^{kip1}$ may act as an important repressor of the genes expressed in activating pregranulosa cells.

This dataset provides the foundations for characterising a genetic network that regulates follicle activation. These findings will reveal key insights into how the ovarian reserve is established and how this is dysregulated in female infertility disorders.

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Defining the Impact of Endocrine Disruptors on Reproductive Health

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Reproductive health is rapidly declining with differences in sexual development (DSDs) now some of the most common abnormalities seen at birth. Many of these DSD are attributed to impacts to the normal development and hormonal output from the developing fetal gonad. In particular, the rapid rise in male DSDs and concomitant drops in male fertility have occurred too quickly to be caused by gene mutations. Instead, these diseases must have their origins in the environment. Many DSDs can now be at least partially attributed to our exposure to endocrine disrupting chemicals (EDCs) which impact hormonal signalling within our bodies. In my lab we have sought to understand the normal hormonal pathways directing reproductive development and examined how EDCs disrupt these to cause disease. We have uncovered novel roles for hormones and disturbingly broad impacts of EDCs in developmental abnormalities and disease legacies that can persist even into unexposed generations.

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Mapping of the human endometrium tissue using spatial transcriptomics

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Aims

Endometriosis is a growth of endometrial tissues outside the uterine cavity, causing chronic pain and infertility. The gold standard for diagnosing endometriosis is by having a invasive laparoscopy surgery.

We have generated dense single-cell and spatial reference maps of the human uterus

We dissect the signalling pathways that determine cell fate of the epithelial lineages in the luminal and glandular microenvironments

Methods

All patients presented to our minimally invasive gynaecologic surgery at Royal Brisbane women hospital. We processed the Eutopic (n= 24) and ectopic tissues (n= 10) by Snap freezing in liquid nitrogen and subsequently stored at −80 °C. The tissues were cut onto visium slides according to the 10x genomics Visium Spatial tissue preparation guide. Alignment and Qc was done using space Ranger, UMAPs and images were generated using loupe browser.

Results

The Eutopic tissues used were characterised into different mensural cycle by H&E staining and multiplexed together. The H&E stained slides of the ectopic lesions were reviewed to confirm the presence of endometriosis lesions. We have generated a spatial reference map of the human uterus to deconvolute bulk data from endometrial cancers and endometriotic lesions, illuminating the cell types dominating in each tissue.

Conclusions

This technique will help develop a platform for future development in the area of endometriosis to create treatment for common conditions including endometriosis and endometrial carcinoma.

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Does out-of-season spawning affect smoltification in chinook salmon (*Oncorhynchus tshawytscha*)

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Chinook salmon (*Oncorhynchus tshawytscha*) parr undergo a physiological transformation called smoltification that pre-adapts these fish for their transition from freshwater rivers to the saltwater ocean. While this occurs once annually and at a specific season in salmon in the wild, out-of-season spawning of farmed salmon imposes seawater transfer onto parr at unusual time points and under differing environmental conditions (e.g. temperature, photoperiod). This study is examining whether differences are occurring in smoltification between individuals from three different cohorts, i.e., an in-season (reaching target smolt weight between Oct and -Dec), a late-season (Dec-Jan) and an out-of-season cohort (Apr-Jun). Fish were sampled at commercial hatcheries every third week throughout their freshwater growth phase from around 4 g to their saltwater -transfer weight of 30 g; a final sampling was done at 60 -days post transfer at saltwater grow-out pens. We determined biometric parameters including length, body weight, standard growth rate, condition factor (K) and hepatosomatic index (HSI). Additionally, we assayed for blood plasma osmolality, for gill mRNA levels of both subunits (α1a –freshwater, α1b – saltwater) encoding the salt-regulating enzyme Na+/K+-ATPase-α1 (α1a – freshwater, α1b – saltwater) and for gill Na+/K+-ATPase activity. Initial observations indicate that the biometric parameters of the out-of-season cohort were more variable than those of the in-season cohort. Additionally, the presence of abnormal livers (white colouration and “crumbly” texture) across almost all sampling sessions has indicated the need for analysis of the metabolic function of these affected fish. We anticipate that our findings will assist the New Zealand salmon farming industry with making of informed decisions in the management of chinook salmon juveniles prior to seawater transfer.

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Exploring Potential Genetic Contributions to Gender Incongruence

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Gender Incongruence (GI) is a marked and persistent incongruence between an individual’s gender and their chromosomal/anatomical sex. Individuals who identify as a member of the opposite sex are known as transgender. Twin studies suggest there is a substantial heritable component to GI, but the genetic mechanisms are currently unknown. Efforts to identify specific genetic contributions have focussed on the hypothesis that variation in sex steroidogenesis genes in those who experience GI may alter the sexual differentiation of neuroanatomy. Candidate gene studies have investigated whether variants in sex steroidogenesis genes are overrepresented in transgender individuals. Here, we aimed to investigate the potential genetic contribution to GI with two methods. Firstly, we conducted a meta-analysis of candidate gene studies in transgender individuals. Of five genes in the sex steroidogenesis pathway (androgen receptor, estrogen receptor alpha and beta, aromatase, and *CYP17*), meta-analysis confirmed a significant association between variants in *ESR1* ($p = 0.0077$) and GI amongst natal males (n = 670 transgender women, 669 cisgender men). Secondly, we conducted the first genome-wide association study in transgender individuals. Although no significant associations were identified in this preliminary study (n = 52 transgender women, 84 cisgender controls), the most associated SNP ($p = 1.738 \times 10^{-5}$) was in *HDAC9*, a global regulator of transcription. *HDAC9* plays a crucial role in the development of sexually dimorphic regions of the brain that regulate sex steroidogenesis and sexual behaviour, and which have been shown to be altered in transgender individuals. By studying the mechanisms by which GI occurs, we also implicitly study how gender identity develops. These preliminary results suggest that variants in genes involved in governing sex steroidogenesis and sexual brain differentiation may contribute to GI, and thus, that gender identity may develop out of the sexual differentiation of the brain.

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A model to dissect sex differences in disease and drug response

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Discerning hormonal versus genetic contributions to sex bias in Parkinson's Disease: the "Four Core Genotype" Rat Model

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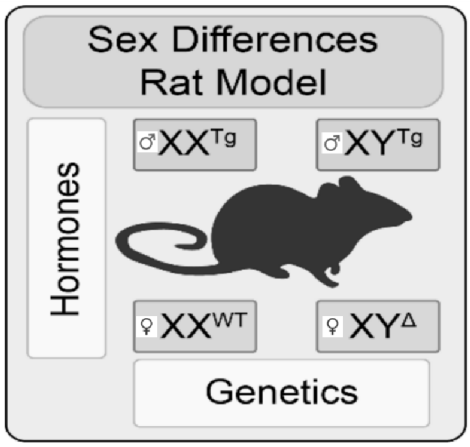
Many diseases show sex differences in incidence, progression or age of onset. Sex differences imply that one sex has hormones (gonadal testosterone and oestrogen) or sex chromosomes (X,Y) that protect from or exacerbate disease risk relative to the other sex. Parkinson's disease (PD) is caused by the progressive death of dopaminergic neurons in the substantia nigra that control the voluntary movement affects twice as many men than women. Men with PD also experience steeper decline and greater neuronal degeneration and denervation and mitochondrial damage. There is no cure for PD, or drug that prevents neurodegeneration. Prevailing dogma is that estrogen is neuroprotective in females. In rat models, we identified male exacerbate factor SRY. Therefore, there is a need to understand the relative effects of hormones and/or sex chromosomes to disease risk.

Our "Four Core Genotypes (FCG)" rat model (Abstract 85805 and Figure) allows us to measure simultaneously the effect of sex hormone or sex chromosome and interactive effects in any rat model of physiology, disease or drug response. To explain sex differences, rats have advantages over mice especially they have better performance in behaviour tests and their larger size are preferred for procedures like stereotactic surgery and telemetry. Following a single intranigral injection of 6-OHDA or rotenone or vehicle into FCG rats [n=30 rats per group]. Motor function will be assessed weekly by the limb use asymmetry and amphetamine-induced rotation tests. At the end of the weekly behavioural tests, rat brains (n=5 per group) will be isolated and processed for measurement of nigrostriatal gene expression by RNAseq, protein expression (Western blot), immunohistochemistry and stereology to quantifying the loss of dopamine neurons on right versus left side of the nigra.



FCG Model. Genotype XY^{Δ}, the testis-determining gene, Sry was deleted from the Y chromosome of Sprague Dawley rats using CRISPR to produce rats with ovaries i.e. XY females. Genotype XX^{Tg}, an Sry transgene was inserted onto an autosomal chromosome producing rats with testes i.e. XX males. Genotype XY^{Tg}, Sry gene was deleted from Y chromosome and an an Sry transgene was inserted onto an autosomal chromosome producing rats with testes i.e. XY males.

To study the effects of sex chromosomes (Genetics) the FCG model compares XX vs. XY rats with the same type of gonads, twice (in rats with testes in XX^{Tg} and XY^{Tg} rats, or in rats with ovaries in XX^{WT} and XY^{Δ} rats). The FCG model also examines the effects of gonadal sex hormones (Hormones) by comparing rats with testes vs. ovaries with the same type of chromosomes, twice (in XX rats, XX^{Tg} gonadal males vs. XX^{WT} gonadal females; or in XY rats, XY^{Tg} gonadal males vs. XY^{Δ} gonadal females).

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Independent association of blood pressure with poor sleep health and shift work in the UK Biobank

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Publish consent withheld

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Circulating microRNAs as predictors for dopamine agonists in hyperprolactinemic patients with pituitary adenoma

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The dopamine agonists (DAs) bromocriptine (BRC) and cabergoline (CAB), are used to restore the hypothalamus-pituitary-gonadal axis in patients with hyperprolactinaemia. BRC is preferred over CAB before and during pregnancy owing to the lack of clinical evidence regarding the effects of the latter on maternal and foetal outcomes. Although recent representative guidelines suggest that both drugs can be used during pregnancy, no study has compared their effects on pregnancy outcomes.

We used data from the national health insurance claims database of the Republic of Korea. The risks of pregnancy-related complications and health problems in the offspring of women exposed to DAs during or within a year before pregnancy were analysed based on the classification of DAs and exposure period.

From 2011-2018, 2,728 and 13,506 women were exposed to CAB and BRC, respectively, during or before pregnancy. Exposure to CAB and BRC during pregnancy increased the risk of gestational hypertension by 67% and 100%, and preterm labour by 64% and 37%, respectively. Exposure to CAB before and during pregnancy increased the risk of congenital malformation by 56% and 49%, respectively; however, BRC exposure did not increase risk regardless of the exposure period. Both CAB and BRC exposure during pregnancy increased the risk of preterm birth, low birth weight, and neonatal intensive care unit (NICU) admission.

Although both DAs increased the risk of adverse pregnancy outcomes, only CAB increased congenital malformation, suggesting the significant evidence that BRC should preferred during pregnancy.

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Models of care for children newly diagnosed with Type 1 Diabetes

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Newly diagnosed children with juvenile Type 1 Diabetes (T1D) typically stay in hospital, which incurs healthcare costs and can be disruptive to families. A range of alternative models of care have been trialled to reduce costs and improve health outcomes and experiences for children and families. This research aims to explore implemented alternative models of care and their effectiveness.

We conducted a literature search of four databases, for peer reviewed studies focused on new diagnosis T1D models of care published from 2010 to 2021 written in English.

A total of eight studies met study inclusion. Model components included hybrid short hospital stays followed by home based, or outpatient clinic visits, for education and monitoring, and early integration of technology, specifically CGM (4T model). Outcomes included HbA1c levels, readmissions, cost, and model preference.

For home based or hybrid short hospital stays, four studies reported no significant differences in HbA1c or readmission rates between patients treated with in- and out- patient care. Cost reductions were observed when patients spent less time in hospital, along with reductions in health service usage. Patients and families preferred out-patient models of care to in-hospital care. Nurses looking after children with newly diagnosed T1D also preferred home-based care models, feeling that it improved relationships with families and opportunities for more effective education. A model based on the "four Ts" (teamwork, targets, technology and tight control) resulted in lower levels of HbA1c compared to a historic cohort.

Home-based or hybrid short-stay models proved cost efficient, did not reduce health education or outcomes, and were acceptable to healthcare professionals, patients and families. Additionally, technology with education support improves

health outcomes. With only eight studies identified, there is a need for further research, especially on the effectiveness, cost-effectiveness and implementation determinants of each model of care.

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Identification of putative cortical granule proteins in zebrafish

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The egg must be pre-equipped with the cellular machinery necessary to facilitate successful fertilization and, in oviparous species, to sustain and protect the embryo throughout development. An integral component of this early life support system is the thousands of maternally derived cortical granules (CG) which initiate the expansion of the perivitelline space upon egg activation/fertilization, facilitate prevention of polyspermy, and provide the embryo with the necessary componentry to develop a system of innate immunity. While the importance of CGs for successful fertilization is often recited, little is known regarding their composition. For this reason, we sought to identify candidate CG proteins from the perivitelline space of zebrafish eggs.

Ovulated eggs were collected from zebrafish and subjected to activation by exposure to water. Perivitelline fluid was aspirated and collected as a pooled sample until approximately 50 µl was acquired (~150 eggs). The pooled sample was processed for LC-MS-based protein profiling and the resulting peptide sequences were subjected to database-dependent protein identification, utilising UniProt's zebrafish protein database.

Our preliminary analysis detected 78 proteins, each represented by at least two unique peptides, in the perivitelline fluid of zebrafish eggs, of which 41 proteins were identified as candidate CG proteins. Seven proteins have been selected for further interrogation by immunohistochemistry, quantitative PCR, and/or antibody-mediated knock-down to further characterize zebrafish CG and shed further light on their functional significance during fertilization and early embryogenesis.

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Impact of long-term captivity on interrenal physiology in New Zealand shortfin eel *Anguilla australis*

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The steroid hormone cortisol is released as the final product of the stress response in teleost fish and is essential in aiding survival. When an animal perceives a stressor, substantial amounts of cortisol are produced and released into the blood, reallocating resources to various tissues and improving the response to the stressor. While high plasma cortisol levels can be beneficial for short durations, prolonged elevated levels of cortisol, produced in response to a chronic stressor such as captivity, can negatively impact various functions including growth and reproduction. The effect of long-term captivity on interrenal physiology was investigated in pubertal New Zealand shortfin eels (*Anguilla australis*). Wild-caught fish were held in tanks for up to eight months, with periodic terminal sampling of individuals at different timepoints. Plasma cortisol levels were compared between groups and the interrenal physiology of eels was analysed by measuring the nuclear diameters of the interrenal cells. Immunoreactivity of the interrenal cells to key enzymes involved in cortisol biosynthesis, cytochrome P450 side-chain cleavage (Cyp11a1) and 11β-hydroxylase (Cyp11b), was also analysed. As captivity length progressed, a significant reduction in immunoreactivity for interrenal tissues stained for either Cyp11a1 or Cyp11b was observed, alongside a concurrent decrease in interrenal nuclear diameters. Unusually, plasma cortisol levels did not differ significantly between the groups, suggesting a potential reduction in cortisol metabolism or increase of cortisol-binding globulin in the plasma to compensate for the apparent reduction in interrenal activity. This study provides valuable preliminary information on the impacts of chronic stress on interrenal physiology and localised cortisol production in *A. australis*. As chronic stress is known to negatively impact various tissues and functions, including reproduction, understanding the impacts of long-term captivity as a chronic stressor is vital to further aquacultural development of fish such as the shortfin eel.

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Switching on/off steroid production using light.

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Steroid hormones regulate many crucial physiological processes such as reproduction, response to stress, salt balance and metabolic processes. Any alteration in their production or activity can have major pathophysiological implications. Steroid production occurs in the gonads or adrenals and is regulated by the hypothalamic-pituitary axis. The luteinising hormone (LH) or the adrenocorticotropic hormone (ACTH) bind respectively to their receptor present on the surface of Leydig cells in the testis or adrenal cells, leading to the activation of key enzymes in the steroidogenic pathway. Herein, we want to assess

whether the steroidogenic pathway can be activated using photoactivated genes at specific wavelengths, bypassing the necessity of LH or ACTH binding to their receptors.

We employed an in vitro approach and transfected steroidogenic cell lines and adrenal cells (MLTC1: Leydig cells and Y1: Adrenal cells) with control and optogenetic constructs. Cells were exposed to different regimens of light (time and mode: pulsatile versus constant). Our data demonstrates that following transfection of the optogenetic construct, light exposure increases levels of steroids in the media, as measured by mass spectrometry. Transcript levels of key steroidogenic genes, including StAR, were significantly increased following light exposure only in cells transfected with the optogenetic constructs.

These novel findings provide a proof of concept as to the efficiency of optogenetic tools to improve endogenous steroid profiles. This data offers a potential refinement over current therapy approaches with less side effects, treatment burden and better dose management.

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Chronic inhibition of arcuate nucleus GABA neurons in a preclinical model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the leading cause of anovulatory infertility and is associated with a hyperactive reproductive axis that may be driven by enhanced GABA activity in the brain (1). Research in a prenatally androgenised (PNA) mouse model of PCOS has identified increased arcuate nucleus (ARN) GABA neuron innervation of gonadotropin-releasing hormone (GnRH) neurons (2). While chronic activation of ARN GABA neurons in healthy mice can drive a PCOS-like phenotype, it is unclear whether inhibition of this population can ameliorate PCOS pathology.

To investigate the impact of chronic inhibition of ARN GABA neuron activity on reproductive function in the PNA mouse model of PCOS, a Cre-dependent viral vector for the inhibitory hM4Di DREADD (designer receptor exclusively activated by designer drugs) or an mCherry control was targeted to ARN GABA neurons in adult PNA (PCOS-like) and healthy vehicle control (VEH) vesicular GABA transporter (VGAT)-ires-Cre mice (n=6-7/group). On average, hM4Di was expressed in 41.5±7.9% of the ARN GABA neuron population, and activation of hM4Di by the designer drug clozapine N-oxide (CNO) caused a robust inhibition of the spontaneous firing activity of ARN GABA neurons *in vitro* (p=0.0001; n=11 cells, N=4 mice).

In vivo, CNO (5mg/kg) was chronically delivered via drinking water for three weeks. No effects of CNO were observed in PNA or VEH mCherry-expressing mice. In both PNA and VEH hM4Di-expressing mice, CNO delivery resulted in a rapid, long-lasting increase in bodyweight (p<0.0001) compared to baseline, but did not affect the typical acyclic phenotype observed in hM4Di-expressing PNA mice at baseline, nor impact circulating testosterone levels, pulsatile luteinising hormone secretion, ovarian morphology or GABA-to-GnRH neuron wiring.

These findings suggest that reducing the activity of ARN GABA neurons does not ameliorate pathology in a preclinical model of PCOS.

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Effects of High Fat Feeding in a Novel HIF1α Mutant Mouse Line

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Introduction: Hypoxia inducible factors (HIFs) play a critical role in the response to hypoxia and in obesity and diabetes. However, the literature shows that increasing HIF1α can cause favourable outcomes and can cause exacerbation of diabetes. We created a novel mouse model with a mutation of HIF1α asparagine 813 to glutamine. We hypothesise that mice with this mutation (HIF1α-N813Q) will be protects from adverse consequences of high fat diet (HFD).

Methods: Male HIF1α-N813Q mutants (n=5) and wild-type (WT) littermates (n=8) were studied from 14 weeks of age. Mice underwent glucose tolerance test (GTT), insulin tolerance test (ITT), glucose-stimulated insulin secretion (GSIS) and metabolic cage studies (Promethion). They were then challenged with HFD (45% of calories from lipids) before repeat metabolic assessments.

Results: Baseline glucose tolerance tests were not significantly different. N813Q mice gained less weight over the study (final weight 28.2g in WT versus 26.8g in N813Q, p<0.001 by mixed model test).

Male HIF1α-N813Q mice tended to a smaller deterioration in GTT after HFD compared to their WT littermates (124mmol/L/120min versus 362mmol/L/120min) following 4 weeks of HFD. Interestingly, given their lower weight, N813Q mice tended to eat more and to do less voluntary wheel running.

Conclusions: This initial data suggests that the asparagine to glutamine mutation confers protective effects in mice challenged with an HFD.

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Estimation of breast volume in transgender women using 2D photography: validation of the BreastIdea Volume Estimator in men and transgender women

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Aim: The BreastIdea Volume Estimator (BIVE) is an internet based application that uses an algorithm to estimate breast volume based on 2D images . This technique has previously been validated in a population of cisgender women (1), but not in men or transgender women due to anatomical differences that occur during male puberty. Validation in the chests of men and transgender women will allow use of this technique to estimate breast volume for clinical and research purposes.

Methods: 2D photography (frontal, lateral views) was performed on transgender women prior to commencing (used as reference population for male chests) and 6 months post commencement of feminising hormone therapy. BIVE was used to estimate breast volume by two researchers independently and compared to a gold standard calculation of breast volume using a 3D scanner. The mean absolute difference (MAD) was calculated to assess accuracy. Intraclass coefficients (ICC) were calculated to assess absolute and relative reliability.

Results: The breast volume of 102 breasts of 41 transgender women (median(IQR) age 26 (23-30) years, BMI 24.6 (21.2-29.0) kg/m2) was estimated using the BIVE application and compared to volumes obtained from 3D modelling. For frontal views, the MAD±SD was 8.27±8.01 mL for observer 1 and 9.58±12.78 mL for observer 2. The standard error of measurement was 0.20 mL for observer 1 and 0.93 mL for observer 2. The relative reliability ICC (95%CI) was 0.993 (0.989–0.996) for observer 1 and 0.949 (0.920–0.967) for observer 2. The absolute reliability ICC (95%CI) between observers was 0.980 (0.969–0.987) suggesting good correlation.

Conclusion: The BIVE application can be used to reliably estimate breast volume in cisgender men and transgender women. This provides a low cost and accessible option for clinicians and researchers in the outpatient setting. Limitations include initial user training to use the application proficiently.

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Use of bicalutamide as an androgen receptor antagonist in transgender women

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Aims: Bicalutamide, a potent non-steroidal androgen receptor antagonist without off-target effects, is increasingly being used to treat transgender women. However, its comparative efficacy, effect on serum total testosterone concentration and risk of hepatotoxicity in this population is unclear.

Methods: A cross-sectional analysis of patients treated with bicalutamide by the Austin Gender Clinic and private endocrinologists was performed, comparing serum total testosterone concentration, serum estradiol concentration and liver function tests to historical cohorts treated with spironolactone (n=38), cyproterone acetate (n=21) or estradiol without anti-androgen (n=21).

Results: Fourteen patients treated with bicalutamide were identified, with median age 28 (24-40) years and duration of hormone therapy 21 (15-37) months. Median bicalutamide dose was 25 (25-50) mg daily and median duration of bicalutamide therapy was 6 (3-12) months. Four patients commenced bicalutamide at initiation of gender-affirming hormone therapy, while the remaining patients had previous anti-androgen therapy. Median serum total testosterone concentration was 4.5 (0.5-17.8) nmol/L in individuals treated with bicalutamide for >6 months. On univariate analysis, this was not different from individuals treated with cyproterone acetate (0.8 (0.6-1.2) nmol/L, p=0.26), spironolactone (2.0 (0.9-9.4) nmol/L, p=0.76) or estradiol without anti-androgen (10.5 (4.9-17.2) nmol/L, p=0.47). There was no between group difference in serum estradiol concentration (overall p=0.09) or serum ALT (overall p=0.53).

Conclusion: There was no difference in the serum total testosterone concentration in those treated with bicalutamide compared to cyproterone acetate, spironolactone or estradiol without an anti-androgen. Within the bicalutamide group, there was significant variability in serum total testosterone concentration, perhaps attributable to differences in serum estradiol concentration and duration of hormone therapy. It is unclear if this contributes to meaningful differences in feminisation. There was no evidence of hepatotoxicity in our cohort of patients treated with bicalutamide. Prospective studies are required to evaluate the comparative efficacy and long-term safety of bicalutamide in transgender women.

PATIENT AND PRACTITIONER EXPERIENCES OF POST-FRACTURE CARE AT THE TERTIARY-PRIMARY CARE INTERFACE: A QUALITATIVE STUDY

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AIM

Whilst Osteoporosis Refracture Prevention (ORP) services are an effective model for secondary fracture prevention (1), adherence to long-term therapies remains suboptimal (1-3). The need to improve coordination between tertiary and primary post-fracture care is increasingly recognised (4-7). This study aims to map current service processes and factors influencing integration of post-clinic care, and identify barriers and supports for seamless healthcare.

METHODS

This qualitative descriptive study used semi-structured interviews with multiple stakeholders at two metropolitan hospitals in NSW and surrounding general practices, recruiting until data saturation. Interview transcripts were analysed for emergent themes. A concept map was developed to describe thematic relationships.

RESULTS

Seven ORP clinicians, 11 GPs, and seven patients were interviewed. Six key themes were found to affect the transition of patient care from tertiary to primary care (PC): interprofessional communication issues and role ambiguity posed the greatest threat to seamless post-fracture care. Delayed, absent, inaccessible, or poor-quality communication was a frequent source of frustration for healthcare professionals. ORP clinicians lacked confidence in existing communication systems and desired bidirectional communication with PC. While GPs were confident and sought a greater role in osteoporosis management, ORP clinicians had limited confidence that patients would discuss osteoporosis with their GP and that GPs would action recommendations. For PC follow-up to occur, patients required both a strong GP-patient relationship and a perceived need to engage with PC. Patient understanding of osteoporosis (influenced by patient education, knowledge, beliefs, and behaviours) affected their PC attendance. Limited public awareness of osteoporosis and healthcare policy prioritisation were also seen to contribute to care gaps in the pre- and post-ORP clinic landscape.

CONCLUSION

This study identified key challenges facing stakeholders seeking to improving post-clinic osteoporosis care. Local policies and care pathways are suggested to address information and service delivery issues across the acute-to-primary care divide.

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The efficacy and safety of tyrosine kinase inhibitors in advanced and metastatic thyroid cancer: A systematic review and meta-analysis of Phase III randomised controlled trials

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Aims: To evaluate the efficacy and adverse events (AEs) of tyrosine kinase inhibitors (TKIs) in adults with advanced or metastatic thyroid cancer compared with placebo.

Methods: Database CENTRAL, OVID Medline, Embase and PubMed were searched through February 2022. We included Phase III randomised controlled trials that investigated the efficacy and AEs of TKIs in adults with advanced or metastatic thyroid cancer compared with placebo. Outcomes included objective response rate (ORR) and AEs, including diarrhoea, nausea, hypertension, proteinuria, palmar-plantar erythrodysaesthesia (PPE) and grade 3+ AEs. Pooled effect sizes were meta-analysed using RevMan 5 software through a random effects model.

Results: Six phase III RCTs (1799 patients) were included (1-6). Evaluated TKIs included lenvatinib, vandetanib, sorafenib and cabozantinib. Trials included patients with radioiodine-refractory differentiated and medullary thyroid cancer. Based on very low-certainty evidence, there was a significantly higher ORR in the TKI group compared with placebo (6 trials; 1799 participants; risk ratio (RR) 19.74, 95% CI 4.92- 79.20), mostly driven by partial response. AEs were significantly higher in the TKI group compared with placebo (diarrhoea: 6 trials; 1799 participants; RR 4.28, 95% CI 2.49-7.39; nausea: 5 trials; 1648 participants; RR 2.25, 95% CI 1.70-2.98; hypertension: 6 trials; 1799 participants; RR 4.92, 95% CI 3.53-6.85, and PPE: 5 trials; 1469 participants; RR 15.40, 95% CI 7.21-32.90). There was significantly more proteinuria with lenvatinib compared with placebo (2 trials; 543 participants; RR 12.23, 95% CI 5.61-26.68). TKIs caused significantly more grade 3+ AEs compared with placebo (6 trials; 1799 participants; RR 3.28, 95% CI 2.04-5.28).

Conclusion: This is the first systematic review on the safety and efficacy of TKIs in advanced or metastatic thyroid cancer to meta-analyse phase III RCT level data, and include the most recently published trials (4,6). This review highlights the need to balance the therapeutic effect and toxicity of TKIs.

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Prevalence of and factors associated with dysglycaemia in adult burn inpatients

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Aim/Background:

Severe burns can precipitate hyperglycaemia. (1) Hospitalised patients with burns and diabetes have greater morbidity than those without diabetes, however there is limited information on glycaemia during acute burns admissions. (2, 3) This study examines the extent of dysglycaemia in burns inpatients with known diabetes, and of stress hyperglycaemia in patients without diabetes.

Methods:

Retrospective cohort study at a tertiary centre of acute hospitalisations from burn injuries. Patients were categorised based on medical history as having known diabetes (n=30) or no known diabetes (n=260). Burn injuries assessed as percentage total body surface area affected (%TBSA). Glycaemic measures included blood sugar level (BSL), hyperglycaemic episodes (BSL 11.1mmol/L) and dysglycemic days (BSL 4 or 16). Length of stay (LOS) was another outcome.

Results:

Admitted patients with known diabetes experienced significantly higher BSLs (9.7 vs 9.0 mmol/L, p<0.001) with a greater proportion experiencing hyperglycaemia (28.0 vs 17.2%, p<0.001) and dysglycaemic days (22.4 vs 8.06%, p<0.001), compared to those without known diabetes despite significantly lower TBSA (1.0 vs 14.8%, p<0.001). Patients with stress hyperglycaemia (N=14) had BSLs comparable to those with known diabetes (admission 10.3 vs 11.5 mmol/L; during admission 9.9 vs 9.9 mmol/L), but more severe burns (15.6% vs 1.0% TBSA) and longer LOS (18 vs 7 days, p<0.001). Presence of known diabetes and extent of burns were predictors of longer LOS In the cohort as a whole (both p<0.001).

Conclusions:

In patients with known diabetes, relatively small burn injuries may result in hyperglycaemia, whereas in burns patients generally both the presence of diabetes and extent of burns are predictors of longer LOS. Stress hyperglycaemia occurs in patients with substantive burn injuries, resulting in promptly elevated BSLs and longer LOS. There is scope to improve inpatient management of BSLs and to investigate whether this would improve longer term clinical outcomes following burns.

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Prediction models for new fragility fracture using penalized regression and artificial intelligence in Korean women aged 50 years or older and men aged 60 years or older

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The osteoporotic fracture could be predicted by clinical risk factors, bone mineral density (BMD), and bone turnover marker (BTM). This study was conducted on 6 University Hospitals in Korea with subjects of women aged 50 years or older and men aged 60 years or older retrospectively after approval by the Institutional Review Boards. We developed prediction models for new fractures based on age, sex, height, weight, previous fracture, current smoking, glucocorticoids, rheumatoid arthritis, secondary osteoporosis, femoral neck BMD, lumbar spine (L1~4) BMD, and total alkaline phosphatase (TALP). Fracture Risk Assessment Tool (FRAX) scores with the World Health Organization had been calculated as Korean. Among the first collected 28,508 subjects, 18,708 participants had been included after applying the exclusion criteria for the purpose of this study. A total of 971 new fractures occurred during the 1.35 years of mean duration of follow-up. The whole dataset was randomly split into training and test sets in a 7:3 ratio. In this study, we applied 3 penalized regression models (Lasso, Ridge, Elastic-Net), 2 machine-learning models (random forest; RF, extreme gradient boosting machine; XGBoost), and 1 deep-learning model (deep neural network; DNN). To evaluate the performance of models, we used accuracy and area under the receiver operating characteristics (AUROC) curve. The accuracy in test sets was 94.50% with a cut-off of 0.5 for all models. The AUROC in test sets were 0.734 for Lasso, 0.736 for Ridge, 0.734 for Elastic-Net, 0.544 for RF, 0.737 for XGBoost, and 0.732 for DNN.

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Time-in-range: A more intuitive measure of glycemic control in type 2 diabetes than HbA1c

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Table 1. CGM characteristics in the HbA1c subgroups

Parameters	HbA1c <8% (n= 18)	HbA1c 8-10% (n=16)	HbA1c >10% (n= 18)	p value
Average glucose (mg/dl)	138 (115- 157)	170.5 (150- 195.5)	232.5 (197- 273)	<0.001 [a]
Glucose management indicator	6.45 (5.6- 7.1)	7.55 (6.85- 8.4)	9.70 (8.5- 11.1)	<0.001 [a]
TIR (%)	82 (68- 88)	62.50 (40.5- 78.5)	18.5 (2- 38)	<0.001[a]
TAR (%)	14 (1- 29)	37.5 (20.5- 55.5)	81.5 (59- 98)	<0.001[a]
TBR (%)	0.5 (0- 7)	0 (0- 1)	0 (0)	0.025[a]
TBR > 4% or level 2 TBR > 1%	6 (33.3)	1 (6.3)	1 (5.6)	0.06 [b]
CV (%)	27.04 (23.13- 31.29)	27.34 (23.32- 31.53)	20.55 (16.41- 27.87)	0.025[a]
CV ≥ 36%	2 (11.1)	1 (6.3)	0 (0)	-

Table 2. CGM characteristics in the TIR subgroups

Parameters	TIR <40% (n=19)	TIR 41- 80% (n=18)	TIR >80% (n=15)	p value
Average glucose (mg/dl)	234 (203- 273)	162.5 (157- 180)	136 (115- 143)	<0.001[a]
Glucose management indicator	9.8 (8.7- 11.1)	7.3 (7.1- 7.9)	6.4 (5.6- 6.6)	<0.001 [a]
SD	49 (44- 63)	50 (41- 56)	32 (26- 34)	<0.001 [a]
CV%	22.27 (16.55- 28.19)	30.76 (26.11- 35.03)	23.78 (19.12- 26.96)	0.003 [a]
TAR (%)	82 (68- 98)	32.5 (22- 44)	6 (0- 18)	<0.001 [a]
TBR (%)	0 (0)	0 (0-1)	0 (0- 2)	0.360 [a]

Data expressed as median (IQR)/ n (%) Statistical tests used- a: Kruskal Wallis test, b: Fisher's Exact test (Freeman-Halton Extension), Statistical significance at p< 0.05. Abbreviations- TIR: Time in range, TBR: Time below range, TAR: Time above range, CV: Coefficient of variation

Assessment of glycemic control is shifting from HbA1c towards continuous glucose monitoring (CGM). We aimed to assess the correlation between HbA1c and time-in-range (TIR), and their relationship with coefficient-of-variation (CV%) and time-below-range (TBR) in Type-2 diabetic (T2DM) patients.

Clinical and laboratory evaluation was followed by CGM (Medtronic IPRO®2, Enlite sensor) for atleast 48-hours in 52 T2DM patients (mean age: 52.6±7.5 years) on stable lifestyle and pharmacotherapy for atleast 3-months. Median diabetes duration was 6.5 years (2-11). All patients were on oral anti-diabetic drugs, with 13.5% additionally on insulin. Median CGM readings were 831 (802-1069.5), with adequate cross-calibration with capillary-blood glucose [MAD- 9.75% (7.2-12.65)].

Median HbA1c and TIR were 8.8% (7.7- 11) and 59% (25-81) respectively. HbA1c had a negative correlation with TIR (ρ=-0.722, p<0.001) by Spearman's rho(ρ) analysis; every 10% increase in TIR corresponding to a 0.59% reduction in HbA1c. HbA1c had a negative correlation with TBR (ρ=-0.396, p=0.004) and CV% (ρ= -0.312, p= 0.024), while TIR did not

have a significant correlation with either variable (ρ= 0.244, p= 0.081 and ρ= 0.145, p= 0.306 respectively). Between-group analysis of HbA1c and TIR-tertiles also demonstrated this dichotomy. Higher TBR and CV% were noted in the lowest HbA1c-tertile (<8%) (p= 0.025 each), while there was no significant difference in TBR between the TIR-tertiles (p=0.36), and CV% was lower in the highest (TIR>80%) and the lowest (TIR<40%) TIR-tertiles (p= 0.003).

Thus, the "well-controlled" territories for HbA1c and TIR have divergent implications because of glycemic variability (GV) and hypoglycemia, inspite of the linear relationship between them. HbA1c suffers the bane of being an average, and directing antihyperglycemic therapy to optimize HbA1c frequently translates into higher GV and TBR. Targeting TIR instead may serve as a more intuitive measure of glycemic control and may help minimize GV and TBR-related morbidity, with long-term prognostic implications.

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The Clinical Utility of PET scanning in Medullary Thyroid Cancer

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Background: Somatostatin receptor (SSTR) functional imaging with PET-CT has broadened the diagnostic and staging capabilities for medullary thyroid cancer (MTC). ^{68}Ga-DOTATATE is a radiotracer with a high affinity for type 2 somatostatin receptors (SSTR2) expressed in many but not all MTCs. Correlation between ^{68}Ga-DOTATATE-PET/CT avidity and *in-vitro* SSTR2 immunohistochemistry has been established in some neuroendocrine tumours (NETs) but not MTC. The utility of ^{68}Ga-DOTATATE-PET/CT and ^{18}F-FDG-PET/CT imaging in predicting MTC prognosis is also unknown.

Methods: In this single centre retrospective study, 37/99 (37%) of MTC patients underwent ^{68}Ga-DOTATATE-PET/CT imaging; of these, 13 (35%) had contemporaneous ^{18}FDG-PET/CT. ^{68}Ga-DOTATATE-PET/CT and ^{18}FDG-PET/CT scans were assessed by two experienced nuclear medicine physicians. SUVmax, SUVmean, metabolic tumour volume (MTV) and total lesion activity (TLA) were assessed for both PET radiotracers. Tumours archived in formalin-fixed paraffin-embedded blocks were constructed into tumour microarrays and immunohistochemistry (IHC) for SSTR2A and Ki67 were scored.

Results: SSTR2A expression was measured by IHC in the primary tumour; 37/99 (37%) had at least some and 62/99 (63%) had no detectable SSTR2A expression. There was no difference in overall survival, calcitonin doubling time or age between SSTR2A +/- tumours. Of the 37 patients with ^{68}Ga-DOTATATE-PET/CT, 32/37 (86%) had avid disease, most commonly in nodal metastases. Ki67 in the primary tumour significantly correlated with ^{68}Ga-DOTATATE MTV (p=0.004) and TLA (p=0.007). SSTR2A IHC did not correlate with ^{68}Ga-DOTATATE avidity. Comparison with ^{18}FDG-PET showed 6/13 patients had ^{18}FDG>^{68}Ga-DOTATATE avidity, 3/13 concordant and 4/13 ^{68}Ga-DOTATATE>^{18}FDG avidity. Disease-specific deaths were only seen in the ^{18}FDG >^{68}Ga-DOTATATE avid cohort. Shorter survival was associated with TLA>20 (p=0.04) but not with RET alteration status, calcitonin, or ^{68}Ga-DOTATATE SUVmax or mean.

Conclusion: Assessment of TLA from ^{68}Ga-DOTATATE PET/CT may predict survival. SSTR2A IHC did not correlate with ^{68}Ga-DOTATATE avidity. Metastatic disease may be optimally assessed by concurrent ^{18}FDG and ^{68}Ga-DOTATATE imaging.

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Trabecular Bone Score declines significantly post allogeneic bone marrow transplant independent of DXA T-score

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Background: As outcomes from allogeneic bone marrow transplantation (BMT) improve, monitoring for longer-term complications becomes increasingly important. Decline in bone health post BMT is well-established, although the pathophysiology may be unique[1]. Trabecular bone score (TBS) is opportunistically acquired during DXA scans, assesses vertebral micro-architecture and has established utility in fracture risk prediction. As glucocorticoid use in BMT subjects is known to cause vertebral fragility, TBS may be a useful adjunct to triage patients for anti-resorptive treatment.

Aims: To compare the change in bone mineral density (BMD) and TBS in patients pre and post BMT.

Methods: All patients who underwent BMT and had a DXA scan performed between 2019 and 2021 at the Royal Melbourne Hospital, including a pre BMT and post BMT DXA were included. Patient characteristics and DXA values were collected from the electronic medical record and TBS iNsight was used to calculate TBS.

Results: 207 patients were identified, of which 50 had scans pre and post BMT. 35 of these 50 patients (70%) were male and the mean BMI was 27.3 ± 5.29kg/m^2. 26 (52%) had related donors, 8 (16%) underwent total body irradiation and 33 (66%) had graft versus host disease. There were statistically significant decreases in T-scores and TBS post BMT (*Figure 1*), with no significant correlation between relative declines in T-score and TBS (*Table 1*). Separately evaluating the 82 DXA scans performed post BMT, there was a non-statistically significant increase in FRAX 10-year fracture risk when the calculation was adjusted for TBS (*Figure 2*).

Conclusions: Bone density and TBS decline significantly post transplantation. TBS provides a distinct, complementary method for assessing fracture risk post BMT and should be considered in this population to triage patients for anti-resorptive therapy.

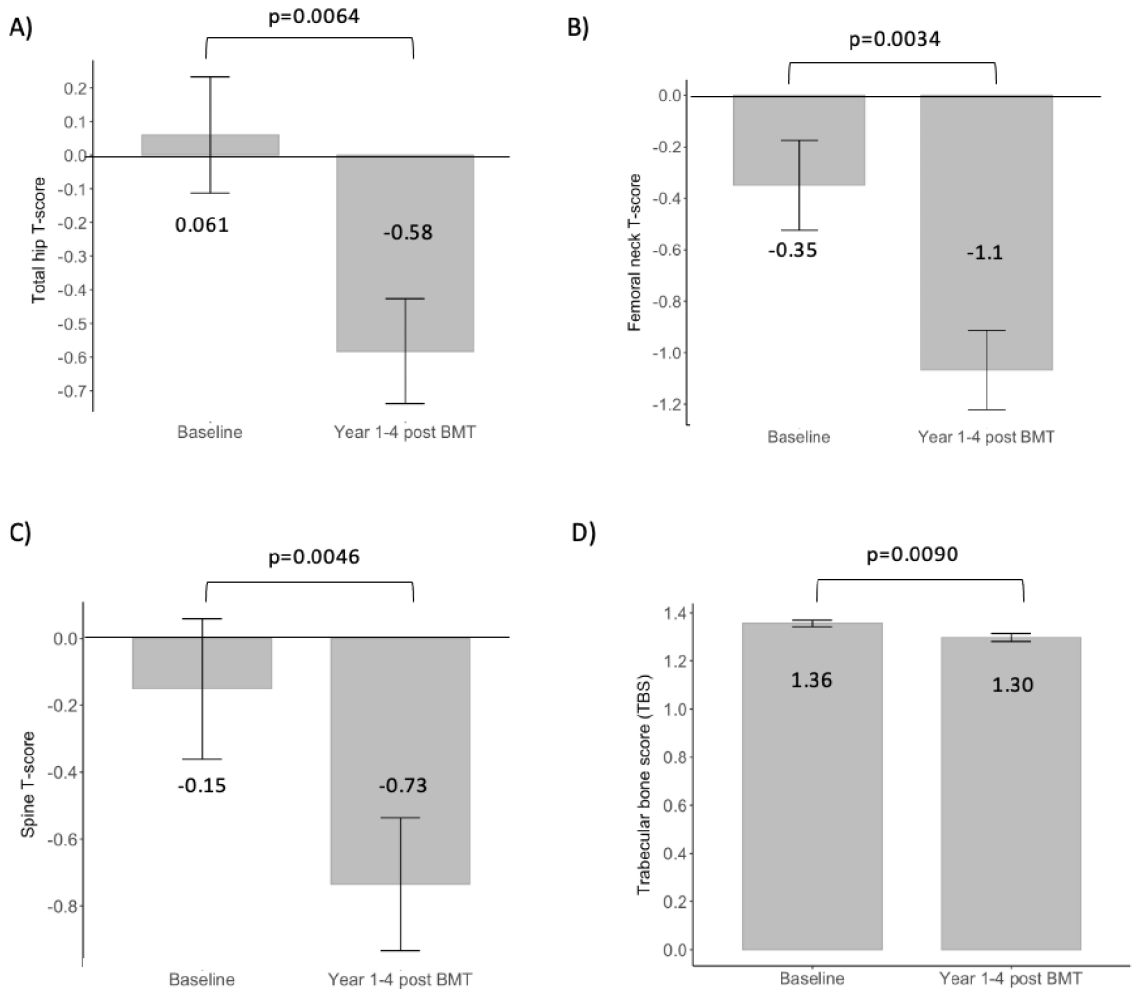


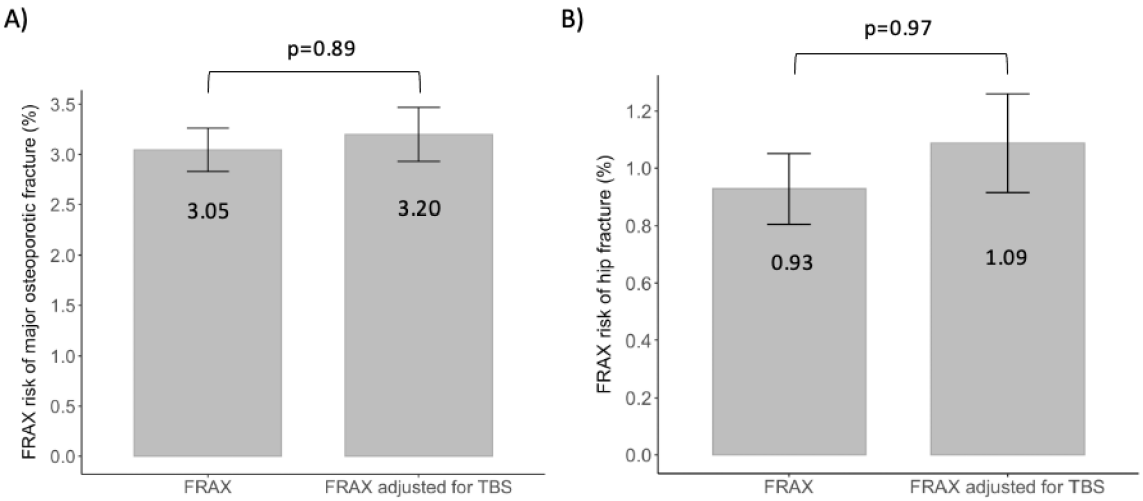
Figure 1. Change in mean T-score and trabecular bone score (TBS) in the years following bone marrow transplant (BMT) in the A) total hip, B) femoral neck, C) spine, and D) TBS.

Mean values as depicted, p-values calculated using the Mann-Whitney test for total hip and femoral neck T-scores (non-normally distributed) and the two sample t-test for spine T-scores and TBS (normally-distributed).

Table 1. Correlation between relative percentage decline in TBS and T-scores between pre and post BMT DXAs measurements

	Comparison with TBS	
	Spearman's rho	**p-value**
Total hip T-score	0.23	0.11
Femoral neck T-score	0.19	0.18
Spine T-score	0.049	0.74

Percentage decline calculated as difference between post and pre BMT value, divided by pre BMT value.



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Increased fracture risk in young adults with Type 1 Diabetes Mellitus (T1DM): An emerging area of need

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Publish consent withheld

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Screening for primary aldosteronism in people with hyperparathyroidism: a multicentre cohort study

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Given the established associations between parathyroid hormone (PTH) excess and hypertension[1-4], as well as the relationship between primary aldosteronism (PA) and hyperparathyroidism (hyperPTH)[5-7], we sought to investigate the proportion of patients with hyperparathyroidism who meet the Endocrine Society criteria for PA screening. We will compare patients with hypercalcemia to those with normocalcemia, and examine the relationships between PTH, calcium, potassium and blood pressure.

This multi-centre retrospective cohort study included patients who attended outpatient endocrinology and bone health clinics at three tertiary health services in Victoria, Australia between 2015-2019. Patients were included if they had parathyroid (PTH) level above the lab specific reference range and were excluded if they had a documented secondary cause of hyperparathyroidism, including Vit D <50nmol or eGFR <60ml/min. Medical records were used to collect demographic, blood pressure and PA screening data as well as biochemistry.

Of 275 patients included in our analysis, hypertension was present in 51.6%, with 62.4% in patients with hypercalcemic hyperPTH compared to 35.5% in those with normocalcemic hyperPTH. In the overall cohort, 15.6% had a guideline indication for PA screening, including 21.8% in those with hypercalcemic hyperPTH and 6.4% in those with normocalcemic hyperPTH. Only 9.3% (4/43) of those with an indication were screened and one was diagnosed with PA. The most common indication for screening was a history of hypertension and hypokalaemia, 38.9% (14/36) in hypercalcaemic hyperPTH vs 28.6% (2/7) in normocalcaemic hyperPTH. We found a positive relationship between serum corrected calcium and systolic blood pressure (p=0.001). There was no clear relationship between PTH and blood pressure or potassium.

PA screening should be considered in those with hyperparathyroidism and hypertension. However, the role of routine screening is patients with hyperparathyroidism is not clear because hypercalcemia per se is a cause of hypertension. Future prospective studies are required.

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Evaluating diurnal glycaemic profiles in patients with COVID-19 treated with dexamethasone: a retrospective observational study

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Background: COVID-19 and dexamethasone predispose patients to hyperglycaemia, an independent risk factor for severity and mortality in COVID-19 patients. Data is scarce on diurnal glycaemic variation in COVID-19 patients

on dexamethasone which could provide key insights into best prevention and management of hyperglycaemia in this population.

Methodology: We conducted a retrospective observational study on 144 patients managed with morning 6mg dexamethasone for COVID-19 at St George Hospital between August 2021 and January 2022. We measured blood glucose levels (BGLs) pre-breakfast, pre-lunch, pre-dinner and bedtime. New diabetes was defined as HbA1c >/= 6.5% or >/= 2 separate episodes of BGL >/= 11.1 mmol/L. Patients admitted to intensive care during dexamethasone course were excluded. We did not analyse influence of diabetes treatment on BGLs.

Results: Median age was 60 years and 55% were male. Out of the patients with known/newly diagnosed diabetes, 42/43 had T2DM. Incidence of newly diagnosed diabetes was 27% (27/101). Median BGL profile for patients without diabetes was: 6.3 mmol/L pre-breakfast, 7.4 mmol/L pre-lunch, 8.0 mmol/L pre-dinner and 7.5 mmol/L bedtime. In patients with known or newly diagnosed diabetes, median BGL profile for those with HbA1c <7.0% was: 7.6 mmol/L pre-breakfast, 11.1 mmol/L pre-lunch, 12.6 mmol/L pre-dinner and 11.0 mmol/L bedtime. Median BGL profile for those with HbA1c 7-10% was: 7.7 mmol/L pre-breakfast, 13.0 mmol/L pre-lunch, 14.4 mmol/L pre-dinner and 13.3 mmol/L bedtime. Median BGL profile for those with HbA1c > 10% was: 12.2 mmol/L pre-breakfast, 13.9 mmol/L pre-lunch, 14.4 mmol/L pre-dinner and 13.9 mmol/L bedtime. Peak BGL in all cohorts occurred pre-dinner.

Conclusions: We found baseline glycaemic control impacted severity and duration of hyperglycaemia. Only patients with HbA1c >10% experienced persistent hyperglycaemia overnight. HbA1c may guide expected diurnal glycaemic variation in patients with COVID-19 on dexamethasone, which may inform approaches to glycaemic treatment in these patients.

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Dimeric [Cys25]PTH(1-34) regulates the expression of genes related to calcium homeostasis in bone and kidney differentially from monomeric PTH(1-34)

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Real world data of the Control IQ system in a paediatric population in a single Paediatric Diabetes Centre.

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Diabetes technologies are evolving rapidly. Automated insulin delivery systems are designed to improve diabetes management and there is emerging data on their efficacy in the real world paediatric population. We aimed to assess the efficacy of the Control IQ™ (CIQ) system following commencement in our Paediatric Diabetes clinic. We included all patients with type 1 diabetes mellitus from our clinic on Tandem® t:Slim X2™ pump with Dexcom® G6 sensor using sensor > 70% of the time who commenced CIQ (n=3 early release program 18th March and n= 37 following national launch 15th April 2022 to 12th August 2022). Pump uploads were completed and analysed through glooko/diasend® to compare two weeks of data at baseline, 2 weeks, 6 weeks, 3 months and 6 months post-commencement of CIQ for average sensor glucose (Av SG), coefficient of variation (CV), time in range (TIR: 3.9 to 10mmol/L), time below range (TBR:<3.9mmol/L), time above range (TAR: 10.1mmol/L to 13.9mmol/L), Very high > 13.9mmol/L and total daily insulin dose. SPSS was used for statistical analysis for ANOVA to compare the variables at different time points ($p<0.05$ was considered as statistically significant). We analysed a total of 40 patients, 35 have been using CIQ for 6 weeks and 28 for 3 months. The results are reported in the table 1. In our real world data, we found that percent TIR increased, Av SG, TAR >10.1mmol/L and Very high >13.9mmol/L decreased at 3 months of CIQ. We plan to continue gathering data prospectively.

Table 1:

	Baseline (n=40)	2 weeks (n=40)	6 weeks (n=35)	3 months (n=28)	(p) ANOVA
Av SG mmol/L	9.23 (1,59)	8.70 (1.11)	8.58 (1.13)	8.67 (1.10)	0.106
TIR 3.9 to 10mmol/L (%)	62.03	70.20 (11.2)	70.76 (11.1)	71.14 (10.1)	0.004
CV	0.37 (0.051)	0.36 (0.052)	0.37 (0.048)	0.37 (0.048)	0.571
TBR <3.9 mmol/L (%)	2.23 (2.31)	1.77 (1.56)	1.94 (1.83)	1.89 (1.62)	0.739
TAR >10.1 mmol./L (%)	22.33 (7.28)	19.08 (5.04)	18.79 (5.40)	18.36 (5.51)	0.018
Very high > 13.9 mmol/L (%)	13.62 (11.54)	8.46 (6.72)	8.41 (7.76)	8.64 (6.66)	0.019

The values are expressed mean (SD) and the p-value is in comparison to the baseline.

Continuous glucose monitoring identifies barriers to optimal glycemic control in patients of type 2 diabetes mellitus

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Continuous glucose monitoring (CGM) helps delineate dysglycemic patterns including glycemic variability (GV). We aimed to identify CGM-metric related factors affecting optimal glycemic control in Type 2 diabetic (T2DM) patients.

We enrolled 52 T2DM patients on stable lifestyle and oral anti-diabetic drugs (OADs) for atleast 3 months. Clinical and laboratory evaluation was followed by CGM (Medtronic IPRO®2, Enlite sensor) for a minimum of 48 hours.

Mean age of study participants was 52.6 ± 7.5 years. Median diabetes duration and HbA1c were 6.5 years (2-11) and 8.8% (7.7- 11) respectively. All patients were on OADs, with additional 13.5% on insulin. Median CGM readings were 831 (802-1069.5), with satisfactory glucometer cross-calibration. HbA1c had a positive correlation with average glucose ($\rho=0.764$, $p<0.001$), and negative correlation with time-in-range ($\rho=-0.722$, $p<0.001$) by Spearman's rho (ρ) analysis. Area-under-curve (AUC) analysis revealed fasting hyperglycemia as the major contributor to HbA1c overall, but the contribution of postprandial hyperglycemia increased with improving glycemic status, increasing from 16.1% (10.2- 21.1) to 33.4% (17.6-40.1) in subgroups with HbA1c>10% and <8% respectively. Time-below-range, TBR ≥4% was observed in 8 (15.4%) patients; 75% of these hypoglycemic episodes were asymptomatic. Coefficient-of-variation (CV%) had an AUC of 0.793 (95% CI:0.654-0.931, $p=0.009$) for predicting a TBR ≥4%, with cut-off of 26.4% having 100% sensitivity and 63.6% specificity. Higher TBR and CV% were noted in the lower HbA1c tertiles, with consistent negative correlation with HbA1c ($\rho=-0.396$, $p=0.004$ and $\rho= -0.312$, $p= 0.024$ respectively), implying higher GV with nearing HbA1c targets.

Postprandial hyperglycemia, asymptomatic hypoglycemia, higher CV% are the key areas requiring focus in T2DM patients with HbA1c nearer to "target" values. CGM can help to identify these barriers and therapy tailored accordingly to achieve

optimal glycemic control. Accurately quantifying GV using CGM could well be the “third pillar” on which the future of glycemic management rests.

Table 1. CGM derived parameters in the HbA1c tertiles

Parameters	HbA1c <8%	HbA1c 8-10%	HbA1c >10%	p value
TBR (%)	0.5 (0-7)	0 (0-1)	0 (0)	0.025[a]
CV (%)	27.04 (23.13- 31.29)	27.34 (23.32- 31.53)	20.55 (16.41- 27.87)	0.025[a]
AUC-PP (mg/dl/unit time)	5911 (3619- 7481)	6805 (5800- 8765)	2374 (1173- 3409)	<0.001

Data expressed as median (IQR). Statistical test used: Kruskal Wallis test, Statistical significance at $p< 0.05$

Abbreviations: AUC- area under the curve, AUC-F: Fasting hyperglycemia, AUC-PP: Postprandial hyperglycemia, CV: Coefficient of variation, TBR: Time below range

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PMID: 19885298; PMCID: PMC2769808.

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Incidental Adrenal Masses: Incidence and Evaluation

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Increasing sensitivity of radiological imaging has led to an increase in the finding of incidental of adrenal masses. Prevalence of these 'incidentalomas' vary upon modality of detection and patient cohort. Several organisation guidelines exist in the suggested evaluation of these lesions. We aim to evaluate the incidence and investigation of incidentalomes discovered at our organisation over a two-year period and compare this to established standards.

This retrospective audit identified patients aged over 18 years-old using key word search criteria within radiology reports from CT studies from 2019 and 2020 undergoing CT Chest, CT Chest/Abdomen/Pelvis and CT Renal/CT KUB. Search terms included adrenal adenoma, incidental adrenal lesion, incidentaloma, myelolipoma, adrenal lesion, and adrenal mass. In addition, data the electronic medical record and medical records were interrogated to gather data on follow-up and co-morbidities. Patients with known history of active malignancy, suspected adrenal pathology or previous identified adrenal adenoma were excluded.

A total of 38 881 CT studies were performed during the 2-year period of interest. 1464 studies were identified using key search criteria. When removing duplicates, false positive results, and applying exclusion criteria a total 274 studies were included in the final analyses. Incidence of incidentaloma 0.7 % with median age 70 years old (Range 29-97) and 48.5 % male. 18.1% of patients were referred for biochemical evaluation and 25.9% referred to specialist physician, surgeon, or general practitioner. Final diagnoses were made in 28 patients including non-functional adenoma (24), subclinical Cushing's syndrome (3), and primary aldosteronism (1).

Our audit demonstrated a need for further education in evaluation and follow-up of incidental adrenal masses with 82% cases not evaluated with biochemistry and 75% not referred for follow-up. Several cases missed screening who had clinical factors that would have warranted biochemistry for primary aldosteronism, a known condition under recognised.

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The current landscape of adrenal vein sampling in Australia

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Primary aldosteronism (PA) is increasingly recognised as the most common endocrine cause of hypertension, with a 14% prevalence described in treatment-naïve hypertensive patients (Libianto 2022 MJA). PA is surgically curable if caused by the unilateral subtype. Accurate subtyping requires adrenal vein sampling (AVS), a technically challenging and time-consuming procedure. Anecdotal evidence suggests limited AVS availability around Australia with heterogenous test protocols and long waiting lists, during which time patients may accrue morbidity from aldosterone excess.

The objective of this study was to describe the current AVS landscape in Australia and identify existing gaps. Endocrine Unit Heads across Australia were invited to complete a survey exploring AVS access and methodology.

Of 46 unit heads contacted, 42 responded (91%) from all states. AVS was provided at 22/42 sites (52%), concentrated in Victoria, NSW and Queensland. One AVS centre was identified in Tasmania, WA and ACT, and none in the Northern Territory. Over half the centres performed ≤10 procedures/year from 2018-2021, and only 1 site consistently performed >50 procedures/year. The survey revealed significant variation in AVS methodology (including cannulation timing, ACTH stimulation and rapid cortisol assay use) and cut-offs for AVS interpretation (Table 1). Reported success of adrenal vein cannulation varied from 50-100% (mean 83%, median 90%), with greater success in centres with dedicated interventional radiologists and higher volume.

With hypertension affecting 34% of Australian adults (~6.8 million) and PA anticipated to affect 14% of hypertensive people, it is apparent that demand for AVS exceeds current availability. Variation in AVS protocols is likely to negatively impact efforts to address this gap. Protocol harmonisation may improve the consistency of success and allow standardized interpretation of results.

A survey of radiologists performing AVS is underway to complement existing data and enable formation of a working group to optimise AVS access and performance around Australia.

Table 1: Variations in AVS techniques and interpretation				
			n	%
AVS cannulation (n=22)	Sequential		17	77
	Simultaneous		4	18
	Unsure		1	5
ACTH stimulation use (n=22)	Yes - before AVS		8	36
	Yes - during AVS with collection of pre- and post-ACTH samples		9	41
	No - not used at all		2	9
	Variable - depending on radiologist		3	14
Rapid cortisol assay use (n=22)	Yes - always		9	41
	Yes - occasionally		2	9
	No		11	50
Method of measuring cortisol and aldosterone	Cortisol (n=22)	Immunoassay	19	86
		LCMS/MS	3	14
	Aldosterone (n=22)	Immunoassay	15	68
		LCMS/MS	7	32
Selectivity index cut-off to indicate successful cannulation (ratio of adrenal-to-peripheral vein cortisol concentration)	Without ACTH stimulation (n=21)	SI > 4	1	5
		SI > 3	3	14
		SI > 2	13	62
		N/A	4	19
	With ACTH stimulation (n=20)	SI > 4	3	15
		SI > 3	13	65
		SI > 2	3	15
		Other: SI > 5	1	5
Lateralisation index cut-off to indicate lateralisation of aldosterone excess (aldosterone to cortisol ratio in the dominant adrenal vein divided by that in the nondominant adrenal vein)	Without ACTH stimulation (n=22)	LI > 4	7	32
		LI > 3	3	14
		LI > 2	7	32
		N/A	4	18
		Other: LI > 2.5 + contralateral suppression	1	5
	With ACTH stimulation (n=20)	LI > 4	16	80
		LI > 3	2	10
		LI > 2	1	5
		Unsure	1	5

Serum estradiol concentrations with estradiol 0.06% gel in transgender individuals

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Aims: Standard estradiol formulations used in menopausal hormone therapy are typically administered to transgender and gender diverse individuals seeking feminisation, though there are currently limited data evaluating transdermal estradiol formulations in gender-affirming hormone therapy regimens. We aimed to assess the serum estradiol concentrations achieved with estradiol 0.06% gel (EstroGel) in trans and gender diverse individuals.

Methods: A retrospective cross-sectional analysis was undertaken of transgender and gender diverse individuals treated with estradiol gel at Endocrine clinics in Melbourne, Australia. Serum estradiol concentration was measured via immunoassay. Primary outcomes were estradiol gel dose and serum estradiol concentration.

Results: Eighty-one individuals treated with estradiol gel were included. Median age was 29 years (23-40), and duration of feminising hormone therapy was 29 months (17-48). Median serum estradiol concentration was 396 pmol/L (233-681) on 1.5 mg (1.5-2.25) (equivalent to 2 pumps) estradiol gel daily. Thirty-seven (46%) individuals achieved serum estradiol concentrations within the recommended target range of 250-600 pmol/L. There was a weak positive correlation between estradiol gel dose and serum estradiol concentration (r=0.23, p=0.04). Compared to a group of 259 individuals treated with oral estradiol, median serum estradiol concentration achieved was higher in individuals treated with estradiol gel (396 vs. 328 pmol/L, p<0.01). Thirteen individual laboratory results with supraphysiological serum estradiol concentrations (2000-6000 pmol/L) and documentation of skin contamination with estradiol gel at venepuncture site were excluded.

Conclusions: Estradiol 0.06% gel achieves serum estradiol concentrations in the recommended range in Australian consensus guidelines though there is significant interindividual variability, with a weak correlation between estradiol gel dose and serum estradiol concentration. Estradiol 0.06% gel represents an alternative estradiol formulation for trans and gender diverse individuals seeking feminisation.

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Men with Klinefelter syndrome and idiopathic hypogonadotropic hypogonadism have deteriorated bone microstructure

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Publish consent withheld

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Does a history of Sodium-Glucose Transport-2 inhibitor use influence the diagnostic criteria used in the diagnosis of diabetic ketoacidosis in patients with type 2 diabetes A retrospective case-control study.

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Aims

SGLT2-inhibitors are associated with DKA with lower-than-expected glycaemia in patients with diabetes. Consequently, neither hyperglycaemia nor ketonuria can be reliably used in the diagnosis of DKA in these patients (1). The obsolescence of these parameters to guide clinicians presents a diagnostic challenge. The recently published JBDS and the older AACE/ACE guidelines both provide for euglycaemia and the use of capillary ketones as a diagnostic criterion for DKA (2,3). The other criteria used include: HC03 >15mmol/L, anion gap >10 and pH <7.3. This retrospective study compared diagnostic criteria employed to diagnose DKA across two centres.

Methods

All patients (165) with T2DM presenting with DKA between 2015 – 2022 were identified from the electronic medical record and divided into those currently receiving SGLT2i at time of diagnosis and those who were not (no-SGLT2i). Clinical characteristics, biochemical parameters and thresholds used to make the diagnosis of DKA were recorded. Statistical analysis was performed.

Results

Significantly fewer cases in the SGLT2i group met criteria for DKA compared to the non-SGLT2i group according to both AACE/ACE (56% vs 72%, *p = 0.035*) and JBDS guidelines (63% vs 82% *p = 0.009*). Diagnostic cut-offs and biochemical parameters between groups varied ([pH <7.3 67% vs 96%, *p = 0.036*], [HC03 <15mmol 43% vs 74%, *p = 0.000*]) pH (7.23 vs 7.17, *p = 0.006*), HbA1c (9.33% vs 11.6%, *p = 0.000*). Differences in the use of statins (32.6% vs 39.7%, *p = 0.001*) and Metformin (89% vs 63%, *p = 0.000*) was observed. There was no significant difference in capillary ketone level between groups.

Conclusion

A history of SGLT2i use may result in the over-diagnosis of diabetic ketoacidosis in patients with type 2 diabetes

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Optimising DYnamic StudieS in EndocrinologY (ODYSSEY): An Endocrinology nurse initiative.

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Background: Endocrinology clinical nurse roles are not established in many metropolitan hospitals in Australia. The Royal Melbourne Hospital (RMH) is a quaternary centre managing adult endocrine and pituitary diseases which until recently has not had access to an Endocrinology nurse to assist with coordinating endocrine dynamic investigations. In May 2021, new measures were implemented to optimise dynamic investigations. These included training an Endocrine Grade 4b Registered Nurse to perform and coordinate dynamic investigations (0.4 FTE), formalising protocols for dynamic investigations, and formalising presentations of dynamic investigation data by the Endocrinology registrar to two Endocrinologists at a fortnightly meeting.

Aim: To determine if a revised model of care of endocrine dynamic investigations incorporating an Endocrinology nurse improves patient outcomes.

Methods: We audited the number of endocrine dynamic investigations performed at baseline (May 2020 - April 2021) compared with following intervention (May 2021 – April 2022).

Results: At baseline, 37 dynamic tests were performed including 10 glucagon stimulation tests (GSTs) and 6 adrenal vein sampling procedures (AVS). Seven patients had adult growth hormone deficiency (AGHD) and five commenced growth hormone (GH) replacement. Following AVS, 2 did not lateralise and were medically managed. 4 lateralised, with 3 patients undergoing successful adrenalectomy with 1 waiting for adrenalectomy. Following intervention, 76 dynamic tests were performed, including 32 GSTs and 11 AVS. 26 had AGHD; 13 received GH treatment education from the endocrine nurse, and 2 were enrolled in a GH clinical trial. Following AVS, 4 did not lateralise and were medically managed. 6 lateralised, with 4 patients undergoing successful adrenalectomy and 2 patients waiting for adrenalectomy.

Conclusion: Following the institution of an Endocrinology nurse, dynamic investigations performed doubled with improved hormonal treatment initiation and staff satisfaction. This experience may be of value to hospitals which are seeking to justify funding of an Endocrinology nurse role.

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The Identification and Management of Adult Growth Hormone INsufficiEncy (IMAGINE): an Endocrinology nurse initiative.

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Background: Endocrinology clinical nurse roles are not established in many metropolitan hospitals in Australia. The Royal Melbourne Hospital is a quaternary centre managing adult endocrine and pituitary diseases, including adult growth hormone deficiency (AGHD). Growth hormone (GH) replacement is now available on the pharmaceutical benefits scheme, however GH commencement is resource intensive: screening eligible patients, performing a GH stimulation test, completing the QoL-AGHDA questionnaire and educating patients about treatment. In May 2021, an endocrinology nurse initiative was implemented to optimise AGHD management. This included training an Endocrine Grade 4b Registered Nurse to screen our Pituitary Database for potential patients, coordinate GH stimulation testing and provide GH treatment education.

Aim: To determine if an Endocrinology nurse initiative improves the detection and management of AGHD and patient outcomes.

Methods: We audited the number of GH stimulation tests performed and the number of individuals diagnosed with AGHD who commenced GH therapy following intervention (May 2021 – April 2022) compared with at baseline (May 2020 – April 2021). Baseline and current satisfaction scores for Endocrinology registrars were also assessed (completed dissatisfied (1) – completely satisfied (5)).

Results: At baseline (May 2020 – April 2021), 10 glucagon stimulation tests (GSTs) were performed, and 7 patients had AGHD; 5 commenced GH therapy. After introduction of an Endocrinology nurse (May 2021 – April 2022), 32 GSTs were performed and 26 had AGHD. 13 were commenced on GH replacement therapy, with 2 individuals enrolled in a GH clinical trial. The introduction of an Endocrinology nurse improved satisfaction scores of the Endocrinology Registrars from 2/5 at baseline to 4/5.

Conclusion: Introduction of an Endocrinology nurse role to improve the detection and management of AGHD has improved rates of testing, diagnosis, treatment, and staff satisfaction. Ongoing funding for an Endocrinology nurse is paramount for providing quaternary level care for patients.

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The association between physical activity and self-rated health status in patients with type 1 and type 2 diabetes

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Poor self-rated health is associated with reduced adherence to therapy (1) and poorer glycaemic control for patients with diabetes (2). Engagement in physical activity (PA) improves self-rated health (3-5), however, less is known about this association in people with diabetes. This study aims to investigate the association between PA and self-rated health in adults with type 1 (T1D) and type 2 diabetes (T2D).

Data from 9,061 adult patients with T1D (n=2694; 52.4% females, mean age 43 years) and T2D (n=6367; 44.2% females, mean age 63 years) were analysed from the 2014 to 2018 biennial cross-sectional Australian National Diabetes Audit. PA was self-reported and categorised as sufficient (≥150min/week moderate and/or vigorous PA), insufficient (some activity but <150min/week), or participant being sedentary (no weekly moderate and/or vigorous PA). Self-rated health was measured using EuroQol visual analogue scale (0-100) based on patients' subjective assessment of their health on the day of the survey. The association between PA and self-rated health was explored using multivariable linear regression adjusted for age, sex, diabetes duration, smoking status, difficulty following recommended diet, HbA1c, and forgetting to take medications. B and 95% confidence intervals (CI) were reported.

Being sufficiently active was reported by 63.2% of T1D and 39.3% of T2D patients. Compared to individuals with T1D who reported being sedentary, those reporting sufficient and insufficient PA had a higher self-rated health score: 10.87 (7.78-13.97), p<0.001 and 5.13 (1.91-8.37), p=0.002, respectively. Compared to individuals with T2D who reported being sedentary, those reporting sufficient and insufficient PA had a higher self-rated health score: 12.33 (10.76-13.90), p<0.001 and 7.18 (5.69-8.68), p<0.001, respectively.

In both individuals with T1D and T2D, greater engagement in PA was associated with higher self-rated health. Our findings suggest that promoting integrated PA as part of holistic diabetes management may help improve patients' overall health status.

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Do-it-yourself continuous glucose monitoring (DIY-CGM) in people with type 1 diabetes - a qualitative study

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In New Zealand, real-time continuous glucose monitoring (CGM) is not funded, and cost presents a significant barrier to access. A do-it-yourself conversion of intermittently scanned CGM (DIY-CGM) is a cheaper alternative. This qualitative study aimed to enhance understanding of user experiences with DIY-CGM amongst adults with type 1 diabetes (T1D).

We conducted 12 semi-structured interviews of participants who were RT-CGM naïve when recruited into a crossover randomised controlled trial (RCT) investigating the effect of DIY-CGM on glycaemic control. Participants had a mean age of 42.2(+/-14.3 years), with an average Hba1c of 58.9mmol(+/-9.9) and time in range(TIR) of 59.8%(+/-14.8). The majority were pump users(58%), male(58%) and all were European.

The DIY-CGM intervention consisted of a bluetooth bridge connected to a Flash Glucose Monitor,thus adding CGM functionality . sampling was used to recruit participants following RCT study completion. Interviews were transcribed and

analysed using NVivo 12 Pro (QSR International) software. Themes and subthemes were identified using thematic analysis.

Participants perceived using DIY-CGM improved both glycaemic control and quality of life. Alarm and trend functionality allowed participants to perceive reduced glycaemic variability overnight and following meals. There was a high degree of trust in DIY-CGM. Challenges while using DIY-CGM included signal loss during vigorous exercise, alarm fatigue, and short battery life. Most participants intended to continue using DIY-CGM or other commercial real-time continuous glucose monitoring (rtCGM) at the end of the study.

This study suggests that DIY-CGM is an acceptable alternative method of glucose monitoring.

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Health-related quality of life is impaired in pre-operative patients with functioning and non-functioning pituitary lesions in an Australian cohort.

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There are a number of lesions that can arise in the sellar and suprasellar space; the most common of which are pituitary neuroendocrine tumours[1], benign neoplasms, reported to occur in 20% of the population[2] with increasing incidence and prevalence[3]. Other lesions include Rathke's cleft cysts, often asymptomatic remnants of embryological development, which can account for up to 3% of pituitary mass lesions[2].

Pituitary neuroendocrine tumours can be non-functioning or hypersecreting and like Rathke's cleft cysts may also cause compression of surrounding structures requiring surgical intervention. Patients with hypersecreting or compressive lesions can develop symptoms that can affect their quality of life and there are multiple scales clinicians use to measure this impact. One of these is the Short Form-36 (SF-36) scale, a 36-question general quality of life scale that be used across different diseases; measuring quality of life in both physical and mental health domains[4]. This scale measures quality of life compared to calculated norm-based scores from population in the United States of America from 1998.

There are a number studies investigating the quality of life in patients with treated pituitary neuroendocrine tumours[5]. However, there are few studies investigating the effect on quality of life of pre-treated pituitary neuroendocrine tumours[6,7] and no studies comparing the effect of different pituitary neuroendocrine tumours or Rathke's cleft cysts in an Australian cohort using norm-based scores calculated from an Australian population[8].

We retrospectively analysed the SF-36 questionnaires of 229 patients attending a pre-operative outpatient appointment and calculated their physical and mental quality of life scores against Australian norm-based scores. We also investigate the effect of gender or age group, in each tumour type, on SF-36 scores to identify patients at higher risk of impaired quality of life.

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Growth hormone (GH) replacement therapy (GHRT) in patients with adult GH deficiency (AGHD) aged ≥60 years: data from NordiNet® IOS and the ANSWER Program

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Aims: Data on effectiveness and safety of GHRT in older patients with AGHD are limited. We compared real-world GHRT outcomes in older (≥60 years) versus middle-aged (35–<60 years) adults.

Methods: NordiNet® IOS (NCT00960128) and ANSWER (NCT01009905) were non-interventional studies investigating long-term effectiveness and safety of GHRT with Norditropin®. Safety was assessed in the full analysis set (FAS) from both studies (non-GH-naïve patients included). The effectiveness analysis set (EAS) was from NordiNet® IOS only (GH-naïve patients; ANSWER EAS included patients previously treated for ≤6 months). Serious adverse reactions (SARs) and non-serious adverse reactions (NSARs) with a suspected causal relationship to GHRT, and serious adverse events (SAEs) not considered related to GHRT, are presented as incidence rates per 1000 patient-years and as incidence rate ratios (IRRs) for older versus middle-aged adults.

Results: Baseline characteristics are shown (table). Mean GH exposure was greater in women than men, and in middle-aged than older women (FAS), increasing slightly over time in all groups. Baseline IGF-I SD score (SDS) was slightly higher in older women, but not men (EAS). Mean IGF-I SDS increased from <0 to ≤1.24 with GHRT. Mean changes in BMI (EAS) and HbA_{1c} (EAS and FAS) were small and similar between age groups in both sexes.

No statistically significant differences were observed between age groups regarding incidence rates for NSARs (5.66 vs 5.38; IRR[mean, 95%CI] 1.051[0.604;1.831]) and SARs (1.00 vs 2.52; IRR 0.396[0.119;1.324]). As expected, SAE incidence rate (considered unrelated to GHRT) was higher in older patients (16.64 vs 9.04, IRR 1.840[1.291;2.622]). Similarly, IRRs of patients ≥75 years (n=59) versus middle-aged patients were only significant for SAEs (23.09 vs 9.04; IRR 2.553[1.113;5.855]).

Conclusion: These data suggest similar clinical outcomes with GHRT in patients with AGHD aged ≥60 compared with 35–<60 years without additional risk of adverse drug reactions in older patients.

Table: Baseline characteristics

	EAS		FAS	
	35–<60 years (n=545)	**≥60 years (n=214)**	**35–<60 years (n=1696)**	**≥60 years (n=652)**
Female, %	45.9	39.3	52.4	43.3
Age, years	48.51 (6.98)	67.16 (4.89)	48.43 (7.05)	67.09 (5.13)
GH dose, mg/day	0.24 (0.16)	0.20 (0.10)	0.32 (0.24)	0.26 (0.18)
IGF-I SDS	−0.94 (1.40)	−0.82 (1.36)	−0.58 (1.53)	−0.27 (1.54)
BMI, kg/m^2	29.29 (6.09)	28.95 (4.58)	30.50 (7.26)	29.42 (5.39)
Duration of follow-up, years	5.37 (4.28)	5.28 (3.92)	5.19 (4.50)	4.65 (3.86)

Data are mean (SD) except for sex.
BMI, body mass index; EAS, effectiveness analysis set; FAS, full analysis set; GH, growth hormone; IGF-I, insulin-like growth factor-I; SD, standard deviation; SDS, standard deviation score.

Investigating serum 21-deoxycortisol for therapeutic monitoring of classic congenital adrenal hyperplasia

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Biochemical monitoring of classical 21-hydroxylase-deficient congenital adrenal hyperplasia (CAH) mostly relies on measurement of serum 17-hydroxyprogesterone (17OHP) and androstenedione (A4) concentrations. However, high biological variability, particularly of 17OHP, limits interpretation of results and makes therapeutic adjustments based on single measurements challenging. Recently, 21-deoxycortisol (21DF), has been proposed as a more reliable marker of disease control owing to its adrenal-specific origin and lower biological variability.

Our objective was to compare the intra-individual variability of serum 17OHP, A4 and 21DF, all quantified by liquid chromatography and tandem mass spectrometry (LC-MS/MS) in a cohort of patients with classical salt-wasting CAH.

32 adults (9 males and 23 females) with salt wasting CAH were identified from a single institution. All LC-MS/MS quantified 17OHP, A4 and 21DF measurements performed at PathWest QEII Laboratory between 01/01/2018 and 04/05/2022 were collated for each patient. 20 patients had 2 or more 21DF measurements. Of these, 40 % had 5 or more measurements. The biological component of each patient's intra-individual coefficient of variation (CVI) was calculated for each analyte. The intra-individual variability was compared between the analytes.

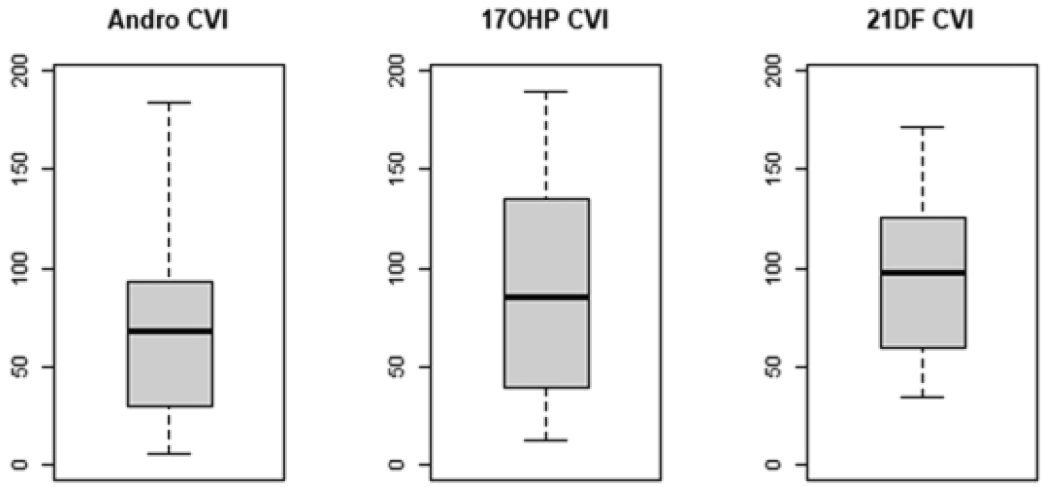
There was strong statistical evidence of lower biological variability (CVI) for A4 than 17OHP (mean difference = -24.1, p=0.003). Other pairwise differences were not significant (A4 versus 21DF p=0.156; 21DF versus 17OHP p=0.104).

The intra-individual variability of LC-MS/MS-quantified 21DF was similar to A4 in adult patients with salt-wasting CAH. Larger prospective studies are required to determine the clinical utility of 21DF measurement for therapeutic monitoring of CAH.

Table: Results of paired Wilcoxon tests comparing the distribution of CVI between each pair of analytes

CVI comparison	A4 – 21DF	A4 – 17OHP	21DF – 17OHP
Mean difference	-15.4	-24.1	-14.3
P-value	0.156	0.003	0.104

Figure: Boxplots of CVI for the 3 analytes.



After-hours management of hyperglycaemia at Hornsby Hospital: A clinical audit

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Background:

Inpatient hyperglycaemia, especially after hours, is frequently managed sub-optimally. Attending Junior Medical Officers (JMOs) are often not part of the patient's admitting team and are unfamiliar with the clinical circumstances.

A clinical audit was conducted to determine current JMO response to after-hours hyperglycaemia.

Aims:

1. To critically appraise the after-hours management of inpatient hyperglycaemia at a local health district hospital
2. Identify potential areas for improvement
3. Provide a structured framework to optimise patient care and safety.

Methods:

A record of all inpatient Clinical Reviews at Hornsby Ku-ring-gai Hospital from January 2022 to March 2022 was obtained. Clinical reviews for all episodes of hyperglycaemia (blood glucose level (BGL) >20mmol/L) that occurred from 1600 hours to 0800 hours were examined for: clinical assessment, glucose management, management escalation, patient monitoring, and follow-up/handover.

Results:

Dysglycaemia was the second most common reason for all clinical reviews. Among these, 55% (134/243 reviews) were for hyperglycaemia, with 71% (95/134) of these reviews occurring after hours. Blood ketone levels (BKLs) were only documented in 64% of reviews. BKLs were not documented for 25% of patients with type 1 diabetes. Patients were physically reviewed in 39% with the majority reviewed remotely. Most patients were managed with a prescription of rapid-acting insulin on an ad-hoc or stat dose basis. This resulted in insulin ''stacking'' in 10 patients, with one patient receiving 22 units cumulative dose of rapid acting insulin in a four-hour time frame. Only 16% of clinical cases were discussed with the after-hours Medical Registrar. A plan for monitoring BGLs was communicated in 82% of cases, although significant variability existed in these plans.

Conclusion:

This audit identified significant areas for improvement in the after-hours management of inpatient hyperglycaemia. Further education for JMOs and nursing staff regarding the appropriate management of hyperglycaemia in hospital settings, especially after hours is needed.

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Body fat reduction during the first year after bariatric surgery in Sri Lankan adults

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Introduction

Bariatric surgery is an extremely effective intervention to achieve weight loss in higher grades of obesity. This is the first prospective study assessing body fat reduction after bariatric surgery among Sri Lankans, to the best of our knowledge.

Objective

We aimed to assess the BFP reduction in obese Sri Lankan patients undergoing bariatric surgery.

Methods

We followed up 50 obese patients who underwent bariatric surgery at Colombo South Teaching Hospital, Sri Lanka for 1 year. Body fat percentage was assessed by bioelectrical impedance analysis. Percentage body weight loss (%BWL) and Percentage body fat loss (%BFL) were calculated as body weight loss /pre-operative body weight and body fat loss/pre-operative body fat respectively.

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/m^2 respectively. Body weight loss verses body fat loss (±SD) at 1 month, 3 months, 6 months, 9 months and 12 months were 8.7 (±3.9) vs 5.5 (±4.8) kg, 16.5 (±5.0) vs 11.0 (±6.0) kg, 22.9 (±5.4) vs 15.2 (±6.1) kg, 27.4 (±7.3) vs 18.6 (±5.0) kg and 29.6 (±8.9) vs 21.7 (7.3) kg. Mean %BWL verses %BFL at the above follow up periods were 7.8 (±2.7) % vs 10.7 (±7.1) %, 15.1 (±2.5) % vs 21.2 (±6.8) %, 21.5 (±3.2) % vs 30.4 (±7.0) %, 25.7 (±4.7) % vs 37.2 (±6.6) % and 28.0 (±6.0) vs 40.6 (±9.5) % respectively.

Conclusions

Bariatric surgery achieved early and impressive weight loss in Sri Lankan obese patients, with %BWL and %BFL progressively increasing to approximately 30% and 40% at 1 year respectively. Overall, %BFL was 35-45% higher than %BWL at all follow up time intervals. Bariatric surgery appears to be very effective in body fat reduction among obese Sri Lankans.

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Variability of percentage body weight loss after bariatric surgery among Sri Lankan adults

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Introduction

Bariatric surgery is the most effective intervention for body weight loss in terms of degree of excess body weight loss. Body weight loss after bariatric surgery shows individual variation. This is the first prospective study assessing body weight loss after bariatric surgery among Sri Lankans, to the best of our knowledge.

Objective

We aimed to describe the degree of variability of body weight loss after bariatric surgery among obese Sri Lankans.

Methodology

We followed up 50 obese patients who underwent bariatric surgery at Colombo South Teaching Hospital, Sri Lanka for 1 year. Percentage body weight loss (%BWL) was calculated as body weight loss /pre-operative body weight and was categorized as <20%, 20-25%, 25-30%, 30-35% and >35%.

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/m^2 respectively. At 6 months after bariatric surgery 37.1%, 51.4% and 11.4% of patients showed PBWL of <20%, 20-25% and 25-30% respectively. None of the patients had a %BWL of >30% at 6 months. At 12 months after bariatric surgery, 11.5%, 23.1%, 23.1%, 30.8% and 11.5% showed %BWL of <20%, 20-25%, 25-30%, 30-35% and >35% respectively.

Conclusions

%BWL following bariatric surgery shows considerable individual variation among Sri Lankan adults. Approximately two thirds of the patients who underwent bariatric surgery achieved %BWL of >20% at 6 months after bariatric surgery. By 12 months after surgery approximately 40% of patients achieved %BWL of >30% while almost half of the patients achieved a %BWL of 20-30%. Interestingly, %BWL of <20% and >35% was recorded in approximately 10% of the patients respectively. This variability in body weight loss should be taken into consideration when selecting patients for bariatric surgery and counselling patients prior to surgery.

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Performance of total testosterone (tT) measured using immunoassay compared with total testosterone measured by LC-MS/MS (liquid chromatography/ mass spectrometry) in the identification of women with polycystic ovary syndrome (PCOS)

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Aims

To determine whether identification of women of PCOS differs between the use of tT measured by LC-MS/MS or a commercially available immunoassay.

Methods

A sub-group of participants in the Grollo-Ruzzene Study of Young Women's Health were invited to a sub-study which involved a pelvic ultrasound, a blood sample for serum tT by both LC-MS/MS and immunoassay (Abbott Architect Gen II) and questions about their menstrual cycle. Participants were classified as having PCOS according to Rotterdam criteria, with the LC-MS/MS tT as the reference.

Results

Of 1121 potential participants, 240 wished to participate and met the inclusion criteria and 168 had a blood sample and ultrasound. None of the 15 women with tT above the LC-MS/MS reference range were also above the reference range of the immunoassay, and no women within the LC-MS/MS reference range had an elevated immunoassay tT. Most participants with an LC-MS/MS tT just above the cut-off were considerably below the immunoassay cut off.

Replacing LC-MS/MS tT results with immunoassay tT, reduced the number of women classified as having PCOS from 31 to 26 due to 3 participants being reclassified as polycystic ovary morphology only and 2 to menstrual dysfunction only. In addition, 2 participants with all 3 criteria were reclassified as only having 2 criteria for PCOS. None of the 137 women not classified as having PCOS by LCMS were reclassified as having PCOS by the immunoassay.

Conclusion

With limited availability of LC-MS/MS, which is considered gold standard for measurement of tT, clinicians should be aware that measurement of tT by the Abbott Architect Gen II may under-identify women with PCOS.

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The utility of rested prolactin sampling in the evaluation of hyperprolactinaemia

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Introduction

Serum prolactin may be elevated by venepuncture stress(1). We investigated the utility of a rested prolactin sample, obtained through an indwelling venous cannula, in preventing overdiagnosis of hyperprolactinaemia.

Methods

Patients at our institution undergo serial prolactin sampling through an indwelling venous cannula, usually over 40 minutes, when investigating hyperprolactinaemia. We retrospectively reviewed all serial prolactin sampling performed during a three-year period. Patients taking interfering medications were excluded. Macroprolactin interference was excluded. The main outcome was normalisation of serum prolactin during serial sampling.

Results

103 patients with documented hyperprolactinaemia (range 360-1690mU/L) were included in the analysis. 50 had a normal prolactin at the start of serial sampling, 10 had an initially elevated prolactin that normalised during serial sampling, and 43 had sustained hyperprolactinaemia. The final prolactin was lower than the initial prolactin in 82 patients (80%, $p<0.001$), suggesting a prevalent effect of venepuncture stress. 49 patients (98%) with a normal prolactin at the start of serial sampling also had a normal prolactin at the end of serial sampling, suggesting the initial prolactin did not provide information additional to that provided by the final (rested) prolactin. Referral prolactin level was only modestly predictive of the likelihood of normalisation on serial sampling (AUC 0.65 females, 0.88 males, $p<0.05$ for both).

Conclusion

Serum prolactin is frequently elevated by the stress of venepuncture. Confirmation of hyperprolactinaemia in a rested sample, obtained from an indwelling venous cannula, prevents inappropriate investigation with significant associated costs and potential for unnecessary treatment.

We have changed our testing protocol for hyperprolactinaemia on the basis of these results, obtaining a single (rested) sample after 40 minutes rather than multiple (serial) samples, which we believe to be a novel approach. This reduces cost, simplifies interpretation of results, and mirrors the practice of obtaining a single rested sample for plasma metanephrines(2).

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Adiposity and other factors predicting recovery of bone density at 12 months post GDM pregnancy

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Gestational diabetes mellitus (GDM) represents an intersection between pregnancy and T2DM; both states impacting bone density (BMD) and structure. We aimed to identify factors influencing bone health after GDM pregnancy. In a longitudinal, observational study of women post-GDM, anthropometric measurements, biochemistry, DXA and lifestyle questionnaires were completed at 3 and 12 months postpartum. Data from 101 women were analysed.

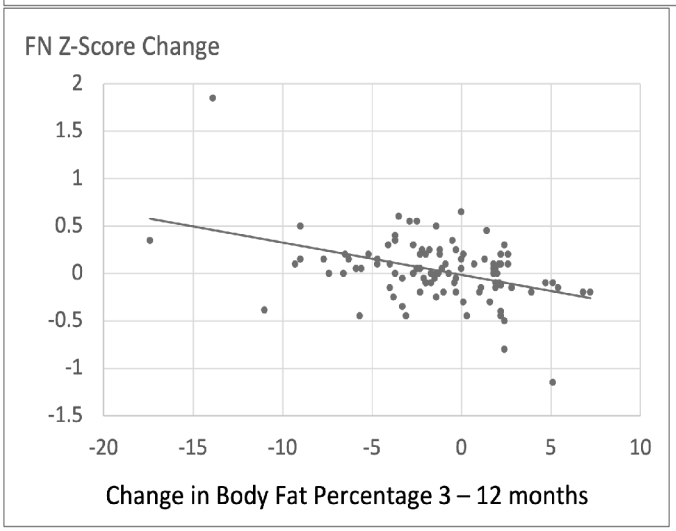
At 3 months postpartum, mean Z-scores for the lumbar spine BMD (LS) (-0.49 ± 1.00 [SD]), femoral neck (FN) (-0.29 ± 0.91), and TBS (-0.51 ± 1.08) were below the expected mean of zero. At 12 months postpartum, BMD and TBS Z-scores improved but remained below zero: LS -0.27 ± 1.02, FN -0.26 ± 0.92, and TBS -0.40 ± 0.97.

At 12 months, despite positive correlation between BMI and absolute BMD, there was inverse relationship between BMI and Z-scores at the LS (r=-0.2, p0.04). Furthermore, we observed a significant negative correlation between total-body fat percentage and Z-scores at LS (r=-0.31, p=0.001) and FN (r=-0.23, p=0.02), and between central fat percentage and Z-scores at LS (r=-0.28, p=0.005) and FN Z-score (r=-0.21, p=0.04). Change in total-body fat% from 3 to 12 months was negatively correlated with change in LS Z-score (r=-0.275, p=0.006) and FN Z-score (r=-0.412, p<0.001). Similar relationships were observed for change in BMI, but not change in central fat%.

At 12 months, women with vitamin D>50 nmol/L had significantly higher LS BMD (1.22 vs 1.15 g/cm2, p=0.004) and LS Z-score (-0.02 vs -0.73, p=0.0006) but there was no FN difference. There was no significant relationship between change in BMD Z-scores and calcium intake, vitamin D, exercise intensity or diabetes status at 12 months. Breastfeeding significantly impacted change in FN absolute BMD.

No consistent associations with TBS Z-scores were observed.

Conclusion: increased adiposity predicts slower recovery of BMD post GDM pregnancy.



Thyroxine and advice about dairy intake in pregnant and postpartum women: misconceptions around drug counselling

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Pregnancy and breastfeeding are times of increased dietary calcium requirement. Dairy foods are an important source of calcium and other nutrients. We became aware that some pregnant women taking thyroxine were being advised by pharmacy prescribing software to avoid dairy products for 2 hours after thyroxine administration, out of keeping with current evidence.

We surveyed 38 consecutive women who attended the Alfred Health Endocrinology in Pregnancy Clinic and were taking thyroxine, to determine their dairy intake and whether they had received advice to avoid dairy for specific time periods after their thyroxine dose.

Thirty-one participants were pregnant and 7 postpartum, 6 of whom were breastfeeding. Median duration of thyroxine use was 7.8 months (range 0.1-10.2 years). Only 13 of 35 women (37%) met the recommended 2½ serves of dairy/day(1). One woman met the higher recommendation of 4 serves/day(2). Thirteen participants (34%) reported being instructed to avoid dairy with thyroxine; 8 of which had been given a time frame (1-2 hours).

We found that in 2019 the Australian Pharmaceutical Formulary (APF) Handbook(3) recommended dispensing of thyroxine with a label stating 'Do not take dairy products, antacids or mineral supplements within two hours of each dose of this medicine' despite the lack of evidence for this recommendation. We wrote to the medical dispensing software companies and the APF New Drugs Advisory Group to advise of this inaccuracy. This resulted in the APF amending their recommendation to remove mention of dairy products from this label(4).

Hypothyroidism requiring thyroxine replacement is common in pregnancy and postpartum, a period when maternal calcium requirements are concurrently high. We found most pregnant and postpartum women taking thyroxine do not meet the recommended intake. As such, it is important that thyroxine labelling does not pose a barrier to adequate dietary calcium intake in this population.

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Testosterone concentrations in men: Individual Participant Data meta-analyses from the Androgens In Men Study.

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Background

Different factors modulate circulating testosterone in men, impacting interpretation of reference ranges. By conducting an individual participant data (IPD) meta-analysis of major cohort studies measuring testosterone accurately, we aimed to clarify sex hormone concentrations and factors associated with these in men.

Methods

Systematic literature searches (June-December 2019) identified prospective cohort studies of adult men with total testosterone measured using mass spectrometry. IPD data were requested. Cross-sectional analyses related total testosterone, sex hormone binding globulin (SHBG), luteinising hormone (LH), dihydrotestosterone (DHT) and estradiol concentrations to sociodemographic, lifestyle, and health factors. Summary curves and summary effect estimates with 95% confidence intervals (CIs) were obtained using two-stage random-effects IPD meta-analyses (PROSPERO: CRD42019139668).

Findings

Summary estimates were obtained from 11 studies (25,364 adult men). There was a non-linear association of testosterone with age, with negligible change among 17-70 year olds (per SD increase age -0.27 nmol/L, CI -0.71,0.18) and decreasing testosterone with age for men >70 years (-1.24 nmol/L, CI -1.61,-0.87). Testosterone was inversely associated with BMI (per SD increase BMI -2.42 nmol/L, CI -2.70,-2.13). Testosterone concentrations were lower for men who: were married/de facto (-0.57 nmol/L); undertook ≤75 minutes vigorous physical activity/week (-0.51 nmol/L); former smokers (-0.34 nmol/L); had hypertension (-0.53 nmol/L), cardiovascular disease (-0.35 nmol/L), cancer (-1.39 nmol/L), or diabetes (-1.43 nmol/L); all CIs <0.0. SHBG increased with age and decreased with BMI, while LH increased non-linearly with age. DHT and estradiol were less prominently associated with these factors.

Interpretation

Multiple factors are associated with variation in male testosterone and SHBG concentrations, with evidence of primary impairment of testicular hormone production after age 70 years. Interpretation of testosterone results in individuals needs to account for these factors, especially age >70 years, BMI, diabetes and cancer. Further research is needed to determine health impacts of declining testosterone in older men.

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Hypoglycaemia in Eating Disorders

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Eating disorders are characterised by a persistent disturbance of eating-related behaviour that results in the altered consumption or absorption of food, and significantly impairs physical health or psychosocial functioning.[1] Hypoglycaemia is common in eating disorders. An US cohort study of adult inpatients with severe anorexia nervosa (AN) found that a blood glucose level (BGL) < 3.3mmol/L occurred in 44% of patients and a BGL < 2.2 mmol/L occurred in 12% of patients.[2] Severe derangement of liver enzymes predicted the development of mild hypoglycaemia.[2]

Hypoglycaemia may have deleterious effects on patients' ability to perform high risk tasks safely, especially when there is impaired hypoglycaemia awareness. The pathophysiology of hypoglycaemia in eating disorders is multifactorial. Dietary restriction and excessive exercise results in the depletion of hepatic glycogen stores and impaired hepatic gluconeogenesis.[3] Impaired glucagon secretion has also been described.[4] Refeeding can also result in hypoglycaemia, particularly in those with prolonged and severe malnutrition. An Australian cohort study of inpatients at a specialist eating disorders unit found that, following a mixed meal, 22% of patients recorded a postprandial BGL <3.5 mmol/l and 3.9% of patients recorded a postprandial BGL < 2 mmol/L.[5] Only low BMI significantly predicted postprandial hypoglycaemia.[5]

There is limited case report data to suggest that postprandial hypoglycaemia may occur with refeeding after starvation as a result of exaggerated insulin secretion during the very early phase.[6] Early studies in people with AN produced inconsistent results regarding insulin sensitivity[7, 8], and the glucose and insulin response post-glucose load has been shown to vary based on OGTT results prior to refeeding.[9] There is limited data to suggest that insulin clearance is significantly increased in people with AN[10].

This presentation will include a critical review of the existing literature on hypoglycaemia and eating disorders, and we will also discuss usual practice and management strategies in the context of existing guidelines.

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Durvalumab-induced hypoparathyroidism

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A 64 year old man with Stage III non-small cell lung cancer treated with Durvalumab (August 2018-August 2019) presented with diarrhoea, carpopedal spasm and prolongation in QTc interval. There were no contributing medications, including proton pump inhibitor, anti-resorptive agents and diuretics. There was no past history of any surgery or radiotherapy to the head and neck regions, nor was there a family history of autoimmune disorders. Investigations revealed marked hypocalcemia hyperphosphatemia, normomagnesemia, mild Vitamin D deficiency, acute renal impairment and a suppressed parathyroid hormone [PTH] level (Table-1). Repeat testing when normocalcemic (2.31 mmol/L) showed persistently low PTH (0.8 pmol/L). Mild hypocalcemia coincided with the commencement of Durvalumab and had persisted until presentation to hospital. He was diagnosed with Durvalumab-induced hypoparathyroidism. Treatment included intravenous and oral calcium, cholecalciferol and calcitriol.

Increasing use of immune checkpoint inhibitors (ICI), such as Durvalumab that targets programmed death-ligand 1 (PD-L1) has reduced self-tolerance and exposed discrete group of immune-related adverse effects (irAE) [1]. Endocrinopathies are common, but hypoparathyroidism is a rare irAE. Only case reports have described its occurrence, and none have been reported among patients treated with Durvalumab [1-3]. Hypocalcaemia has been reported in a small proportion of patients treated with Durvalumab [4].

Unlike other irAEs, endocrinopathies often require lifelong treatment with permanent hormone replacement for unclear reasons [5]. Persistence of hypoparathyroidism despite discontinuation of immunotherapy has been previously recognised

[6]. The mechanism of ICI-related hypoparathyroidism remains unclear. It is postulated that anti-parathyroid and CASR-activating autoantibodies are implicated in its pathophysiology [7]. Further research is needed to elucidate the role of these antibodies and whether ICI-associated hypoparathyroidism is a destructive autoimmune process like in other endocrinopathies.

Further assessment of hypocalcemia is warranted among patients treated with ICI. The aforementioned autoantibodies are not commercially available for use in routine clinical practice.

Table 1. Laboratory investigations

	Baseline prior to Durvalumab (Aug 2018)	At completion of Durvalumab (Aug 2019)	On admission in September 2021	At Discharge in September 2021
Corrected calcium (mmol/L)	2.46	2.03	1.63	2.61
Magnesium (mmol/L)	0.86	0.90	0.74	0.92
Phosphate (mmol/L)	1.11	1.34	1.94	0.64
Vitamin D level (nmol/L)		48	38	-
Parathyroid hormone level (pmol/L)		2.2	1.1	0.8
TSH (mIU/L)	0.71	2.63	1.23	-
Creatinine (umol/L)	76	117	219	117
eGFR (mL/min/1.73m2)	>90	57	26	56

Deterioration in glycaemic control in a patient with a pancreatic mass: a prompt to consider Glucagonoma

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Glucagonoma is a rare functioning pancreatic neuroendocrine tumour (P-NET), with an incidence of 0.01-0.1 per million per year (1). We report a case in a 64-year-old man with a 15-year history of well-controlled type 2 diabetes who presented with significant deterioration in glycaemic control with associated intermittent diarrhoea, weight loss, intermittent aphthous ulceration and a migratory rash.

CT pancreas and ^{68}Ga-DOTATATE PET demonstrated a 20 mm DOTATATE-avid pancreatic tail lesion with 2-3 sub-centimetre DOTATATE-avid lesions in the uncinate process without metastatic disease (Figure 1). Pancreatic polypeptide level was elevated at 759 pmol/L and fasting glucose was 16.2 mmol/L with a markedly increased glucagon level of 508 pg/mL (reference range 40-140), consistent with glucagonoma.

He underwent laparoscopic distal pancreatectomy and splenectomy. Histopathology revealed a 19 mm pancreatic well-differentiated (Grade 1) neuroendocrine tumour with a Ki-67 index of 2%. Immunoperoxidase staining was positive for synaptophysin and glucagon, consistent with a glucagonoma. Glycaemic control improved in the immediate postoperative period with resolution of skin rash and diarrhoea.

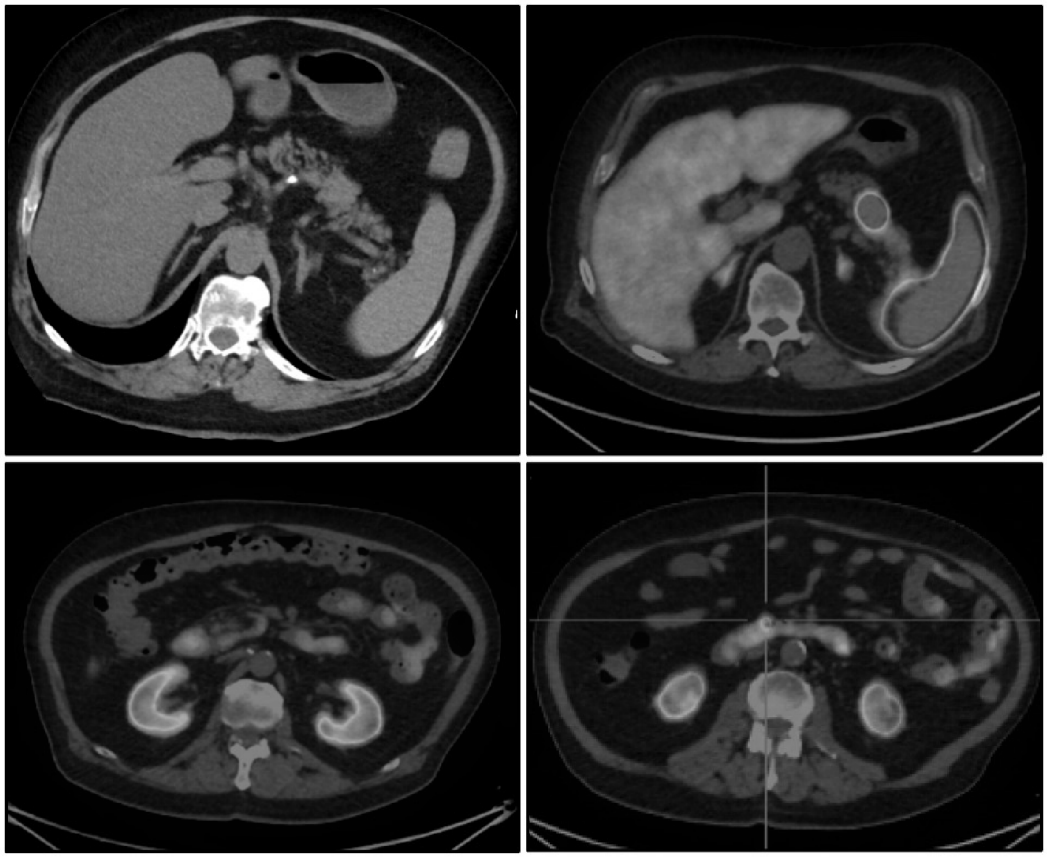
Four months postoperatively he required recommencement and rapid uptitration of insulin therapy. The glucagon level decreased to 317 pg/mL one month post operation and 331 pg/mL after six months. Given his ongoing increased insulin requirements, elevated glucagon and remaining pancreatic lesions, endoscopic ultrasound with possible microwave ablation is planned.

First line therapy for locoregionally confined glucagonoma should be surgical resection or radiotherapy ablation where technically feasible. Treatment options for advanced disease include somatostatin analogues, mTOR inhibitors or chemotherapy, with peptide receptor radionuclide therapy emerging as a promising option for metastatic disease (2).

Our case highlights the clinical, biochemical and imaging features of glucagonoma. Poorly controlled diabetes is frequently encountered in clinical practice, thus detailed history of associated symptoms, particularly in patients with pancreatic

lesions, may prompt consideration of this rare condition.

Figure 1.



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High dose phenoxybenzamine in a young patient with phaeochromocytoma and likely marijuana withdrawal: A case report

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Background: Surgical resection of phaeochromocytomas requires careful planning. The Endocrine Society guidelines recommend combined alpha and beta-adrenergic blockade to reduce peri-operative morbidity and mortality. Phenoxybenzamine is the preferred agent for alpha-adrenergic blockade with a recommended starting dose of 10mg BD, and a maximum suggested dose of 1mg/kg/day (1). There is limited evidence for the use of phenoxybenzamine above this dose.

Case: We present the case of a 23-year-old Caucasian male with a 5cm left-sided phaeochromocytoma and severe hypertension (BP>200/130mmHg), complicated by grade 3 hypertensive retinopathy, macroalbuminuria and left ventricular impairment. He was symptomatic with palpitations, diaphoresis, and headaches. His 24-hour urine normetanephrine was 30.2 umol/24hr (0-3.5) and plasma normetanephrine was 7290 pmol/L (<570). Plasma metanephrine and 3-methoxytyramine were not elevated. Ga-68 DOTATATE-PET/CT confirmed the absence of metastatic disease. The patient engaged in recreational drug use consuming marijuana 1.5g daily, occasional LSD and ketamine.

Despite reaching the recommended 1mg/kg/day of phenoxybenzamine, he continued to have significant hypertension with no postural change. Marijuana withdrawal was considered as a potential contributor to his hypertension, and PRN benzodiazepines were administered. Phenoxybenzamine was progressively uptitrated to a dose of 120mg every 6h with additional Amlodipine 10mg every 12h and Metoprolol 100mg every 12h, to achieve the pre-operative BP 100/70mmHg with a significant postural drop. He was also treated with oral and intravenous sodium chloride prior to surgery. The peri-

operative course was uneventful with successful surgical resection, discontinuation of all antihypertensive agents post-operatively and no post-operative hypotension.

Conclusion: This case demonstrates high doses of phenoxybenzamine may be required in young adults with a shorter duration of hypertension and greater arterial compliance, and the challenges of managing marijuana withdrawal in the setting of peri-operative adrenergic blockade for phaeochromocytoma. It highlights the safe administration of high dose phenoxybenzamine in a unique circumstance and the importance of individualised care.

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Polyuria and Acute Myeloid Leukaemia – an Unusual Cause of Central Diabetes Insipidus

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Background: Central diabetes insipidus (DI) occurs due to inadequate secretion of antidiuretic hormone (ADH) from the neurohypophysis resulting in hypotonic polyuria and hypernatraemia.[1] Acquired central DI can occur due to disturbance of the neurohypophysis such as trauma, infection, ischaemia or malignancy. Acute myeloid leukaemia (AML) can be a rare, acquired cause of central DI.[2]

Case: A 70-year-old Caucasian female was admitted with febrile neutropenia in the context of a 2-week history of polyuria and polydipsia, drinking up to 4L water daily. Initial blood film demonstrated >80% blasts, and the diagnosis of AML was confirmed by bone marrow biopsy. Cytogenetic analysis identified the inv(3q) and monosomy 7 karyotype. PET scan and biopsy also demonstrated leukaemic infiltration of the left submandibular lymph node. However, CSF cytology x3 did not identify any malignant cells. The diagnosis of DI was confirmed following overnight water deprivation, resulting in morning (0835) serum sodium 154mmol/L, serum osmolality 306mmol/L, urine sodium 37mmol/L, urine osmolality 146mmol/L and copeptin 3.2pmol/L. MRI Brain identified absence of the posterior pituitary bright spot with mild abnormal enhancement of the infundibulum and hypothalamus, which was suggestive of infiltration. Treatment was commenced with desmopressin. The dose required for stabilisation of urine output and serum sodium rapidly increased from 200mcg nocte to 200mcg mane/1000mcg nocte. She was treated with FLAG-Ida chemotherapy and intrathecal methotrexate, which was complicated by suspected cytarabine syndrome and colitis. Unfortunately, the patient developed neutropenic sepsis with progressive type 1 respiratory failure, and died upon transition to comfort care.

Conclusion: This case demonstrates the unusual situation of AML-induced central DI. The presence of inv(3q) and monosomy 7 chromosomal abnormalities are associated with the development of central DI, and is a poor prognostic marker.

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Hypocalcaemia in malignancy: A case of prostate cancer with osteoblastic metastases

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Background: Prostate cancer is the most common non-skin cancer in Australian men.[1.2] Metastatic prostate cancer may be associated with osteoblastic lesions that consume calcium for bone formation.[3]

Case: A 77-year-old Caucasian male was admitted with gastrointestinal bleeding on a background of bowel telangiectasia, castrate-resistant metastatic prostate cancer (BRCA2-associated) and oesophageal cancer in remission. Blood tests

identified a new onset asymptomatic hypocalcaemia that was not present on admission, with corrected calcium 1.92mmol/L, ionised calcium 0.99mmol/L, phosphate 0.62mmol/L, magnesium 0.88mmol/L, PTH 23.3pmol/L, eGFR>90mL/min, 25OH vitamin D 18nmol/L, and ALP 174U/L. Whilst he had received subcutaneous Denosumab 120mg, his serum calcium levels were initially within normal range and only declined 30 days following administration. Serum calcium levels remained low despite cessation of PRBC transfusions, replacement of 25OH vitamin D deficiency, hypocalciuria (calcium-to-creatinine ratio <0.1mmol/mmol) and treatment of prostate cancer with Docetaxel and androgen deprivation therapy with Leuprorelin and Enzalutamide. PSMA-PET and CT chest, abdomen and pelvis confirmed the presence of extensive sclerotic bony metastases with multiple pathological fractures. Further investigations identified significant elevations in PSA 2,162ug/L (0.3-7.5), bone formation marker P1NP 185ug/L (15-115), and bone resorption marker urine DPD 5.9nmol/mmol (2.3-5.4), which is consistent with bone formation of osteoblastic metastases. Treatment with Caltrate 1200mg TDS, calcitriol 0.5mcg QID and cholecalciferol 5000IU daily was required to achieve and maintain normocalcaemia. Due to poor functional status, he was not suitable for further anti-cancer therapy. He was re-admitted with gastrointestinal bleeding and experienced a cerebrovascular accident. Unfortunately, his condition deteriorated, and he died upon transition to comfort care.

Conclusion: This case demonstrates osteoblastic metastases as an atypical cause of hypocalcaemia. Added contributors such as vitamin D deficiency with anti-resorptive usage need to be considered. Without effective prostate cancer treatment that could reverse osteoblastic metastases, there will be ongoing hypocalcaemia and requirement for high dose calcium replacement.

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Cushioning the blow of ectopic ACTH syndrome

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A 77-year-old male presented with rapid onset peripheral oedema and proximal myopathy. Computed tomography revealed diffusely enlarged adrenal glands, liver metastases, and a small spiculated lung lesion. Cushing's syndrome was confirmed with markedly elevated 24-hour urine free cortisol levels. The 8mg dexamethasone suppression test indicated an ectopic source of cortisol. FDG-PET revealed low grade uptake in the lung and hepatic lesions. Ga68-DOTATATE PET was negative. Core biopsy of a liver lesion confirmed metastatic neuroendocrine tumour (NET) with immunohistochemistry suggesting a primary lung origin. Metyrapone and ketoconazole successfully reduced 24-hour urine free cortisol but multiple complications of hypercortisolism still developed. The absence of DOTATATE uptake eliminated the possibility of peptide receptor radionuclide therapy (PRRT). Other management strategies of ectopic ACTH syndrome (EAS) were considered and bilateral adrenalectomy was ultimately performed. The metastatic NET was managed with active surveillance and remains stable after eight months.

EAS is a rare condition that reflects excessive ACTH secretion by NETs, most commonly by well-differentiated pulmonary NETs (1,2,3). Management of metastatic EAS requires consideration of the tumour risk against the hormonal risk. Options for tumour control are limited in metastatic EAS as there is minimal evidence for targeted therapies although PRRT may be effective (1,2,3). Management of hypercortisolism includes pharmacologic treatment, which is limited by tolerability and efficacy, or bilateral adrenalectomy, which is effective but associated with post-operative morbidity and permanent glucocorticoid requirement (1,2,4). In several large case series, bilateral adrenalectomy was required for most patients with metastatic disease and was associated with better survival (3,4,5,6). In conclusion, EAS is most commonly due to pulmonary NETs which often have an excellent tumour prognosis due to their indolent nature. In such cases, management of hypercortisolism is a priority and bilateral adrenalectomy may be required, particularly in patients with metastatic disease to provide effective long-term control.

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Intraoperative Surprise - A Case of a Bladder Paraganglioma

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We describe a case of a 63-year-old Chinese female presenting with a year long history of headaches, palpitations and lower abdominal fullness on a background of hypertension. Initial investigations were suggestive of malignancy with elevated tumour markers; CA125 58 U/ml (N: <30) and CA19.9 126 U/ml (N: <39). A pelvic ultrasound revealed a hypervascular bladder wall mass measuring 22 x 15 x 16mm, concerning for a transitional cell carcinoma. MRI pelvis further characterised the lesion to have extended outside the bladder wall and demonstrated diffusion restriction.

An elective laparoscopic total abdominal hysterectomy, bilateral salpingo-oophorectomy, cystoscopy and TURBT was organised. At the time of the biopsy the blood pressure spiked to 210/120mmHg from 120/80mmHg which was managed with intravenous hydralazine. Serum metanephrines and normetanephrines were ordered and were elevated at 640pmol/L (N: <447) and 1920pmol/L (N: <1160) respectively. Histopathology confirmed the diagnosis of a bladder paraganglioma. A PET Gallium-68 DOTATE scan found no significant DOTATE activity within the bladder wall or any metastatic disease. The patient was pre-operatively stabilised on phenoxybenzamine and propranolol commenced three days later. A cystoscopy and robotic assisted partial cystectomy was performed successfully 10 days later with minimal handling of the mass. The patient had an excellent post operative recovery with remission of her hypertension and palpitations.

Bladder paragangliomas are extremely rare neuroendocrine tumours accounting for less than 0.06% of bladder tumours and less than 1% of all phaeochromocytomas and paragangliomas (1). The most common symptoms include painless haematuria, hypertension and micturition attacks (2). Due to the rarity of these tumours diagnosis is often difficult. CT and MRI may play a role in assisting with diagnosis preoperatively such that a catecholamine crises may be avoided intraoperatively. On MRI these tumours have T2 hyperintensity, slight T1 hyperintensity compared to muscle and marked restricted diffusion on DWI (3).

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Severe hyponatraemia with cerebral oedema after Pfizer BNT162b2 mRNA vaccination against COVID-19

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Background: Severe hyponatraemia can lead to serious neurological complications including coma, seizure and death. Hyponatraemia and the Syndrome of Inappropriate Antidiuretic Hormone (SIADH) has been previously described in cases of COVID-19, however there have been few reports post vaccination. We describe a case of severe hyponatraemia post second Pfizer BNT162b2 mRNA vaccination against COVID-19.

Case presentation: A 48-year-old previously well woman presented to the emergency department with severe headaches and confusion one day after she received her second Pfizer COVID-19 vaccination. She reported thirst and increased fluid intake in response to feeling unwell but no more that 2.5L. Vital signs were normal. Laboratory investigation revealed a serum sodium of 113mmol/L, potassium of 3.4mmol/L, urea 3.5mmol/L and serum osmolality of 266mmol/kg. TSH, random cortisol and C-reactive protein levels were normal. She was found to be in urinary retention and developed marked polyuria post in dwelling catheter insertion. Following this she underwent spontaneous and rapid correction of serum sodium without intervention. Retrospective analysis showed an inappropriately high copeptin of 4.4pmol/L.

Conclusions: It is important to be cautioned and aware of hyponatraemia as an immediate side effect of COVID-19 vaccination. The exact mechanism is unknown and further research is required to understand the acute endocrine effects which may arise in response to COVID-19 vaccination.

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Adrenal tuberculosis – an old disease worth remembering.

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A 60-year-old female was referred for a left adrenal 35-mm mass detected on imaging for weight loss over one year, associated with anorexia, nausea and abdominal discomfort. Her background included hypertension and seronegative arthritis on prednisolone. Biochemical evaluation showed no adrenal hormone hypersecretion and left adrenalectomy was performed due to concern of malignancy. Subsequent histology showed necrotising granulomas and FDG-PET/CT revealed FDG-avidity in contralateral adrenal, thoracic and right cardiophrenic lymph nodes. She emigrated from Burma to Australia 30 years ago. Prednisolone was ceased and empirical rifampicin, isoniazid, pyrazinamide, ethambutol was initiated for tuberculosis. One week later, she was admitted with an adrenal crisis. Primary adrenal insufficiency was confirmed on Short Synacthen test and elevated ACTH. Adrenalectomy likely reduced reserve, then rifampicin CYP3A4-induction increased cortisol clearance. Retrospectively, she had two years of skin hyperpigmentation, thought to be lichen planus pigmentosus, then melasma. This improved following hydrocortisone replacement, and in hindsight was related to primary adrenal insufficiency. After eight months of anti-tuberculous treatment, there was resolving FDG-activity of right cardiophrenic and thoracic lymphadenopathy suggesting treatment response. Moderate activity in the right adrenal with normal appearance on CT persists.

Since Thomas Addison first described primary adrenal insufficiency from adrenal TB, its incidence has declined in developed countries.[1] In patients presenting with either adrenal masses or insufficiency, it should still be considered as a differential in patients with appropriate demographic and risk factors. Diagnosing adrenal TB may not be straightforward if adrenal resection is not indicated or extra-adrenal sites not obvious or biopsy-amenable. TB in the adrenal may also not necessarily display imaging abnormality. FDG-PET/CT is sensitive in evaluating activity, extent, and treatment response in pulmonary TB, however use for monitoring treatment response in extra-pulmonary disease is unclear.[2,3] There are only rare case reports of recovery of adrenal function following anti-tuberculous treatment.[4]

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Another stroke of bad luck in a case of premature ovarian insufficiency

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Premature ovarian insufficiency (POI) is defined as menopause prior to the age of 40. Women who experience POI are at increased risk of reduced bone density, cardiovascular disease, mood disorders, cognitive impairment, sexual dysfunction and have a shorter life expectancy (1). A recent cross-sectional study has suggested women with POI may experience more severe symptoms than women who undergo natural menopause (2). Women with POI should be offered menopausal hormone replacement therapy (MHT) up to the age of 50 years for relief of symptoms resulting from low oestrogen and be counselled on the role of MHT on bone and cardiovascular protection (1,3).

We present a case of a 37-year-old who presented with irregular periods for three years prior associated with vasomotor (VSM) and genitourinary symptoms (GSM). Workup demonstrated high gonadotropins and low oestradiol. Screening investigations for known causes of POI were unremarkable. Medical history included a left middle cerebral territory infarct at 16 weeks of pregnancy (5 years prior) associated with narrowing of the left angular artery; previous migraines with visual and sensory aura; mild dyslipidemia; and a family history of venous thromboembolism and ischaemic heart disease. Post stroke the patient had been advised to avoid further pregnancies and the oral contraceptive pill.

We will review evidence and rationale for use of MHT in the context of severe menopausal symptoms and prior stroke in a young patient with POI, and the importance of collaboration with other specialties in the workup of this complex case. Finally, we will discuss alternative treatment in the form of non-hormonal based therapies.

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An atypical presentation for pancreatic neuroendocrine tumours and new diagnosis of Multiple Endocrine Neoplasia Type 1 with a likely pathogenic variant c.459C/G

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Multiple Endocrine Neoplasia Type 1 (MEN-1) is an autosomal dominant condition associated with tumours of the pancreas, parathyroid and pituitary gland [1]. Amongst pancreatic neuroendocrine tumours, 60-90% are non-functioning [2,3]. We discuss the atypical presentation of MEN-1 with a perforated viscus in the context of newly diagnosed pancreatic neuroendocrine tumours and primary hyperparathyroidism.

A 33-year-old Caucasian male presented with a duodenal perforation complicated by peritonitis and septic shock. PTH-dependent hypercalcaemia (corrected calcium 3.09mmol/L and PTH 73.1pmol/L) and ureteric calculi were incidentally identified in the context of longstanding hypercalcaemia, recurrent ureteric calculi and a multigenerational family history of primary hyperparathyroidism. Investigations identified a 21mm parathyroid lesion, multiple FDG-avid bony lesions most consistent with Brown's tumour, a left forearm T-score -3.3 SD consistent with osteoporosis and multiple Ga-68 DOTATATE-PET avid pancreatic lesions.

He was initially treated with a left parathyroidectomy and partial thyroidectomy followed by a completion parathyroidectomy. Histopathology confirmed left superior and right parathyroid adenoma with right parathyroid hyperplastic nodules. Endoscopic Ultrasound guided biopsy confirmed the presence of Grade 1 Pancreatic Neuroendocrine Tumours. There were elevations of serum chromogranin A 1147 mcg/L (<102), glucagon 306pg/mL (<208), pancreatic polypeptide 228pmol/L (<=55) and gastrin 65pmol/L (5-55) in the context of proton pump inhibitor usage. However, he had no clinical features of a VIPoma, insulinoma or glucagonoma, and his serum gastrin levels were not grossly elevated despite his presentation with a duodenal perforation. Genetic studies confirmed a variant c.459C/G in exon 3 of the MEN1 gene consistent with MEN1 syndrome.

This case demonstrates the presentation of duodenal perforation which led to a formal diagnosis of pancreatic neuroendocrine tumours and MEN1 syndrome. The hypercalcaemia likely has exacerbated the gastrin production and resulted in the duodenal perforation. It highlights the importance of considering MEN diagnosis in young onset primary hyperparathyroidism to prevent potential neuroendocrine related complications.

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Cushing's disease presenting as an embolic stroke in a young patient with patent foramen ovale

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Introduction

Cushing's syndrome has been linked with increased incidence of cardiovascular and venous thromboembolic disease[1,2]. Here we report a case of a young woman presenting with embolic stroke with previously undiagnosed patent foramen ovale (PFO) and Cushing's disease.

Case Description

A 35-year-old female presented with stroke-like symptoms including sudden-onset left hemiparesis and visual field loss. Her background medical history included type 2 diabetes mellitus (short duration), hypertension, being overweight (BMI 26) and PCOS. MRI confirmed multi-territory cerebral infarcts and CT Angiogram (Neck/Brain) was unremarkable. Thrombophilia screen and stroke workup, including prolonged Holter monitoring, were also unremarkable apart from a large PFO on echocardiogram.

During her hospital admission, she was noted to have clinical features of Cushing's syndrome and subsequent outpatient biochemical testing (table 1) indicated ACTH-dependent Cushing's disease. She was commenced on therapeutic anticoagulation which was changed to dual antiplatelets for three months after the PFO closure with Amplatzer Talisman PFO Occluder.

Pituitary MRI showed microadenoma and Cushing's disease was confirmed with petrosal sinus sampling, arranged after PFO closure. She was commenced and maintained on metyrapone until trans-sphenoidal resection of pituitary microadenoma six months after cardiac procedure to allow safe discontinuation of antiplatelets.

She had resolution of biochemical and clinical Cushing's disease after pituitary surgery. She remains on replacement hydrocortisone 18 months later with good neurological recovery and no further cardiovascular, venous or metabolic complications.

Discussion and Learning Points

Our case highlights the importance of considering Cushing's syndrome as a cause of acquired thrombophilia in younger patients with cardiovascular disease. The pathogenesis involves both over-activation of coagulation cascade (resulting in low APTT) and reduced fibrinolytic activity[1]. Our patient had previous diagnoses of PCOS, Type 2 Diabetes Mellitus and

hypertension, which in retrospect were all likely related to pathological hypercortisolism and went into remission following pituitary surgery.

	Test #1	Test #2	Test #3	Test #4	Reference Range
Overnight Dexamethasone Suppression Test (nmol/L*)	276				>50
24-hour urinary free cortisol (nmol/day)	1200	1600			<900
Midnight Salivary Cortisol (nmol/L)	10	8.6	11	12.1	<5.7
ACTH (pmol/L)	14.3	12.1			<10

Table 1: Initial biochemical tests supporting diagnosis of ACTH-dependent Cushing's disease

Time		ACTH (pmol/L)	IPS: Peripheral ACTH Ratio
11.57	L Petrosal Sinus 1	9.5	1.1
	R Petrosal Sinus 1	9.9	1.1
	Peripheral 1	8.8	
11.58	L Petrosal Sinus 2	9.8	1.1
	R Petrosal Sinus 2	432	47
	Peripheral 2	9.2	
12.00	L Petrosal Sinus 3	10.3	1.1
	R Petrosal Sinus 3	16.3	1.7
	Peripheral 3	9.7	

Table 2: Results of Inferior Petrosal Sinus Sampling
Interpretation: The central: peripheral ACTH ratio of right petrosal sinus 2 is significantly greater than 2, whilst the central: peripheral ratio is < 2 in all 3 left sided samples. Conclusion: Consistent with central ACTH production and assuming no anomalous venous drainage is supportive of right sided ACTH pituitary secretion.

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Langerhan Cell Histiocytosis following Selpercatinib use in Metastatic MTC

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Background: RET alterations are found in 60% of sporadic medullary thyroid cancers (MTCs). RET specific tyrosine kinase inhibitors (TKI), selpercatinib and praseltinib, have improved progression free survival in metastatic patients with tolerable adverse effects. Langerhans cell histiocytosis (LCH), an unrelated clonal neoplasm of myeloid dendritic cells, is usually driven by alterations in the MAPK pathway. We report an MTC patient who developed LCH whilst being treated with selpercatinib and postulate that inhibition of RET may have led to paradoxical activation of its downstream pathway and driven the progression of LCH.

Clinical Case: A 22 year old female non smoker presented with widely metastatic MTC with serum calcitonin of 25,600ng/L. After debulking surgery a somatic *RETM918T* alteration was identified and she was commenced on selpercatinib. She had a partial response with minimal adverse effects. 24 months after treatment, 3-4mm lung nodules were identified on imaging. Although asymptomatic, the nodules doubled in size in 8 weeks. ^{18}FDG-PET scan showed low avidity. Core biopsy revealed an inflammatory infiltrate rich in eosinophils with lesser numbers of histiocytes including neoplastic Langerhans cells. Molecular testing identified a complex BRAF mutation *BRAF p.(V600_K601>D)* at a variant allele frequency of 4.76% in keeping with expected neoplastic cellularity and confirming the diagnosis of pulmonary LCH. Although this BRAF alteration has not previously been reported in LCH, it has been reported in melanoma where it is responsive to BRAF/MEK inhibition. As the patient is asymptomatic, she was commenced on inhaled steroids and close monitoring.

Conclusion: This is the first case report of RET kinase inhibition and concurrent LCH. We speculate treatment with a RET specific TKI may activate dormant LCH driven by a BRAF mutation. We recommend continued vigilance for the possibility that RET inhibition may activate other underlying neoplasms with mutations in the MAPK pathway.

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Extreme catechol-O-methyltransferase pathway activity resulting in giant but silent composite phaeochromocytoma

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Introduction: Clinically silent phaeochromocytoma (PCC) are increasingly recognized but are typically small with relatively low levels of metanephrines. However, upregulation of catechol-O-methyltransferase (COMT), the enzyme that inactivates catecholamines to their metabolites in PCC, can cause clinical silence despite significant size and biochemical activity demonstrated by elevated metanephrine and normetanephrine levels. Composite PCC comprise PCC and a second tissue sharing embryological origin from the neural crest, including ganglioneuroma. While fewer than 100 cases have been described, they demonstrate similar natural history and prognosis to other PCC. Composite tumours are less frequently associated with most germline mutations causing PCC but more strongly associated with neurofibromatosis type 1.

Case: We present a 62-year-old woman with a clinically silent giant PCC, incidentally discovered on investigation for pulmonary embolism. Further imaging revealed a 15 cm adrenal mass and a second 7 cm pelvic mass. Prior to endocrine referral a biopsy of the adrenal mass suggested neuroblastoma. No haemodynamic compromise ensued with biopsy. Biochemical findings summarised in Table 1 confirmed a PCC with metanephrine excretion 23-fold normal despite normal catecholamines. The patient has no clinical or familial features of NF1 or other hereditary syndromes associated with PCC.

Surgical resection was uneventful after 6 months anticoagulation and preoperative alpha blockade. Histopathology showed a composite PCC/ganglioneuroma with discrete areas of both tumours within the same mass. Metanephrines and normetanephrines normalised 6 weeks post-operatively and have remained normal. Later resection of the pelvic mass revealed an unrelated ovarian teratoma. Genetic testing is yet to be performed as she does not qualify by local criteria.

Conclusion: Extreme activity of COMT, beyond typical for PCC, is likely to have caused intratumoural inactivation of catecholamines and facilitated asymptomatic growth of this tumour to its giant size. Composite PCC are rare but have a similar clinical presentation and management to other phaeochromocytoma.

	Normal Range	Value
Plasma Metanephrine	(<500 pmol/L)	**2749**
Plasma Normetanephrine	(<900 pmol/L)	**5537**
Plasma 3-Methoxy-Tyramine	(<150 pmol/L)	148
24-hour urine Noradrenaline	(<450 nmol/24hr)	435
24-hour urine Adrenaline	(<150 nmol/24hr)	128
24-hour urine Dopamine	(<3500 nmol/24hr)	7436
24-hour urine Metanephrine	(<1.3 umol/24hr)	**30.5**
24-hour urine Normetanephrine	(<3.0 umol/24hr)	**28.2**
24-hour urine 3- Methoxy- Tyramine	(<2.7 umol/24hr)	4.3

Table 1: Preoperative biochemical evaluation of adrenal medulla

The importance of using complementary imaging modalities A case of metastatic insulinoma not identified on GA-68 DOTATATE PET

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Insulinoma is the most common functioning pancreatic neuroendocrine tumour, yet remains uncommon with 4 cases per million person years[1]. Metastatic insulinoma is exceedingly rare, comprising only 6% of insulinomas[2]. Localisation of neuroendocrine tumours can be performed via Ga-68 DOTATATE-PET, a functional imaging modality where the tracer binds to Somatostatin subtype 2 receptors, which are often expressed in insulinomas[3].

A 64-year-old Caucasian male was investigated for recurrent severe episodes of feeling unwell which satisfied Whipple's Triad for hypoglycaemia. Biochemical investigations after a limited fast demonstrated a formal plasma glucose level of 1.7mmol/L with inappropriately detectable C-peptide 0.40nmol/L (0.4-1.5), insulin 8mU/L (0-20) and pro-insulin 48.5pmol/L (<13.3), which suggested an insulinoma. Ga-68 DOTATATE-PET scan identified focal uptake in the body of the pancreas (SUV 9.7) but nil metastatic lesions. CT Abdomen and MRI Pancreas confirmed a pancreatic lesion, but also identified multiple arterial enhancing liver lesions. Surgical resection of the pancreatic and liver lesions were performed with histopathology of the lesions confirming the presence of a Grade 2 Neuroendocrine Tumour. Post-operative MRI identified multiple persistent liver lesions, but repeat Ga-68 DOTATATE-PET scan only identified a single avid liver lesion (SUV 27.2). Treatment with diazoxide, dexamethasone, cornstarch, Flash #2 glucose monitoring and Sandostatin LAR delivered an initial improvement in glycaemia. A glucagon stimulation test showed safety in its emergency administration. Ongoing therapy is being guided by a multidisciplinary team specialising in Neuroendocrine tumour care, with planned Lutate therapy.

This case demonstrates the unusual situation where Ga-68 DOTATATE PET localised the primary insulinoma lesion, but not metastatic liver lesions. Repeat Ga-68 DOTATATE PET was subsequently able to identify a large metastatic liver lesion, although several other liver lesions remain undetected. This case highlights the importance of serial anatomical and functional imaging for the diagnosis and localisation of metastatic neuroendocrine tumours.

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SDHB mutation presenting with multifocal paraganglioma in a young woman

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Background:

Phaeochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumours (NET) arising from adrenal medulla chromaffin cells and extramedullary neural crest cells[1]. We report the case of a woman with multifocal paraganglioma due to *SDHB* mutation, and her carrier daughter found to have an Organ of Zuckerkandl paraganglioma during long-term screening.

Case history:

A 34 year old female with no significant medical history presented with one year history of headaches, tinnitus, syncope, vomiting and unintentional weight loss. Her father had a neck paraganglioma excised 7 years ago. Para-aortic and bladder masses were found on computed tomography and biopsies confirmed paraganglioma. Magnetic resonance imaging showed a 13x16x35mm base of neck tumour. Normetadrenaline was elevated (11,000pmol/L; reference <900). All three lesions were MIBG avid. Partial cystectomy and excision of the para-aortic lesion confirmed NET on histology. Skull base lesion was treated with stereotactic radiotherapy.

Results:

Genetic testing confirmed a diagnosis of SDHB mutation related multifocal paraganglioma. Yearly plasma metanephrines were negative and periodic positron emission tomography (PET) showed diminishing activity in the base of skull lesion. *SDHB* mutation was found in her sister (asymptomatic), nephew (who had paraganglioma surgically removed), son and daughter (age 9 and 11 years). Annual plasma metanephrine screening in the daughter became elevated (9730pmol/L; reference <550) at age 19 years. A PET avid Organ of Zukerkandl mass (55x45mm) was removed laparoscopically, confirming paraganglioma.

Conclusion:

Up to 40% of PPGL arise from germline mutations and approximately 20% of PPGL patients carry a germline mutation in an *SDHx* gene[2]. The first international consensus (2021) on screening and follow-up of asymptomatic *SDHx* mutation carriers recommended that children and adults should be regularly screened clinically, biochemically and with imaging according to the proposed protocol[2]. Surveillance improves outcomes for *SDHB* mutation carriers, with evidence for smaller tumours, reduced risk of metastases and lower mortality[3].

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Ectopic ACTH and CRH secretion as a rare cause of ACTH dependent Cushing's

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A 29-year-old male is referred with persistent biochemical and clinical ACTH-dependent hypercortisolaemia after transsphenoidal resection of a presumed right middle fossa pituitary tumour with no adenoma identified on histology. He describes a one-year history of progressive Cushingoid symptoms.

Further investigation is undertaken to localise the ACTH source. IPSS is consistent with a pituitary source (Table 1). However, Dotatate-PET identifies four pancreatic NETs without concomitant hormonal secretion (Table 2). He proceeds to hypophysectomy given the impression of Cushing's disease. Unfortunately, post-operative cortisol and ACTH remained elevated: morning cortisol 487 nmol/L, ACTH 37-45 ng/L, and 24 hr urinary cortisol 1045 nmol/day.

Subsequently, CRH serum measurement is obtained and is significantly elevated at 12.4 pg/mL (<2 pg/mL). This, in combination with evidence of persistent ACTH and cortisol excess after total hypophysectomy, was thought to be in keeping with ectopic dual secretion of CRH and ACTH from pancreatic neuroendocrine tumours. Surgical opinion is that the risk of pancreatic biopsy is too high to pursue tissue diagnosis. Medical management is trialled but is unsuccessful, and he proceeds to bilateral adrenalectomy with biochemical and clinical remission post operatively (cortisol 29 nmol/L) (Image 1).

Cases of dual secreting CRH and ACTH neuroendocrine tumours (NETs) are exceedingly rare with only 5 adult cases in the literature (1-5) and 2 associated with primary localised pancreatic lesions (3,5). Differentiation of central and ectopic cortisol secretion can be challenging given that pituitary production of ACTH is maintained and even stimulated by CRH on petrosal sinus sampling (2). Additionally, the CRH assay is not widely available in Australia.

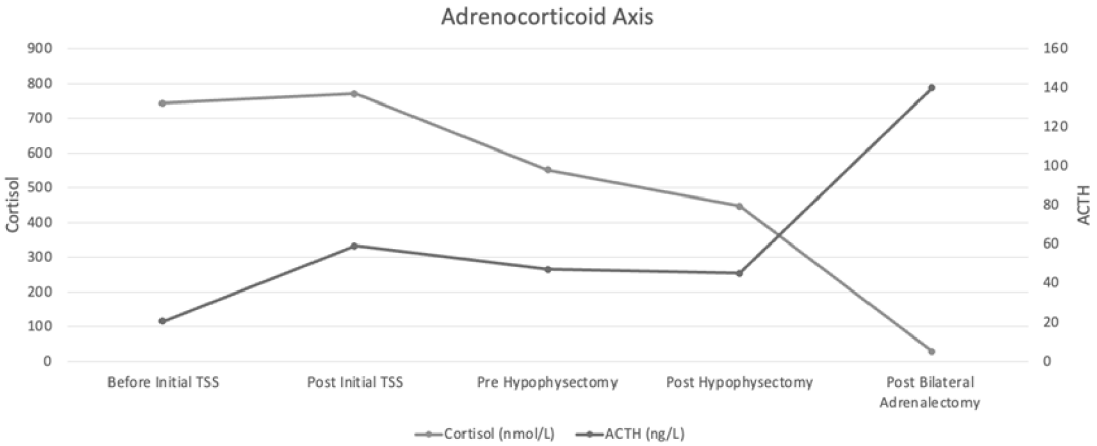
Ectopic dual secretion of CRH and ACTH is a rare cause of Cushing's syndrome which should be considered in cases where clinical, hormonal and imaging findings and treatment response are not consistent with a more common cause of hypercortisolism.

Table 1: Inferior petrosal sinus sampling

Time	Site	ACTH (5 - 60 ng/L)
Pre-CRH Stimulation	Right	49
	Left	81
	Peripheral	47
+ 5 mins CRH Stimulation	Right	117
	Left	115
	Peripheral	44
+ 10 min CRH Stimulation	Right	164
	Left	146
	Peripheral	51
+ 15min CRH Stimulation	Right	193
	Left	162

Table 2: Pancreatic NET Biochemistry

Blood test	**Value**
Chromogranin A RR: <102 ug/L	69
Calcitonin RR: < 4.0 pmol/L	<0.6
Gastrin RR: 6 – 55pmol/L	20
Vasoactive Intestinal Polypeptide RR: 0 – 30 pmol/L	16.6
Insulin RR: 1.9 – 23.8 mU/L	5.9
Glucose RR: 3.0 – 6.0 mmol/L	4.4
Glucagon RR: < 208	272
Pancreatic polypeptide RR: < 228pg/ml	Pending
Plasma Metanephrines RR < 500pmol/L	111



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A patient with an ectopic sphenoid bone TSH secretory adenoma

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Ectopic thyroid stimulating hormone (TSH)oma located outside the sella turcica is exceedingly rare and can be associated with significant diagnostic delay. Clinical presentation depends on anatomical location and size of the ectopic tumour and degree of thyrotoxicosis.

A 71-year-old female presented with goitre and thyrotoxicosis. Initial investigations revealed elevated free thyroxine (fT4) of 21.6 pmol/L and tri-iodothyronine (fT3) of 5.8 pmol/L with inappropriately high-normal TSH OF 3.8 mIU/L. Remainder of the pituitary panel was unremarkable. Assay interference was deemed unlikely, pituitary magnetic resonance imaging (MRI) scan was reported as ‘normal’ and germline sequencing was negative for thyroid hormone receptor ß pathogenic variants. One-year later, total thyroidectomy for enlarging symptomatic goitre and suspicious nodule revealed multifocal microscopic papillary thyroid carcinoma. Post-thyroidectomy, TSH was difficult to suppress despite thyroxine replacement (2mcg/kg/day).

Six-years later, she presented to an ear, nose and throat surgeon with nasal congestion and a sphenoid bone mass was discovered on naso-endoscopy. MRI scan of the head revealed a 3cmx2.3cm mass within the sphenoid bone. Ectopic TSHoma was confirmed on surgical resection with strong diffuse staining for chromogranin A, synaptophysin, Pit1 and TSH. Retrospective review of the initial pituitary MRI scan revealed a 2.3cmx2.2cm sphenoid mass which had initially been missed.

This is the first reported case of an ectopic TSHoma located in the sphenoid bone. Review of all 14 reported ectopic TSHoma cases (1-13) revealed the most common location as nasopharynx (9/14) and most common presentation as inappropriate TSH secretion and nasal congestion with median 2.5-year diagnostic delay. Surgical resection is generally complete without risk for post-operative hypopituitarism.

Ectopic TSHoma, which can be clinically and biochemically indistinguishable from pituitary TSHoma, should be considered in patients with inappropriate TSH secretion when more common differentials are excluded e.g. thyroid hormone resistance or pituitary TSHoma.

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Massive biochemically silent phaeochromocytoma masquerading as non-functioning adrenocortical cancer

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A 71-year-old female presented with acute right-sided chest/flank pain with blood pressure 103/66 mmHg and unremarkable biochemistry. Computed tomography (CT) scan of the chest/abdomen demonstrated right subsegmental pulmonary emboli and a 108mmx99mm heterogeneous lesion inseparable from the inferior vena cava (IVC) suspicious for a haemorrhagic right adrenal mass while the left adrenal gland was unremarkable. She had well-controlled hypertension (amlodipine monotherapy) and no history of diabetes, osteoporosis or episodes of sweats, headaches or palpitations.

Subsequent outpatient adrenal functional screen was unremarkable with normal plasma normetanephrines 430 pmol/L (<1280), metanephrines 90 pmol/L (<447) and 3-methoxytyramine 110 pmol/L (<181), late-night salivary cortisol 3 nmol/L (<8) and aldosterone/renin ratio 21 (<70). Progress CT abdomen showed an 88x64x68mm right homogeneous adrenal mass with resolution of haemorrhage. ^{18}F-FDG-PET/CT scan demonstrated the right adrenal mass had diffuse intense FDG-avidity (SUVmax 20.2) with no other focal abnormal FDG-avidity.

She was re-admitted for open right adrenalectomy for presumed non-functioning adrenocortical cancer. The right adrenal mass was adherent to the IVC and diaphragm requiring partial resection. She had intraoperative and immediate (<24 hours) post-operative hypotension requiring metaraminol. She was covered with hydrocortisone which was later ceased given robust morning cortisol level (283 nmol/L) off glucocorticoid therapy.

Surprisingly, histopathology demonstrated a completely resected 120mm phaeochromocytoma with tumour cells arranged in small nests with areas of sheet-like growth and low mitotic count (1/10 per hpf). There was extensive haemorrhagic and cystic change in the centre of the lesion and the tumour invaded into the peri-adrenal soft tissue and IVC lumen. Tumour cells demonstrated positive staining for synaptophysin and chromogranin-A with Ki-67 index of 5%. Staining for SDHA and SDHB and fumarate hydratase were intact however weakly positive staining for 2SC raised suspicion for fumarate hydratase-deficient phaeochromocytoma.

Results of ^{68}Ga-DOTATATE-PET/CT scan to exclude multifocal/metastatic disease and germline phaeochromocytoma panel will be presented.

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Two rare cases of osteonecrosis of the external ear canal associated with denosumab use for osteoporosis

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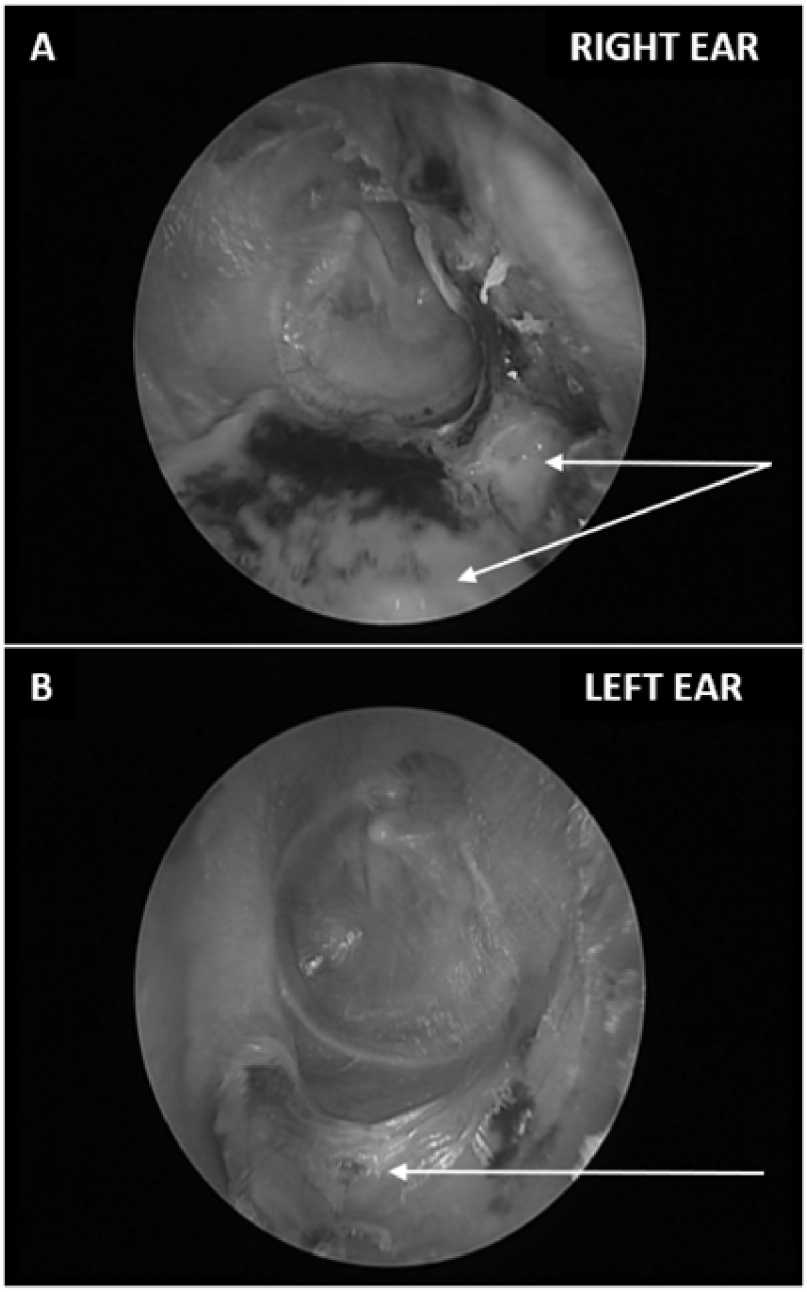
Ear canal osteonecrosis (ECO) is a rare skeletal complication of antiresorptive use. We describe two patients who developed ECO whilst taking antiresorptives for post-menopausal osteoporosis; 1) a 79-year-old asymptomatic bisphosphonate-naïve woman with 2-year denosumab exposure with incidental findings of exposed bone in both ear canals whilst undergoing ear wax clearance, 2) a 64-year-old woman with right-sided otalgia after 5-years risedronate and 5-years denosumab treatment found to have an area of exposed bone in the right ear canal on otoscopy. Neither patient had previous local radiotherapy. In both patients, CT temporal bone scan found no evidence of bone erosion and bone turnover

markers were suppressed. Both patients ceased denosumab and were managed conservatively with topical therapy, with stable otoscopy findings at 12-months with ongoing exposed bone.

ECO is considered to share similar pathogenesis to osteonecrosis of the jaw and has a working definition of exposed bone in the ear canal for >8 weeks without prior local radiotherapy. Twelve other cases have been reported in patients taking antiresorptives for osteoporosis. Bisphosphonates were implicated in 12/14 cases whilst two cases occurred in bisphosphonate-naïve patients on denosumab (including our case). Antiresorptive duration prior to symptom onset or diagnosis ranged between 1.5 years and >10 years (mean 6-7 years). Osteonecrosis was bilateral in 50% of cases and most common symptoms were otalgia (10/12), otorrhoea (5/12) and deafness (3/12). Our patient is the only one described with asymptomatic ECO. Treatment in almost all cases involved topical therapy initially and antiresorptive cessation. Outcomes are varied with some responding well to conservative therapy and others needing more extensive surgical treatment.

ECO is rare and can occur in patients taking bisphosphonates for osteoporosis and denosumab without prior bisphosphonate exposure. Localising ear symptoms in patients on antiresorptives should alert physicians to ECO and prompt early ear/nose/throat (ENT) surgical referral.



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Primary hyperparathyroidism manifest

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Primary hyperparathyroidism is a common endocrine problem characterised by overproduction of parathyroid hormone (PTH) with hypercalcaemia. Solitary parathyroid adenomas account for approximately 80% of cases[1]. PTH overproduction relates to increased parathyroid cell mass with reduced calcium sensing receptor expression leading to blunted negative feedback[2]. The majority of patients have mild hypercalcaemia with an elevated or inappropriately normal PTH level[3].

A 57-year-old female presented with malaise, altered mental status, a one-week history of nausea and vomiting and a two-week history of left hip pain. Corrected calcium was 4.25 mmol/L (2.1-2.6), phosphate 0.8 mmol/L (0.7-1.5), parathyroid hormone 125.1 pmol/L (2.0-9.0), 25-hydroxy vitamin D 21 nmol/L (50-150) and eGFR 38 ml/mn/1.73m^2 (>60). A firm mass was palpated in the region of the left inferior pole of the thyroid. Vigorous intravenous rehydration and calcitonin infusions were delivered with rapid improvement in hypercalcaemia. A CT abdomen pelvis revealed an obstructive 10mm renal calculus at the left vesicoureteric junction. A ureteric stent was placed emergently. Ultrasound of the neck showed two nodules in the left inferior thyroid, one with intensely avid uptake of 99mTc-Sestamibi on Spect-CT. The patient proceeded to a left parathyroid exploration with hemithryoidectomy and central node dissection, histopathology diagnosed an atypical parathyroid tumour with severe cytological atypica without diagnostic features of parathyroid carcinoma. Day 3 postoperative hypocalcaemia developed and was managed with one week of twice daily calcium carbonate and calcitriol.

This case is a florid presentation of primary hyperparathyroidism with both acute and chronic complications. It highlights the utility of the clinical examination in patients with hypercalcaemia. Even in the absence of longer acting anti-resorptives, postoperative hypoparathyroidism with hypocalcaemia was seen, consistent with suppression of the healthy glands. Severe hypercalcaemia with a highly elevated PTH should prompt consideration of parathyroid carcinoma and atypical parathyroid adenomas.

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An Illusive Source of Hypercalcaemia

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A 31 year old unemployed female was asked to present by her gastroenterologist after routine pathology revealed a corrected calcium (CCa) of 3.16 mmol/L and an acute kidney injury with an eGFR of 21 ml/min/1.73m^2 with previously normal pathology. She was initially referred to gastroenterology for vomiting and a seven month unintentional weight loss of 60kg. Investigations included abdominal ultrasound revealing non-alcoholic steatohepatitis (NASH) with a fibroscan suggestive of cirrhosis. Following endoscopy, there was a finding of terminal ileum biopsy showing non-necrotising granulomas.

Other background included absence seizures, idiopathic intracranial hypertension and Darier disease. Her medications included lamotrigine and topiramate. She did not drink alcohol or smoke. She had typical symptoms of hypercalcaemia and no history of nephrolithiasis or fractures. On examination, her blood pressure was 118/78 mmHg with a pulse rate of 65 beats per minute. She had a normal cardiovascular examination and no lymphadenopathy.

Her CCa improved from 3.16 to 2.9 mmol/L with intravenous rehydration as did her renal function from an eGFR of 21 to 35 ml/min/1.73m^2. Parathyroid hormone (PTH) level was reduced (1.4 pmol/L) and subsequent investigations were unremarkable except for elevated angiotensin converting enzyme (ACE). A PTH related protein level is pending. She required intravenous pamidronate on two occasions for rebound hypercalcaemia. A FDG PET scan demonstrated

increased uptake in right cervical chain lymph nodes and subsequent excisional biopsy showed progressive transformation of germinal centres.

This case presents a diagnostic and management dilemma of PTH-independent hypercalcaemia with acute kidney injury, with differentials of lymphoma and sarcoidosis. This is in the setting of NASH with uncertain aetiology, in a previously well 31 year old female. ACE has poor sensitivity and specificity (1) and cautious interpretation is required in liver disease(2). Haematological review is pending, with consideration of chemotherapy and corticosteroids.

Table 1. Diagnostic Investigations for Hypercalcaemia

Investigation	**Results**			
Pathology	**Initial Presentation**	**First IV 30mg Pamidronate Infusion**	**Second IV 30mg Pamidronate Infusion**	**Reference Range (units)**
Corrected Calcium	3.16 → 2.9	3.33 → 2.54	2.55 → 2.49	2.1 – 2.6 (mmol/L)
Phosphate	1.56	1.5	1.24	0.8 – 1.5 (mmol/L)
eGFR	21 → 35	41 → 71	58 → 81	>60 (ml/min/1.73m^2)
Parathyroid Hormone	1.4	1.4	-	1.6 – 6.9 (pmol/L)
25 Vitamin D	44	-	-	50 – 150 (nmol/L)
1, 25 Vitamin D	108	-	-	48 – 190 (pmol/L)
Serum protein electrophoresis	Normal	-	-	
Urine Bence Jones protein	Not detected	-	-	
Free light chains – K/L ratio	0.94	-	-	0.31 – 1.56
ACE	450	421	-	37 – 211 (ug/L)
8am Cortisol	276	-	-	140 – 640 (nmol/L)
TSH	3.7	-	3.6	0.3 – 3.5 (mU/L)
fT4	10.1	-	9.2	9 – 19 (pmol/L)
Quantiferon TB	-	-	Negative	
US guided core biopsy - Right cervical lymph node	No granuloma, atypical lymphoid infiltrate or evidence of metastatic malignancy. Normal flow cytometry.			
Excisional biopsy Right cervical lymph node	Progressive transformation of germinal centres. Flow cytometry raises possibility of a lymphoproliferative disorder.			
Radiology				
Ultrasound neck	Prominent 9mm lymph node in right submandibular region			
CT neck/chest/abdomen/pelvis	Right 16 x 17 mm jugulodigastric lymph node, several right cervical chain lymph nodes up to 10mm. No mediastinal/hilar lymphadenopathy. No renal calculi. Appearance of fatty liver.			
FDG PET CT scan	Pathologically enlarged FDG-avid lymphadenopathy in right cervical chain at levels II and III. Right level II node demonstrates greatest FDG-avidity.			

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Larotrectinib in Metastatic Medullary Thyroid Carcinoma

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A 68-year-old woman was referred for 8 month history of a neck lump, dysphonia and unintentional weight loss of 15kg.

Her background included multinodular goitre (MNG) and Hashimoto's hypothyroidism managed on thyroxine, with subsequent Graves' disease 8 years later with a Thyrotropin receptor antibody level of 7.9 IU/L. Other history included depression and left arm melanoma excision. Her medications included celecoxib and escitalopram. She did not drink alcohol or smoke and was a carer for her husband. There was no family history of malignancy and no history of neck irradiation.

She had a blood pressure of 110/60mmHg with right neck swelling. Pemberton's sign was negative. Cardiorespiratory exam was unremarkable.

Ultrasound demonstrated a 23mm soft tissue mass adjacent to the right thyroid. Computed tomography scan showed mass effect of goitre on the trachea and oesophagus with right cervical lymphadenopathy. FDG PET scan demonstrated an intensely avid lobulated right thyroid lobe mass with right neck and mediastinal nodes. Biopsy confirmed medullary carcinoma with positive calcitonin.

Multiple Endocrine Neoplasia screening was negative. Due to the degree of extrathyroidal extension involving the trachea and common carotid artery, this T4aN1bMx medullary thyroid carcinoma (MTC) was not amenable to surgery. Subsequent investigations included a variant of unknown significance in the RET gene. The tissue was NTRK positive and she enrolled in a clinical trial for Larotrectinib. Following the fifth cycle, imaging demonstrated progressive tracheal compression.

Surgical resection is the mainstay of management for MTC(1). Tyrosine Kinase Inhibitors are an option for unresectable disease(2). This case demonstrates a 68 year old female with an unusual background of Hashimoto's hypothyroidism, subsequent Graves' disease with concomitant MNG, who presented with Stage 4 unresectable MTC and tolerated a Larotrectinib trial but developed disease progression. The prognosis is poor with a 5-year survival rate of 26% for metastatic disease(3).

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Internal hernia following single anastamotic gastric bypass surgery

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Obesity has emerged as a major global health issue[1,2], and metabolic and obesity surgery (MOS) is the most effective treatment for sustained weight reduction and resolution of complications.[3] Single anastomotic gastric bypass (SAGB) surgery involves the creation of a longer gastric pouch that is anastomosed to the jejunum, creating a single tension-free gastrojejunostomy for ingested food to bypass the duodenum, whilst the efferent limb of the gastrojejunostomy receives bile, pancreatic, and proximal intestinal secretions.[4]

A 47-year-old lady presented with diabetic ketoacidosis. She had undergone laparoscopic SAGB eight months prior for complicated obesity in the setting of a type 2 diabetes mellitus (DM) diagnosis. However, she had been re-classified as having type 1 DM six weeks prior to her presentation, with fasting hyperglycaemia, an undetectable C-peptide level and detectable islet cell antibodies. Persistence of her symptoms and mild ketosis despite aggressive fluid resuscitation and insulin-dextrose infusion prompted further investigations. Abdominal imaging demonstrated dilated jejunal bowel loops proximal to the gastro-jejunal anastomosis, with air fluid levels suggestive of a small bowel obstruction. A gastroscopy revealed a tight stricture approximately 20cm into the efferent limb, and laparoscopic assessment showed internal herniation of small bowel through the defect between the anti-rotation suture and the efferent limb. The suture was successfully released to relieve the obstruction.

Efferent loop syndrome is often caused by adhesions, internal hernias, or stenoses secondary to scarring from previous ulcerations.[4,5] It has been rarely reported after SAGB, as the procedure obviates the need for a second anastomosis.[4] However, internal hernias must be considered as a differential diagnosis in non-surgical presentations such as DKA among

patients who have undergone SAGB. Symptoms that persist despite initiation of appropriate treatment in DKA in the setting of past MOS warrant urgent investigations and consideration of explorative laparoscopic assessment, even if radiological findings are unremarkable.

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Two cases of Graves' hyperthyroidism following Pfizer COVID vaccination

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Vaccination reduces the risk of severe health consequences of coronavirus-19 (COVID-19) infection. We report two cases of new-onset Graves' hyperthyroidism suspected to have been precipitated by recent Comirnaty, Pfizer COVID vaccination.

Case 1: A 30 year old woman presented with sweats, rapid palpitations and arthralgia of the upper and lower limb joints and 2 kgs of weight loss within 7 days after a third COVID vaccination. Thyroid function tests showed subclinical hyperthyroidism; TSH: <0.02 mIU/L, free T4: 16.6 pmol/l, and positive TSH receptor antibody: 4.0 U/L (<1.0), anti-thyroglobulin and thyroid peroxidase antibodies. Thyroid ultrasound showed thyroiditis and sub-centimetre nodules. Follow-up clinical picture is most consistent with mild Graves' disease with persisting subclinical hyperthyroidism and positive TSH receptor antibody, without antithyroid medication. Whilst she has multiple sclerosis treated with alemtuzumab, this was more than 5 years ago and thyroid function had been normal before the COVID vaccine, making alemtuzumab less likely to be causative.

Case 2: A 57 year old woman developed rapid palpitations, diarrhoea, tremors and 15 kg of weight loss, 2 to 3 weeks after a fourth COVID vaccination. TSH was 0.01 mIU/l, free T4: >75 pmol/l, free T3: >30.8 pmol/L, thyroid stimulating immunoglobulin 3.39 IU/L (<0.55) and positive anti-thyroglobulin and thyroid peroxidase antibodies. Thyroid ultrasound showed thyromegaly, features of thyroiditis and a 7 mm nodule. Treatment was commenced with carbimazole and propranolol with improvement.

A number of cases of new-onset or relapsed Graves' disease following COVID vaccination have been reported, with the mRNA vaccine Pfizer most commonly associated and median time to symptom onset after vaccination of 7 days (1).

Graves' hyperthyroidism should be considered when hyperthyroid symptoms follow soon after COVID vaccination, particularly with the mRNA Pfizer vaccine.

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Paliperidone induced hypoglycaemia a diagnostic dilemma

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Mr. M. A. is a 36-year-old male with a nomadic lifestyle who was hospitalised with prominent negative symptoms of schizophrenia in August 2021 and started on paliperidone and venlafaxine. A routine metabolic screen in February 2022 revealed a fasting glucose of 2.7mmol/l despite being asymptomatic. He was referred to the closest regional hospital for further investigation of recurrent asymptomatic hypoglycaemia. He completed the full 72-hour fast due to a lack of Whipple's triad and the results supported a negative test.

72 H Fasting Test	0	3	6	12	18	24	30	32	34	36	38	40	42	44	46	52	54	56	58	60	62	64	66	68	70	72	72.10	72.20	72.30
Glucose mmol/l	3.1	3.4	3.3	2.9	3.1	3.1	2.5	2.8	2.8	2.8	2.7	2.7	2.7	2.9	3.8	2.6	2.6	2.7	2.3	2.5	2.6	2.4	2.4	2.5	2.7	2.4	4.0	4.6	4.1
Insulin mIU/l	7.0			6.6	7.2	6.2	6.6	3.4	3.4	4.2	4	4	3.7	5.3	-	3.7	2.5	1.8	3.1	2	1.6	3.0	2.4	7.0	6.6	2	20.4	11.7	9.7
Proinsulin pmol/l	22			9.2	7.7	8	4.4	<3.1	<3.1	<3.1	<3.1	<3.1	<3.1	<3.1	-	TF	TF	<3.1	<3.1	TF	<3.1	<3.1	<3.1	<3.1	5.3	<3.1	10.1	3.5	8.8
C-peptide pmol/l	818			666	737	716	541	464	427	464	535	464	440	531	-	362	314	299	336	290	291	338	315	463	660	319	1330	1033	886
Beta-hydroxybutyrate Mmol/l	0.1	>0.1	>0.1	>0.1	0.1	0.1	0.5	0.6	0.5	0.5	0.8	0.7	0.6	0.8	1.1	1.1	1.1	1.2	1.4	1.3	1.6	2.1	2.4	2.5	3.2	2	2	2	2
Inappropriate endogenous hyperinsulinemia. Plasma glucose < 3.0 mmol/L AND: Insulin ≥ 20.8 pmol/L (= 3.0 mIU/L); C-peptide ≥ 200 pmol/L; Proinsulin ≥ 5 pmol/L; beta-hydroxybutyrate < 2.7 mmol/L; plasma glucose increased by 1.4 mmol/L post glucagon injection																													

Table 1: 72-hour fasting test.

Sulphonylurea screening, insulin antibody, morning cortisol, IGF-1, renal and liver function tests were normal. He is non-alcoholic and eats regularly. There was no family history of hypoglycaemia or pancreatic disorders.

Mr. M.A was transferred to our tertiary service for additional imaging. DOTATATE scans, CT and MRI scans were negative for pancreatic pathology. As hypoglycaemia persisted, he underwent a calcium stimulation test (Table 2), which revealed a 2 to 3 fold increase in insulin levels at the proximal splenic artery at 90s (10.3-->28.3mIU/L), andgastroduodenal artery at 90s (23-->45.6mIU/L). He had started on Diazoxide.

	-120	0	30	60	90	120	180
Splenic artery, distal	450763210	450763227	450763234	450763241	450763258	450763265	450763272
Glucose (mmol/L)	3.5	3.4	3.6	3.5	3.8	3.5	3.7
Insulin (mIU/L)	21.7	19.6	14.1	14.2	18.7	17.3	15.7
C-peptide	1737	1816	1496	1478	1538	1593	1664
Proinsulin	21.1	20.7	17.5	16.9	19.2	20	20.1
Splenic artery, mid	450763142	450763159	450763166	450763173	450763180	450763197	450763203
Glucose (mmol/L)	3.6	3.7	3.5	3.5	3.5	3.7	3.6
Insulin (mIU/L)	11.4	12.9	17.2	18.5	23.3	17	14.2
C-peptide	1422	1497	1669	1826	1920	1611	1532
Proinsulin	16.7	18.8	19.6	11.5	21.8	18.4	18.7
Splenic artery, proximal	450763074	450763081	450763098	450763104	450763111	450763128	450763135
Glucose (mmol/L)	3.6	3.5	3.5	3.7	3.7	3.6	3.8
Insulin (mIU/L)	8.4	10.3	13.6	20.2	28.3	21.5	15.7
C-peptide	1253	1430	1515	1641	1922	1743	1597
Proinsulin	15.9	15.9	16.7	24.4	24.6	23.1	22.1
Right hepatic	450763005	450763012	450763029	450763036	450763043	450763050	450763067
Glucose (mmol/L)	3.6	3.4	3.5	3.5	3.6	3.7	3.6
Insulin (mIU/L)	26	18	19.1	22.3	24.4	20.6	17.8
C-peptide	2020	1747	1780	1976	2041	1760	1740
Proinsulin	23.6	19.8	16.9	26.7	23.6	21.9	22.5
Gastroduodenal	450762930	450762947	450762954	450762961	450762978	450762985	450762992
Glucose (mmol/L)	3.6	3.6	3.5	3.5	3.5	3.6	3.7
Insulin (mIU/L)	27.1	23	21.2	29.3	45.6	29.8	20.6
C-peptide	1879	1684	1817	1929	2313	1854	1643
Proinsulin	26	23.4	22.1	26.6	32.4	27	23.2
Superior mesenteric attempt no. 1	450762862	450762879			450762886	450762916	450762923
Glucose (mmol/L)	3.7	3.6			3.5	3.5	3.6
Insulin (mIU/L)	31.4	15.7			16.3	15.6	22.1
C-peptide	1737	1545			1723	1769	1812
Proinsulin	24.6	21.3			25.5	27.8	25.3
Superior mesenteric attempt no. 2	450762794	450762800	450762817	450762824	450762831	450762848	450762855
Glucose (mmol/L)	3.5	3.7	3.6	3.5	3.4	3.4	3.5
Insulin (mIU/L)	18.7	17.3	19.2	16.5	12.7	9.9	8.1
C-peptide	1778	1668	1762	1623	1568	1257	1319
Proinsulin	24.9	21.6	24.4	22.3	19.1	20.2	22.1

Table 2: Calcium stimulation test.

As there were cases of severe hypoglycaemia with risperidone and risperidone analogue paliperidone, discussions with the mental health team led to switching paliperidone to aripriprazole in May 2022. BGLs over the next 2 months ranged between 3.2 and 11.6 mmol/l. Diazoxide was discontinued and regular corn-starch was commenced.

Discussion:

Several cases of hypoglycaemia caused by antipsychotics have been reported [1,2,3]. A fall in blood glucose is normally rapidly detected, and counter-regulatory mechanisms are recruited. The α2-adrenoceptors on pancreatic β-cells inhibit insulin secretion and seem to be critically important for this counter-regulatory response. The α2-adrenoceptor antagonists increase insulin secretion [2,4]. Risperidone has an antagonistic effect on α2-adrenoceptors[2]. Paliperidone, the primary active metabolite of risperidone, has an affinity for α2-adrenoceptors that is slightly stronger than that of risperidone [1,5].

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A case series of monogenic diabetes from Northern India showing novel variant mutations

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Introduction:

Monogenic Diabetes (MD) is a topic of increasing interest, with new gene defects being discovered as an etiology of MD on a frequent basis.

Case Series:

We present 9 cases of MD with novel mutations involving 9 gene mutations: HNF4A, HNF1A, HNF1B(2), PAX4, BLK, ABCC8, CEL gene and KCNJ11. In this abstract we would like to highlight a few clinical features that we believe will aid in patient care. In our practice we have diagnosed people with MD even in their late 40s and 50s. In the areas where the penetration of genetic testing is less we can diagnose patient in later age contrary to the common mindset which we see in OPD setting.

We were able to successfully discontinue insulin use in a patient who had been on insulin for over 5 years, was in his late 50s, and has the HNF4A mutation. Even after 5 years of diagnosis, one of our HNF1B patients was responding well to sulphonylurea. Also, even after being categorised as a type 1 diabetic for over two decades, a person with negative Type 1 antibodies and no history of ketoacidosis assisted us in diagnosis of MD with PAX4 mutation. A person with a CEL gene mutation developed prominent eye symptoms due to retinal involvement early in the disease's progression.

Conclusion:

- We report 10 mutations in 9 people with MD of novel variants of genetic mutation.
- Also our case series highlights that where there is lesser penetration of genetic testing we might get people with MD in their fourth or fifth decade of age.
- A detailed history of onset of diabetes, family history and antibodies status will help us clinch the diagnosis
- With growing clinical experience newer aspects of management of MD are being discovered.

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Phaeochromocytoma presenting with vascular rupture

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A 42-year-old male with neurofibromatosis type 1 (NF1) characterised by skin neurofibromas and café-au-lait spots presented to the emergency department with sudden onset left neck swelling after coughing. CT angiogram demonstrated a large haematoma secondary to rupture of a left external carotid artery branch. He underwent surgery for repair of the ruptured artery; however, immediately postoperatively, a coughing fit following extubation resulted in re-bleed necessitating repeat surgical intervention. Histopathology of the operative specimen demonstrated arterial vessel with invading neurofibroma. Postoperatively in ICU, despite the use of multiple antihypertensives, including high dose IV labetalol, perindopril, metoprolol, prazosin and GTN patch, the patient’s systolic blood pressure was intermittently >200 mmHg.

Given his background of NF1 and significant hypertension, the patient was investigated for a phaeochromocytoma. Plasma normetadrenaline was 5225 pmol/L (reference range <900 pmol/L) and metadrenaline was 7998 pmol/L (<500 pmol/L). CT demonstrated a right-sided 3.7cm adrenal mass consistent with a phaeochromocytoma. Following Endocrinology review, phenoxybenzamine and diltiazem were commenced with cessation of all other antihypertensive agents. He was discharged day 10 postoperatively and is awaiting phaeochromocytoma resection. In retrospect, he denied any symptoms suggestive of a phaeochromocytoma.

His mother was the index case and his only sibling who also had NF1 died in her 30s of a presumed ruptured aorta.

Discussion

NF1 is an autosomal dominant condition caused by a vast number of pathogenic variants in the NF1 gene. The estimated incidence is ~ 1/1,900–1/3,500 people worldwide, of whom approximately 3% develop a phaeochromocytoma. The less commonly appreciated association between NF1 and vascular abnormalities include rupture and invasion by neural

tumours. Significant hypertension in those with a phaeochromocytoma may represent a phaeochromocytoma crisis, even in those who were otherwise apparently asymptomatic. A high level of suspicion for phaeochromocytoma is therefore crucial in those with genetic predispositions and hypertension.

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Successful long-term use of low dose Tolvaptan in an elderly patient for chronic Syndrome of Inappropriate Antidiuretic Hormone secretion (SIADH)

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Background:

Hyponatraemia is a common electrolyte disorder occurring in up to 15 to 22% of hospitalised patients (1) and is associated with increased morbidity (2) and mortality (3). Syndrome of inappropriate antidiuretic hormone secretion (SIADH) is the most common cause of hyponatraemia (4) and is precipitated by numerous medical conditions and medications (5). However, management of chronic SIADH remains suboptimal with limited effects of fluid restriction and underutilisation of other therapies (6). Tolvaptan, a selective oral vasopressin V2-receptor antagonist, despite its introduction more than a decade ago and its remarkable potency (7), is seldomly used likely due to the risk of overcorrection and liver function test derangement, costs, clinician non-familiarity and paucity of data in its chronic use.

Case:

84 year-old presented with nausea and generally lethargy due to chronic hypotonic hyponatraemia secondary to SIADH in setting of Mycobacterium avium complex infection on rifampicin, ethambutol and clarithromycin. Her biochemistry included: serum sodium 122mmol/L, serum osmolality 255mmol/kg, urine sodium 44mmol/L with a normal thyroid function test and 8am cortisol level. Due to the limited improvement with fluid restriction, salt tablets and intolerance of urea secondary to gastrointestinal side effects, 15mg PO tolvaptan stat was utilised with immediate however short-lived effect. The effect of rifampicin (CYP3A inducer) and clarithromycin (CYP3A inhibitor) on tolvaptan, a CYP3A substrate, was deemed to counteract each other's effects. With tolvaptan 7.5mg PO second daily, hyponatraemia resolved without liver function test derangements and enabled relaxation of fluid restriction improving the patient's the quality of life. Six months later, patient remains normonatraemic (139mmol/L) and is on weaning doses of tolvaptan.

Conclusion:

Long-term low dose tolvaptan may be a safe and effective treatment for chronic SIADH even in elderly comorbid patients. Tolvaptan should be considered for management of SIADH refractory to fluid restriction and second line therapies such as urea.

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Insulin pump safety in prolonged hypoglycaemia

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FW, a 23-year-old female with Type 1 Diabetes Mellitus managed with Dexcom 6 basal IQ technology, was admitted for management post a severe prolonged hypoglycaemic episode associated with status epilepticus. FW had well controlled (HbA1c 7.0%), uncomplicated diabetes with infrequent hypoglycaemic episodes. The presenting hypoglycaemic episode occurred in the context of exercise, alcohol and possible gamma hydroxybutryic acid spiking.

Review of device data revealed prolonged hypoglycaemia (glucose <2mmol/L) for ten hours (Figure 1 and 2). Predictive low glucose suspend activated with resumption of basal insulin every two hours, in alignment with pump safety measures, before again suspending. Despite limited basal insulin overnight, FW remained hypoglycaemic, indicating alpha cell dysfunction or poor glycogen reserve. Ketones were 0.2mmol/L when first recorded hours after her initial management in a rural clinic.

Electroencephalogram recorded status epilepticus, MRI-Brain was consistent with hypoglycaemic encephalopathy with FLAIR hyperintensity and restricted diffusion of the basal ganglia and hippocampi tails with mild cerebral oedema. Interval imaging noted new signal hyperintensity and diffusion restriction in the anterior globus pallidus bilaterally with likely resolving mild cerebral oedema. FW had multiple failed attempts at extubation before decision for tracheostomy. Over the subsequent weeks there was slow and poor neurological recovery resulting in eventual withdrawal of care and death. Whilst organ donation was considered, she was deemed an unsuitable donor as time to death post withdrawal of care exceeded ninety minutes.

This case allows the unique perspective to scrutinize continuous glucose monitoring during prolonged severe hypoglycaemia and assess safety features of insulin pump. Safety of insulin pumps in the areas of software, hardware, wireless communication, human factors, alarms and bolus calculators will be discussed. As will the ethics regarding onus on practitioners who are set up as safety alert contacts. This case highlights the benefits of dual-hormone artificial pancreas system

Figure 1: Day prior to prolonged hypoglycaemic episode

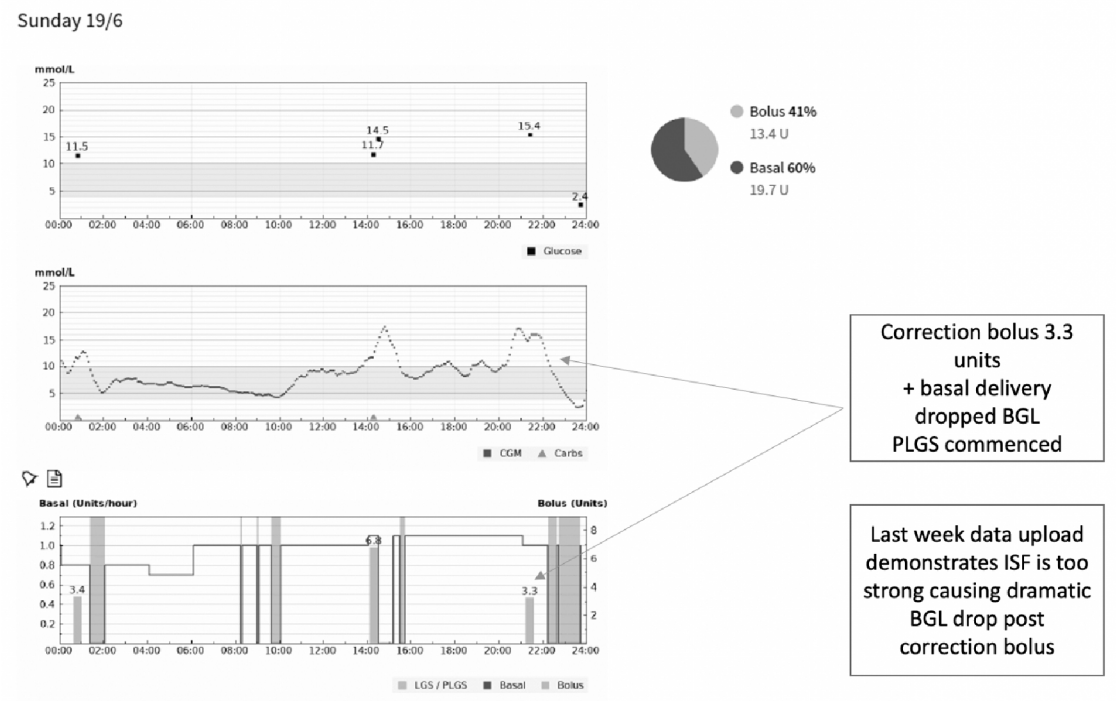
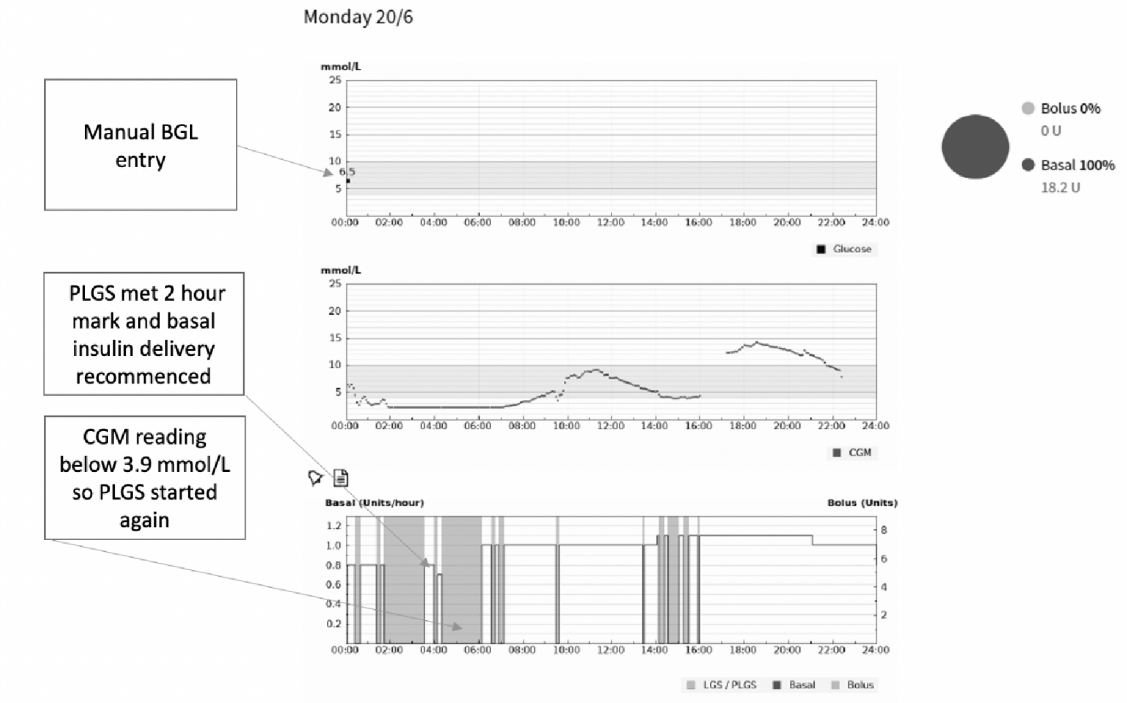


Figure 2: Day of prolonged hypoglycaemia



Hyperfunctioning Thyroid Cancers

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Hyperfunctioning thyroid cancers are rare. Current ATA guidelines recommend against routine biopsies of autonomous thyroid nodules (ATNs)(1). We present two cases of thyroid cancer found in autonomous nodules.

Case 1

A 30-year-old female with subclinical hyperthyroidism was referred for pre-pregnancy planning. Thyroid ultrasound showed two sub-centimetre TIRAD 4 nodules arising in the upper poles bilaterally. Technetium scan showed increased uptake in both nodules. The ultrasound also showed an abnormal right-sided level 3 lymph node. Biopsy of the right upper pole nodule and the lymph node showed papillary thyroid carcinoma with lymph node metastasis. The patient underwent total thyroidectomy and lymph node excision. Final histology showed lympho-vascular infiltration and BRAF positivity. She underwent radioiodine ablation.

Case 2

A 72-year-old female with minimal thyrotoxic symptoms, was found to have suppressed TSH, free T4 25.2pmol/L, free T3 of 7.5pmol/L, and TRAb 4.5U/L. Thyroid ultrasound showed multinodular goitre with a suspicious left upper pole nodule, which appeared irregular, hypoechoic with poor infiltrated margins. Technetium scan suggested that this nodule was autonomous. Subsequent biopsy confirmed papillary thyroid cancer. She underwent total thyroidectomy. Final histology showed a well capsulated intrathyroidal papillary thyroid cancer of 7 mm without lymph node involvement, or vascular invasion. She did not receive radioiodine ablation.

Discussion

Thyroid cancer is not thought to be common in ATNs. There are approximately 60 case reports of thyroid malignancy within ATNs. Current literature suggests the rate of malignancy within ATNs is less than 10%. Low TSH may be protective, and autonomous thyroid cancer cells are usually well differentiated and less likely to metastasise(2).

Conclusion

The vast majority of ATNs are benign. However careful assessment with thyroid ultrasound may identify rare cases of hyperfunctioning thyroid malignancy.

A management dilemma in a co-secreting Growth Hormone and Thyroid Stimulating Hormone pituitary neuroendocrine tumour.

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Pituitary neuroendocrine tumours, also known as pituitary adenomas[1], are benign neoplasms reported to occur in up to 20% of the population[2]. Thyroid stimulating hormone (TSH) secreting tumours are rare and account for 0.5-3% of all pituitary neuroendocrine tumours[3]; with only 20% of these co-secreting growth hormone (GH) or prolactin (PRL) due to their shared Pituitary transcription factor 1 (Pit-1) lineage[3,4,5]. Plurihormonal Pit-1 tumours are also known for their aggressive nature[4].

We report a case of a 34 year old male presenting in 2019 with clinical symptoms of lethargy, insomnia, tremor, palpitations and tachycardia. Biochemistry showed evidence of hyperthyroidism (fT4 29.1pmol/L, fT3 12.6pmol/L) with inappropriately normal TSH (TSH 3.5mU/L,) and elevated IGF-1 77nmol/L (RR14-40nmol/L). Magnetic resonance imaging (MRI) demonstrated a 30 x 27 x 24m pituitary macroadenoma and he subsequently underwent transsphenoidal resection, with histopathology consistent with a plurihormonal PIT-1 positive, TSH and GH-secreting tumour. Post-operative imaging showed evidence of a small 4mm residual tumour and he developed post-operative panhypopituitarism with likely growth hormone deficiency but ongoing TSH hypersecretion.

Though rare, there are previous case reports of co-secreting TSH and GH pituitary neuroendocrine tumours[3,6,7,8,9], cured through surgical intervention alone[3,7], requiring adjuvant use of somatostatin analogs[6,9], concomitant use of a somatostatin analog and Pegvisomant[8] and also a case of resistance to somatostatin analogue after surgery[10]. Adjuvant radiotherapy is an option but has a low remission rate of 60%[8].

In our patient, medical management poses a dilemma; Lanreotide 60mg every 6 months controls TSH secretion and residual tumour size remains stable, but leads to further reduction of GH levels and significantly contributes to fatigue and reduced quality of life. To date he continues to await surgical resection of his residual tumour.

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The Three Ps... Diagnosis, management and surveillance of MEN-1.

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We present the case of a clinical diagnosis of Multiple Endocrine Neoplasia-1 (MEN-1) in a 29 year old woman.

CH is a 29 year old Thai National who relocated to Australia in 2015. Her past medical history included obesity with BMI of 31kg/m^2 and transgender (M to F) on the oral contraceptive pill.

CH was admitted with a perforated duodenal ulcer and found to have hypercalcaemia due to primary hyperparathyroidism (calcium 2.85mmol/L, PTH 15.5pmol/L, and 24 hour urine calcium 14.0mmol/day).

Pituitary MRI demonstrated two cystic hypo-enhancing lesions suggestive of a complex pituitary adenoma. The only significant pituitary hormone abnormality was mildly elevated prolactin at 1002 mIU/L (50-300).

Morning cortisol was 394nmol/L (150-52) and ACTH was 6.6pmol/L (0-12), however 24 hour urine free cortisol was significantly elevated at 1314nmol/L (<250).

A 1mg dexamethasone suppression test demonstrated partial suppression with a cortisol of 109nmol/L (<50nmol/L).

A DOTATATE PET CT demonstrated three discrete prominent foci of increased uptake in the pancreas. The lesions were not seen on pancreas MRI but were visualised on endoscopic ultrasound and in the clinical setting, are highly suggestive of pancreatic neuroendocrine tumours.

Gastrin was elevated at 426pmol/L (6-55), as was chromogranin A at 1076ng/ml (<102), however these may be falsely elevated in the setting of PPI infusion.

Fasting blood glucose levels and other gut hormones were normal.

In summary this case demonstrates a clinical diagnosis of MEN-1 with parathyroid, pituitary and pancreas involvement and potential Cushing's in a 29 year old woman. DNA MEN-1 confirmation is pending.

The case raises the following discussion points:

1. General presentation and incidence of MEN-1.
2. Clinical indication to refer for genetic testing of MEN-1.
3. Genetics and phenotype of MEN-1 including assessment of clinical MEN-1 that is genetic test negative.
4. Assessment and management of gastro-pancreatic complications.
5. Screening and surveillance MEN-1 positive patients.

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Hypocalcaemia and hypophosphataemia following concurrent denosumab injection and ferric carboxymaltose infusion

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We present the case of a 29-year-old woman with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), complicated by recurrent episodes of optic neuritis and an atonic bladder. She had required immunosuppression therapy since diagnosis in 2016, initially with mycophenolate mofetil and azathioprine, followed by the combination of rituximab, methotrexate and prednisolone. In the setting of ongoing flares of optic neuritis the rituximab was transitioned to tocilizumab and she commenced plasma exchange therapy via a right radiocephalic arteriovenous fistula. Given her prolonged high-dose steroid requirement she was commenced on alendronate to reduce her risk of glucocorticoid-induced osteoporosis. This continued from 2017 to 2020, followed by a change to denosumab, which was initiated by her general practitioner in 2021. Two days after her most recent denosumab injection, she received a ferric carboxymaltose infusion for iron deficiency anaemia. She then reported two weeks of perioral and peripheral paraesthesia and muscle spasms, as well as increasing fatigue. Pathology demonstrated a corrected calcium of 1.89 mmol/L (2.15-2.65 mmol/L), an ionised calcium of 1.04 mmol/L (1.15-1.3 mmol/L) and an undetectable phosphate of <0.23 mmol/L (0.75-1.5 mmol/L). Renal function was normal (eGFR >90 ml/min/1.73m^2) and vitamin D was 58 nmol/L (>50 nmol/L). The hypocalcaemia was caused by the denosumab injection, which in turn exacerbated hypophosphataemia via a reactive secondary hyperparathyroidism. However, the primary driver of hypophosphataemia was likely the ferric carboxymaltose infusion causing FGF-23 driven phosphaturia. She was commenced on oral electrolyte replacement, requiring a maximum dose of sodium phosphate monobasic 4000mg BD, calcium carbonate 1200mg BD and calcitriol 0.25mcg BD. She reported resolution of her symptoms, however experienced significant diarrhoea associated with high-dose phosphate replacement. Her electrolyte replacements were weaned over a two-month period and then ceased, with maintenance of normal electrolyte levels. She was advised to avoid ferric carboxymaltose infusions in the future.

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Acromegaly diagnosed 20 years after an initial presentation with symptomatic hyperprolactinaemia

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We present the case of a 61-year-old female who initially presented aged 41 years with a two-year history of infertility and galactorrhoea. She had an elevated prolactin level of 1258 mIU/L (29-513 mIU/L) with an otherwise unremarkable pituitary screen. MRI demonstrated a 1.0 x 0.6 x 0.6 cm left-sided pituitary adenoma. Weekly cabergoline 0.5 mg was commenced, which was continued for three years before being ceased and she was subsequently lost to follow up. Twenty years later, she re-presented with an eight-year history of increased hand and feet size, increased jaw size, and associated arthralgias. She had been commenced on anti-hypertensive therapy in the preceding two years, but denied diabetes, excessive sweating, skin tags, headaches, diplopia or visual loss. Investigations revealed an elevated IGF-1 level of 99 nmol/L (5-32 nmol/L) with preservation of the remaining pituitary axes, and in particular a normal prolactin level of 321 mIU/L. MRI of the pituitary demonstrated a locally invasive 2.3 x 4.3 x 2.8 cm pituitary mass with typical inferolateral expansion, eroding into and replacing the sphenoid sinus and clivus, invasion into the left cavernous sinus with lateral extension to the left internal carotid artery, and enveloping 180 degrees around the right internal carotid artery. The patient underwent endoscopic endonasal resection of the pituitary macroadenoma. Histology demonstrated a sparsely granulated somatotroph adenoma, with no additional pituitary hormone staining. Six months post-operatively the IGF-1 levels had normalised to 28 nmol/L and follow up imaging demonstrated near complete resection of the tumour with only a rind of heterogeneous enhancement remaining, and normal appearance of the remaining pituitary gland and stalk. In summary, our patient was diagnosed with a locally invasive isolated-staining somatotroph macroadenoma 20 years after her initial presentation with symptomatic hyperprolactinaemia, despite the absence of lactotroph staining and normal IGF-1 levels at initial presentation.

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Growing weaker: a case report of the under-recognised complication of vertebral fractures in acromegaly

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Acromegaly is a slowly progressive condition of excess growth hormone (GH) production resulting in the abnormal enlargement of bones and thickening of soft tissues of the body. Osteoporosis is known to be associated with GH deficiency but can also occur in GH excess, manifesting predominantly as fragility vertebral fractures (VFs).

A 32-year-old male was referred for assessment of a minimal trauma T7 VF. A DEXA scan showed lumbar spine osteopaenia (BMD 0.988g/cm^2, T-score -1.9SD) with normal bone density in both hips (mean BMD 1.264g/cm^2, mean T-score +1.3SD). Incidentally, testosterone was low (9.9nmol/L [11.5-32.0]) with inappropriately low FSH and LH, though these normalised pre-operatively without intervention. Clinically, there was large sweaty spade-like hands and mild prognathism, but no other features consistent with active acromegaly. There was no significant past medical, family or medication history.

Further investigations revealed IGF-1 72-83nmol/L (14-41) with failure of suppression with an GH suppression test. An MRI pituitary identified an 8x4mm superior pituitary abnormality displacing the pituitary stalk. He underwent a transsphenoidal sinus hypophysectomy 10 months following his initial presentation, experiencing post-operative complications of both diabetes insipidus and syndrome of inappropriate anti-diuretic hormone at different times.

Subsequent follow up demonstrated resolution of GH excess with normalisation of GH and IGF-1 levels. He was commenced on oral bisphosphonates for osteoporosis and his bone mineral density has remained stable on repeat DEXAs, with no signs or symptoms of active acromegaly.

This case demonstrates that fragility VFs can be the sole presenting complaint in patients with osteoporosis due to underlying acromegaly. GH and IGF-1 excess contributes to increased bone turnover with detrimental effects on cortical and trabecular bone structure[1]. VFs have been reported in up to 60% of acromegalic patients[2] with male predominance[1]. It is associated with duration of active disease[3] with no direct correlation between VFs and BMD[1].

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Metastatic medullary thyroid cancer in a young patient with neurofibromatosis 1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant neurocutaneous disorder, known to have an increased risk of benign and malignant tumours. Medullary thyroid carcinomas (MTC) are rare tumours arising from the parafollicular cells and have been associated with multiple endocrine neoplasia type 2 (MEN2). The association of NF1 and MTC is rare, and are seldomly associated with other endocrine tumours.

A 23-year-old female with a history of chronic necrotising pancreatitis and background of NF1 was found to have an incidental large mediastinal mass (28x47x30mm), confirmed to be of neuroendocrine origin on biopsy. Further investigations with FDG and DOTATATE PET scans demonstrated a bulky thyroid with increased uptake in bilateral cervical chains. She was subclinically hyperthyroid with serum calcitonin 40,206pg/mL [0-10], CEA 19.0ug/L [<3] and normal metanephrines. The remainder of the pituitary panel was normal. A thyroid ultrasound noted a large left inferior pole solid, hypoechoic lesion 20x40x24mm in size with irregular margins and internal punctate echogenic foci of calcification (TIRADS 5). Subsequent biopsies of a left lobe nodule and bilateral cervical chain nodes confirmed metastatic medullary thyroid carcinoma. Calcitonin level on needle rinse at all biopsied sites was >80,000ng/L.

Multidisciplinary team reviews were suggestive of a contiguous thyroid and mediastinal mass likely MTC with significant retrosternal extension and lymph node metastasis. She underwent surgical resection of both masses with histopathology and gene sequencing pending. Adjuvant therapy will be considered dependent on her post-operative course.

This is a rare case of a MTC in a patient with NF1, with only a handful reported in literature to date. NF1 has been linked with thyroid carcinomas such as thyroid C-cell hyperplasia and papillary thyroid carcinoma. We await further results to determine whether there are direct links between the NF1 gene and MTC in our patient, or if co-existing culprit oncogene mutations are present.

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Staged resection of suspected bilateral phaeochromocytoma in a patient with end-stage kidney disease on haemodialysis

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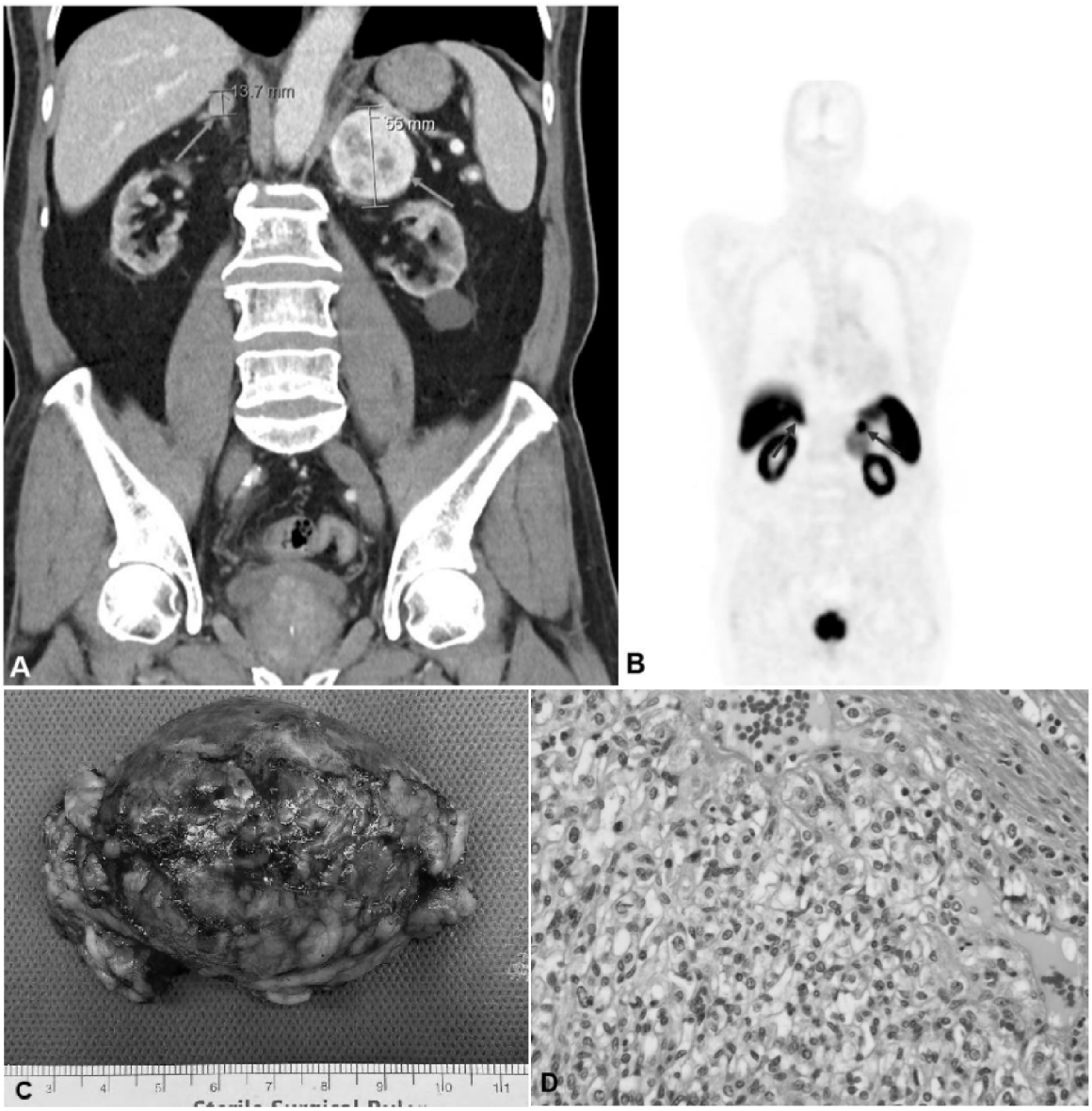
Phaeochromocytoma is a rare finding in end-stage kidney disease (ESKD). Usual treatment complexities are complicated by diagnostic considerations of anuria, dialysis-dependent catecholamine variability and challenges in perioperative fluid management. Plasma metanephrines/normetanephrines can be independently raised in ESKD and haemodialysis, adding further diagnostic challenges. Available case reports of phaeochromocytoma in ESKD have described unilateral lesions. We describe a haemodialysis patient with suspected bilateral phaeochromocytoma, managed with staged surgical resection.

A 73-year-old haemodialysis patient presents with resistant hypertension, on a background of hypertensive nephrosclerosis and Type 2 diabetes mellitus. CT imaging revealed bilateral contrast-enhancing adrenal lesions (left 5.5cm, right 1.4cm) (**Figure 1A**). PET-CT demonstrated corresponding FDG-avid lesions and PET Gallium DOTATATE was suggestive of bilateral neuroendocrine tumours, although findings were non-specific for the right lesion. (**Figure 1B**). In the context of resistant hypertension, the lesions were favoured to be phaeochromocytomas, with the diagnosis supported by markedly elevated plasma normetanephrine levels of 6,600pmol/L (Ref <1,079pmol/L).

Blood pressure (BP) control was suboptimal (systolic BP 170-210mmHg) on four anti-hypertensive medications (irbesartan, lercanidipine, atenolol, and prazosin) and were modified to be specific for phaeochromocytoma. Maximum alpha-receptor blockade was achieved with high-dose phenoxybenzamine (300mg/day) followed by propranolol and nifedipine targetting systolic BP 140-160mmHg.

Considering the high perioperative risks and patient comorbidities, a staged unilateral resection was favoured over simultaneous bilateral approach. The left adrenal lesion was resected laparoscopically (**Figure 1C**) with histopathology confirming phaeochromocytoma (**Figure 1D**). Minimal vasopressor support for BP was required intraoperatively and *no* support postoperatively, consistent with bilateral phaeochromocytoma. Interestingly, normetanephrine levels normalised postoperatively despite persistent hypertension, and the decision was made to manage the right lesion with surveillance.

Permanent hypocortisolism occurs following bilateral adrenalectomies. Our case highlights the benefits of a cautious approach to suspected bilateral phaeochromocytomas with staged surgical resection, particularly in high-risk populations and if there is diagnostic imaging uncertainties.



A) Adrenal CT: bilateral contrast-enhancing adrenal masses (green arrows). **B)** PET gallium Dotatate: bilateral adrenal activity consistent with neuroendocrine tumour (red arrows). **C)** Surgical resection of phaeochromocytoma (7.0cm) **D)** Phaeochromocytoma: classic pattern of small nests (Zellballen) of neuroendocrine cells.

Obstruction to managing a crisis

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Phaeochromocytoma crisis (PCC) is an acute catecholamine-induced state of haemodynamic instability which can lead to end-organ dysfunction associated with significant mortality. Catecholamine-induced ileus is a feature of PCC that is rarely described.

We report a case of PCC with ileus in a 46-year-old male with a newly diagnosed 10 cm phaeochromocytoma, triggered by high dose oral dexamethasone. This manifested as inverted Takotsubo cardiomyopathy, type one respiratory failure and acute pulmonary oedema, further complicated by concurrent ileus which responded poorly to conventional measures. After initial intensive care management, there was interval clinical improvement. However, despite escalating doses of oral phenoxybenzamine exceeding 1 mg / kg over six days, paroxysmal diaphoresis and hypertension requiring glyceryl trinitrate persisted. Inadequate phenoxybenzamine absorption was suspected in the context of ileus. IV phentolamine was used with concurrent phenoxybenzamine up-titration, which ultimately resulted in resolution of ileus and improvement of cardiac function and allowed use of oral alpha blockade in the pre-operative period.

In PCC ileus, it is proposed that excess catecholamines cause decrease in intestinal peristalsis, motility and tone via activation of alpha and beta adrenergic receptors in intestinal smooth muscle (1). Reported cases of ileus are often refractory to conventional therapies including decompression with nasogastric and rectal tubes, laxatives and enemas. Alpha blockade can attenuate catecholamine stimulus and is helpful in catecholamine-induced ileus (1). To our knowledge, the challenges in adequate systemic absorption of oral alpha blockers in ileus are not highlighted in the literature. This case demonstrates PCC with ileus resulting in reduced oral absorption of phenoxybenzamine, requiring IV phentolamine for resolution of ileus and cardiac function.

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Polyuria polydipsia syndrome met with a rare differential diagnosis

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CASE STUDY

LR is a 73-year-old gentlemen who presented with two months of polydipsia and polyuria. His medical history included renal cell carcinoma in remission and he was a current smoker. Examination showed normal visual fields.

A water deprivation test was performed, urine osmolality increased by 20% after desmopressin was given suggestive of partial central diabetes insipidus. He was commenced on desmopressin. Initial pituitary function assessment revealed hypogonadotrophic hypogonadism. Within the next 4 weeks, he developed central hypothyroidism and secondary adrenal insufficiency and was commenced on thyroxine and hydrocortisone.

MRI showed a pituitary mass compressing the optic chiasm, other cerebral lesions were also present suspicious for metastases. CT chest showed a left upper lobe lung mass. Histology from the lung biopsy revealed lung adenocarcinoma. This histology in the context of the rapid progression to panhypopituitarism was highly suggestive of metastatic non-small cell lung carcinoma.

LR received radiotherapy to the pituitary metastases and chemotherapy. Despite a complete metabolic response on staging, he remains on pituitary hormone replacement.

DISCUSSION

Pituitary metastases are rare, neoplasms from almost every tissue have been reported to metastasise to the pituitary with breast and lung accounting for two-thirds of pituitary metastases.

Diabetes insipidus is the most common clinical presentation of symptomatic pituitary metastases. This highlights a predominance of metastasis to the posterior lobe, likely from direct haematogenous spread through the inferior hypophyseal artery. Pituitary insufficiency may be apparent at presentation or present insidiously and patients should be alert to symptoms and be regularly screened.

Water-deprivation test was used to diagnose diabetes insipidus. Copeptin is useful as an initial test to diagnose or exclude central diabetes insipidus. However, it is not widely available and lead time can delay diagnosis. Pituitary metastases should be a considered as a rare differential diagnosis especially in patients presenting with diabetes insipidus.

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A covert case of ectopic ACTH

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Ectopic adrenocorticotropic hormone (ACTH) is a rare cause of Cushing's syndrome, accounting for approximately 5 to 10 % of cases (1). The optimal treatment of ectopic ACTH is surgical resection of the underlying tumour (1).

We present a challenging case of ectopic ACTH in a 74-year-old male with a rapid onset of signs and symptoms. He had a background of frequent and prolonged exogenous steroid use. Initial biochemical testing revealed a significantly elevated low-dose dexamethasone suppression test, 24-hour urinary free cortisol, midnight salivary cortisol, and ACTH. Neuroimaging revealed a 3 mm pituitary adenoma. Dynamic testing with a corticotrophin-releasing hormone stimulation test and high-dose dexamethasone test were suggestive of ectopic ACTH over pituitary disease. Computed tomography (CT) did not identify a source. During this time, the patient had multiple admissions to hospital with worsening glycemic control and hypertension, progressive proximal myopathy, as well as hypokalaemia and sepsis. He was subsequently commenced on medical therapy and eventually had a ^{68}Ga-DOTA-somatostatin analogue positron emission tomography (PET)/CT scan which did not demonstrate any identifiable focus for ectopic ACTH production. The patient declined to have bilateral adrenalectomy, and chose to remain on medical therapy with ongoing surveillance.

We faced many diagnostic challenges with this patient. The history of exogenous steroid use and the finding of a small pituitary adenoma were both red-herrings. We were also unable to localise the source of ectopic ACTH. Conventional imaging studies, such as CT, can localise the tumour in approximately 50 % of cases (2). ^{68}Ga-DOTA-somatostatin

analogue PET/CT has the highest sensitivity for localising covert ACTH-producing tumours (2). Medical therapy can be continued for prolonged periods in patients with covert disease, allowing serial imaging over time to identify the tumour (1). However, in the case of indolent disease, it can take years to decades before the source is identified (1).

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Induction of lactation in a patient with complete androgen insensitivity syndrome

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With rising rates of surrogacy, induced lactation is increasingly relevant, allowing women who did not undergo pregnancy to breastfeed. In this case report, we describe the clinical management of a patient with complete androgen insensitivity syndrome (CAIS) who wished to breastfeed. We review the literature regarding lactation induction using pharmacological and non-pharmacological therapies in arranged mothers – XX women, transwomen (XY) and women with CAIS (XY). To our knowledge, there are only two case reports in the literature describing induced lactation in transwomen and CAIS respectively.

A 32-year-old married woman with CAIS was expecting a child by a surrogacy arrangement. She had been on oestrogen therapy since the diagnosis of CAIS at age 18 years. When the surrogate mother was at 32 weeks gestation our patient expressed a desire to be able to breastfeed the infant. Based on published case reports and review articles on lactation induction, in addition to her regular oestrogen therapy, our patient was commenced on a combination of two actuations of Estrogel daily (to increase circulating oestradiol), a galactagogue and regular mechanical breast stimulation eight weeks prior to the delivery of her baby. On delivery of her child, Estrogel was ceased. The patient produced a small quantity of breastmilk, allowing suckling of the infant for a short time but not sufficient for effective breast feeding.

Induced lactation is possible in chromosomally XY individuals. Based on our experience and the published literature, it is more successful in cis/trans-women who have had progesterone/progestogen exposure to the breast which is important for ductal maturation and lobular formation. We conclude that the addition of a progestogen to our patient's treatment regimen, either as part of her hormone therapy or part of the lactation induction program would have improved her chances of establishing successful lactation.

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A case series of neck of femur fractures occurring in patients with type 1 diabetes and active diabetes-related foot ulcers

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There is an association with type 1 Diabetes (T1DM) and an increased risk of fractures described in the literature. This is likely multifactorial due to abnormal bone mineralisation and/or impacts on bone quality directly resulting from the disease itself, as well as the increased risk of falls associated with diabetes-related complications. While data is limited regarding the bone health of individuals with diabetes-related foot ulcers (DFU), available literature suggests that DFU may be a risk factor for low bone density and insufficiency fractures in this population. It has been noted that bone mineral density at the femur in this cohort is particularly affected. This may be due to interventions that improve wound healing including extended periods of offloading or alternatively may be secondary to the effects of inflammation on bone remodelling.

We identified four patients with T1DM and active DFU previously admitted between 2015 and 2021 to the Multidisciplinary Diabetes Foot Unit (MDFU) at a large Australian Tertiary Hospital who subsequently suffered neck of femur fractures.

The patients ranged from 39-57 years old, all of whom had suboptimal glycaemic control and documented microvascular complications including peripheral and autonomic neuropathy. In some cases, these complications were severe, including gastroparesis requiring parenteral feeding and previous below knee amputation resulting in being non-ambulatory. Additionally, all patients required some form of pressure offloading in the lead up to their fracture.

This case-series highlights a potential relationship between T1DM, active DFU and neck of femur fractures in a cohort of relatively young patients. While there is conflicting data relating duration of diabetes and diabetes control to fracture risk, our patient cohort had poorly controlled diabetes with concurrent microvascular complications. The association between type 1 diabetes, active DFU and future fracture risk warrants further exploration via longitudinal prospective studies.

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A rare case of SOX1 paraneoplastic limbic encephalitis in papillary thyroid cancer

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Introduction

Paraneoplastic neurological syndromes (PNS) are rare immune-mediated nervous system disorders associated with tumours and occur in <1% of cancer patients. SOX1 (Sry-like high mobility group box) is part of a family of transcription factors that play a role in the developing central nervous system. Anti-SOX1 antibodies are onco-neurological autoantibodies associated with various neurological syndromes, including Lambert Eaton myasthenic syndrome, paraneoplastic cerebellar disorder, and paraneoplastic limbic encephalitis. Neurological symptoms can precede cancer diagnosis by up to 4 years. These anti-SOX1 antibody mediated PNS are most commonly reported with small cell lung cancer, where up to 36.5% of patients have positive SOX1 antibodies, but can also occur in non-small cell lung cancers. Very few other cancers have been reported in the literature. Papillary thyroid cancer is the most common thyroid malignancy with an excellent survival rate of >90% and is rarely associated with paraneoplastic syndromes.

Case

We present the case of a previously well 65-year-old gentleman with SOX1 paraneoplastic limbic encephalitis and multifocal papillary carcinoma presenting with rapidly progressive cognitive impairment and neuropsychiatric symptoms. His initial MOCA was 24/30 and ACE was 69/100. Investigations yielded serum positive but CSF negative SOX1 antibodies. PET scan showed an FDG-avid thyroid lesion and he underwent total thyroidectomy confirming a stage 2, BRAF positive, multifocal papillary thyroid cancer with extrathyroidal extension (pT3a, N1, Mx). He was subsequently treated with I-131 dose of 3700MBq and commenced on thyroxine suppressive therapy. His neurological symptoms were refractory to initial methylprednisolone and IVIG however significantly improved four weeks post thyroidectomy and additional immunosuppression. His MOCA and ACE assessment improved to 29/30 and 96/100 respectively after three months.

Conclusions

SOX1 limbic encephalitis is a rare PNS occurring in this case in association with papillary thyroid cancer which responded well to treatment. However, development of further malignancies is still a possibility.

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Cushing's disease: a bumpy course

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Cushing's disease in pregnancy is difficult to diagnose and manage. Pregnancy is a state of physiological hypercortisolism, making reference ranges for cortisol levels and screening tests difficult to establish. There are significant maternal, fetal and obstetric outcomes associated with Cushing's in pregnancy.

We present a case of a 31 year old female (CM) who develops relapsed Cushing's disease in early pregnancy. She had a history of Cushing's disease at age 20 with transphenoidal sinus surgery. Histopathology demonstrated ACTH staining pituitary adenoma.

CM developed clinical features of relapse at age 30 with weight gain and facial rounding and this was biochemically confirmed with elevated 24 hour urinary free cortisol (UFC) of 420mol/24hr (54-319) and raised midnight salivary cortisol of 17nmol/L (RR <4). Around the time of these results she fell pregnant. A non-contrast pituitary MRI demonstrated a 6x4mm microadenoma.

She developed gestational diabetes requiring insulin and hypertension at 6 weeks. Given the early onset of these conditions and the high risk of pre-eclampsia, stillbirth and premature delivery with Cushing's disease in pregnancy, surgical management was planned in second trimester. Petrosal sinus sampling demonstrated a central to peripheral gradient and she underwent transphenoidal sinus surgery at 17 weeks . Histopathology demonstrated adenoma with positive ACTH staining and Ki67 of 6%, with evidence of tumour invasion into bone. Her insulin and antihypertensive requirements stabilised though postoperative cortisol levels remained high. Fetal growth measured in the 15th centile. CM had premature

rupture of membranes at 35 weeks, delivering a healthy infant with uncomplicated delivery. 24 hour UFC have normalised post partum though definitive cure is unlikely. Planning of further pregnancies will represent a challenge.

This case outlines challenges associated with management of Cushing's in pregnancy in regards to interpreting reference values, balancing maternal and fetal outcomes and selecting treatment options.

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Hyperparathyroidism-jaw tumour syndrome: diagnosis and management dilemmas

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Background: Hyperparathyroidism-jaw tumour syndrome (HPT-JT) is a rare genetic syndrome associated with primary hyperparathyroidism (PHPT), fibro-osseous tumours of the maxilla and mandible (30%), and renal (15%) and uterine involvement (50% of females). Given the rarity of HPT-JT there is much controversy on many aspects of this condition which includes its diagnosis, management, follow-up and screening. We present a case of HPT-JT and highlight some of the challenges associated with this syndrome (1, 2).

Case Presentation: A 38-year-old Caucasian man with no prior personal or family history, presented with symptomatic severe hypercalcaemia due to PHPT. His initial biochemistry showed corrected calcium 3.10 mmol/L (2.1 – 2.6), phosphate 0.32 mmol/L (0.75 – 1.5), markedly elevated parathyroid hormone level at 70.4 pmol/L (1.6 – 7.5) and creatinine 86 umol/L (60 – 110). His vitamin D initially was 35 nmol/L but subsequently become replete on supplementation. Localisation studies revealed a large 32 x 30 x 24 mm left inferior parathyroid adenoma and he subsequently had a left inferior parathyroidectomy. His histopathology and immunohistochemistry revealed a PDP9.5 positive and parafibromin deficient atypical parathyroid tumour, which weighed 16 x 7g. He was referred for genetic testing which showed no pathological variant in the CDC73 gene. He has renal stones but no evidence of jaw or renal tumour on screening imaging.

Conclusion: HPT-JT is rare and heterogeneous in nature, and there are many challenges associated with this condition which includes diagnosing HPT-JT with the use of genetic testing and immunohistochemistry, determining the best surgical approach to PHPT which is controversial, and diagnosing parathyroid carcinoma which is commonly associated with HPT-JT. Further research and multidisciplinary care is required for optimal management of patients with HPT-JT.

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A Rare Cause of Hypopituitarism

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JD is a 68-year-old man, who presented with 1-week history of abdominal pain associated with nausea and diarrhoea, secondary to aperient use in the setting of opioid-induced constipation. He was diagnosed with a BRAF-mutated non-small cell lung cancer with liver and bony metastases in June 2021 and completed radiotherapy to his cervico-thoracic spine. He received oral cobimetinib/vemurafenib for 5 months, ceased due to immune-related cardiomyopathy.

In hospital, he developed symptomatic hypotension with a BP of 80/50mmHg. He received hydrocortisone 100 mg intravenously prior to diagnosis of adrenal insufficiency. Results of his endocrine testing are shown in Table 1.

Table 1: Results of endocrine testing before administration of hydrocortisone

Test (all done at 6.30am)	Result	Reference range
ACTH	14 ng/L	<14 ng/L
Cortisol	20 nmol/L	101-536 nmol/L
TSH	0.05 mIU/L	0.4-4 mIU/L
FT4	6.7 pmol/L	8.5-27 pmol/L
LH	0.7 IU/L	3-10 IU/L
FSH	1.0 IU/L	1.6-9.7 IU/L
Testosterone	<0.3 nmol/L	9-35 nmol/L
Prolactin	663.4 mIU/L	97-484 mIU/L
IGF-1	5.2 nmol/L	4-32 nmol/L

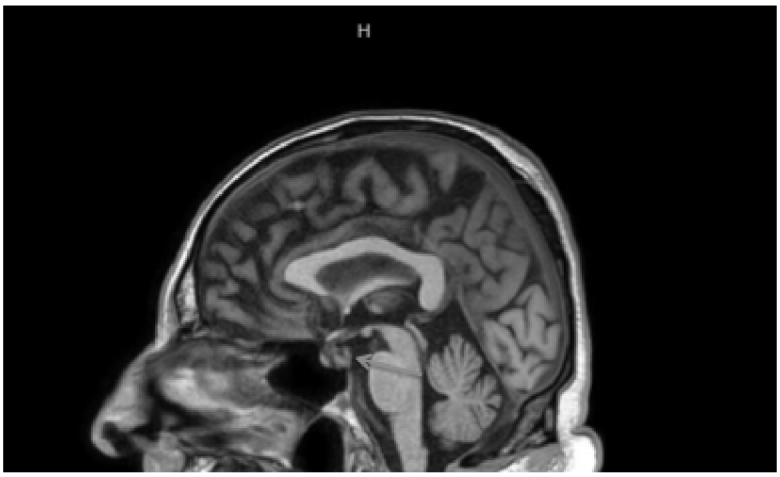

Figure 1: T1 sagittal pre-Gadolinium. Enlarged pituitary infundibulum and thickening and non-tapering enhancement of the pituitary stalk (arrow)

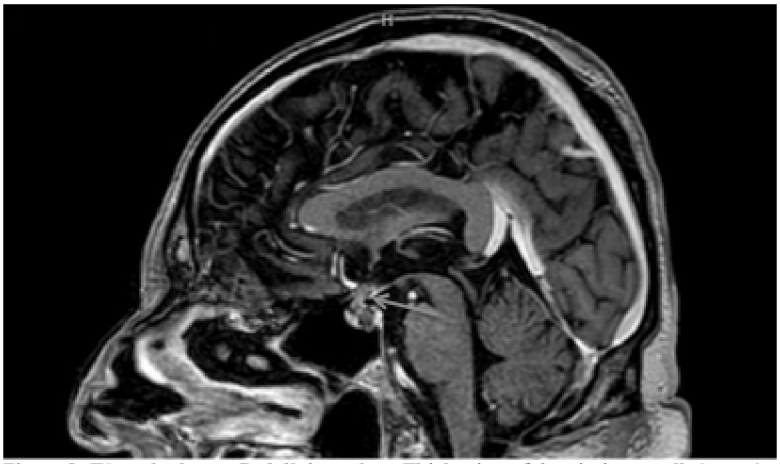

Figure 2: T1 sagittal post-Gadolinium view. Thickening of the pituitary stalk (arrow).

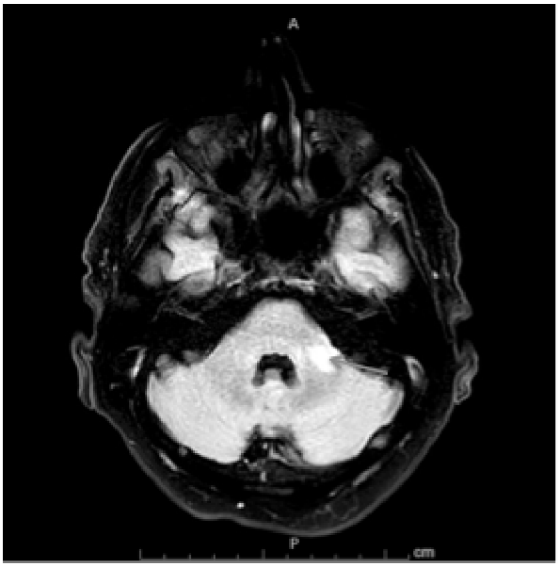

Figure 3: T2 FLAIR Axial View. 4mm hyperintense peripheral nodule located in left brachium pontis (arrow)

Given the features consistent with a pituitary metastasis (figures 1-2), a pituitary biopsy was not required. Due to multiple intracranial metastases (figures 1-3), surgical resection was inappropriate.

Intravenous hydrocortisone was changed to oral hydrocortisone 20 mg mane and 10 mg at 4pm. Thyroxine 100 mcg daily was also started. He was readmitted a week later with progressive lethargy and exertional dyspnoea. Computed tomography pulmonary angiogram did not show evidence of pulmonary embolism. Repeat echocardiogram showed improvement in his ejection fraction. Testosterone replacement was considered, but not started. JD was planned for radiation therapy, however he deteriorated rapidly and passed away in hospital.

Discussion

Breast cancer in women and lung cancer in men are the most common primary cancers associated with pituitary metastases. Adrenal insufficiency was the commonest hormonal dysfunction, followed by central hypothyroidism, hyperprolactinaemia and diabetes insipidus[1].

JD's MRI brain showed features consistent with a pituitary metastasis[1,2]. A biopsy is recommended to confirm the diagnosis. However, a pituitary biopsy was not indicated in his case and it would not have changed the management.

Treatment includes management of the primary tumour and relieve of symptoms associated with mass effect[1]. As surgical resection was inappropriate, radiotherapy to the pituitary stalk metastasis was considered.

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How cryopreserved sperm quality is affected differently after an isolation technique is applied (DGC, swim-up, electrophoretic separation)

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Sperm cryopreservation is being increasingly used as a means of preserving the fertility of patients subjected to chemotherapy and as a method of storing donor spermatozoa prior to artificial insemination or IVF/ICSI. In this clinical context, it will be important to determine the optimal technique for isolating the spermatozoa once they have been thawed. Capturing the highest quality spermatozoa with a minimum of iatrogenic damage should not only enhance the chances of successful conception but also reduce the risk of miscarriage as well as genetic /epigenetic mutations in the embryo. To address this issue, we have cryostored human semen samples using a slow freezing protocol and Quinn's Advantage™ Sperm Freezing Medium. The samples were subsequently thawed and subjected to three types of sperm isolation procedure: swim-up from semen, density gradient centrifugation (DGC), and electrophoretic separation using the Felix™ device. A comparison of sperm quality in the unprocessed semen was also undertaken before and after freezing to provide baseline data on the impact of cryopreservation on sperm biology. Cryopreservation led to the anticipated loss of sperm motility and vitality in association with an increase in lipid peroxidation and DNA damage. Following sperm selection, all three isolation techniques resulted in an increase in sperm motility and in the case of the Felix™ and swim-up procedures, an increase in sperm vitality. Otherwise there were no significant differences between sperm separation techniques with respect to ROS generation or lipid peroxidation. However, there was a major difference in terms of DNA integrity, with the Felix™ device isolating cells exhibiting significantly lower levels of DNA damage than either DGC or swim up. This technique therefore offers some advantages over alternative isolation strategies, in terms of both the quality of the gametes isolated and the time taken to achieve the isolation.

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Ensembled learning for smart sperm analysis in assisted reproduction

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Infertility is a global health issue (1) that affects one in six couples in Australia (2), nearly half of which involve an element of male-factor infertility (3). A semen analysis including the morphological assessment is a routine and essential step in the infertility assessment pipeline. In most clinics, such analysis is still carried out manually leading to subjective results. The fundamental difficulty of sperm morphology classification is ideally suited to machine learning algorithms to automatically analyze and classify sperm images (4). Machine learning-based algorithms have been used to classify images of stained/dead sperm cells based on their head morphology status (5,6). Here, we demonstrate a Meta-classifier algorithm for morphology classification of stain-free live human sperm cells considering head, midpiece, and tail abnormalities. Combining VGG16, VGG19, modified ResNet-34, and DenseNet-161 architectures, an ensemble deep learning model has been developed to generate a comprehensive set of visual features for the morphology classification of sperm cells. The model includes fully automated detection and cropping algorithms to extract individual sperm from 100× magnification images and pass them through the classification algorithm. To prepare our training dataset, a user-friendly software interface is being developed and used by two expert clinicians to independently label the cells based on their morphological characteristics. We achieved a classification rate of 74.5% with 78.5% precision for overall normal/abnormal unstained human sperm images**.** Additionally, our model is capable of classifying cells based on their head, midpiece, and tail abnormalities with classification rates of 76% and 70.5%, respectively. We demonstrate a comprehensive machine learning algorithm capable of classifying sperm based on their head, midpiece, and tail abnormalities, providing a promising opportunity to improve sperm morphology analysis in fertility clinics.

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Cochrane Systematic Review: Day 3 versus day 5 embryo biopsy for preimplantation genetic testing of monogenic defects

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Aims:

To assess the benefits and harms of day 5 embryo biopsy, in comparison to day 3 biopsy, in preimplantation genetic testing for monogenic defects (PGT-M).

Methods:

Searches of electronic bibliographic databases were performed to identify randomised controlled trials. Hand searches of grey literature such as trial registers, relevant journals, Google scholar, and published conference abstracts were also

performed.

We used standard methodological procedures recommended by Cochrane.

The primary review outcomes were live births and miscarriages.

Outcomes were calculated per woman randomised and were reported as odds ratios with 95 % confidence intervals.

Results:

We are uncertain whether day 5 embryo biopsy, compared to day 3 biopsy, has an effect on live births as only one study was included in the analysis and the confidence interval was wide and crossed the line of no effect (OR 1.50, 95% CI 0.26 to 8.82, 1 RCT, 20 women; very low-certainty evidence) . It is also uncertain whether day 5 embryo biopsy has an effect on miscarriages (OR 3.32, 95 % CI 0.12 to 91.60, 1 RCT, 20 women; very low-certainty evidence). Other secondary outcomes which were assessed in this review were viable intrauterine pregnancies, ectopic pregnancies, stillbirths and termination of pregnancies. The evidence was uncertain for all of these outcomes. We could not reach a conclusion regarding gestational age at birth, birthweight, neonatal mortality and major congenital anomaly as no studies reported data suitable for analysis.

Conclusion:

We are uncertain if there is a difference in live births or miscarriages between day 5 and day 3 embryo biopsy for PGT-M. The results should be interpreted with caution, as the evidence was of very-low certainty, and further studies are needed to confirm findings.

Effect of GM-CSF on bovine in vitro oocyte maturation.

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Aims:

Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is found in the reproductive tract and has been shown to have beneficial effects when added to embryo culture media in many species including humans [1, 2]. GM-CSF is also found in the ovarian follicle [3] suggesting it may have a role in oocyte maturation. We have previously shown that the addition of GM-CSF to in vitro oocyte maturation (IVM) media improves oocyte developmental competence resulting in increases in blastocyst rate and cell numbers, and implantation and birth rates following frozen embryo transfer in mice. The present study was undertaken to determine if GM-CSF can improve bovine IVM.

Methods:

Cumulus oocyte complexes (COCs) were aspirated from abattoir derived ovaries and matured in 0 or 10 ng/ml of recombinant bovine GM-CSF (Kingfisher, Assay Matrix) in VitroMat media (ARTLabs Solutions) at 38.5C in 6% CO2, in air. Following fertilisation in Research Vitro Fertilisation media (Cook Medical) in 6% CO2 in air, presumptive zygotes were cultured in Research Vitro Cleave (Cook Medical) and VitroBlast (ARTLabs Solutions in 6% CO2; 7% O2; N_2 balance), and embryo development examined. Day 8 blastocyst cell numbers were also determined by differential staining and fluorescent imaging. Each group contained approximately 40 COCs and the experiment was replicated 10 times. Data was analysed using a paired t-test.

Results:

GM-CSF significantly increased day 8 blastocyst rate (81.1% vs 70.5%), blastocyst inner cell numbers (43.7 vs 38.3), total cell numbers (141.6 vs 128.1), and ICM:TE (0.50 vs 0.44) ratio compared with the control group .

Conclusion:

In conclusion our results suggest that adding GM-CSF to bovine IVM media, improves bovine embryo development.

1. Granulocyte–macrophage colony-stimulating factor promotes human blastocyst development in vitro. C. Sjöblom, M. Wikland, S.A. Robertson ; Human Reproduction, Volume 14, Issue 12, 1 December 1999, (pg. 3069–3076)
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3. Ovarian immune cells express granulocyte-macrophage colony-stimulating factor (GM-CSF) during follicular growth and luteinization in gonadotropin-primed immature rodents. K Tamura 1, H Tamura, K Kumasaka, A Miyajima, T Suga, H Kogo. Mol Cell Endocrinol. 1998 Jul 25;142(1-2): (pg.153-63).

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Podocalyxin may facilitate the formation of compact and hardy spheroids in high grade serous carcinoma

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Background: High grade serous carcinoma (HGSC), the most common form of ovarian cancer, often metastasizes transperitoneally via shedding the of cancer cells as aggregates (cancer spheroids), allowing it to disseminate throughout the peritoneal cavity. Overexpression of transmembrane protein podocalyxin (PODXL) has been associated with poor prognosis of several epithelial cancers including HGSC, and PODXL surface localisation in HGSC has been associated with a significant decrease in disease-free survival.

Aims: To examine PODXL expression in HGSC tissues, ovarian cancer cell lines and ascites-derived primary tumour cells, and to determine the impact of PODXL on HGSC spheroid morphology and compactness.

Methods: PODXL expression was examined by immunohistochemistry in tissues, and by RT-PCR and immunofluorescence (ICC) in cell lines and ascites-derived primary cells. Kuramochi cells, a HGSC cell line with high PODXL expression, were used as a model and PODXL was knocked out (KO) using CRISPR technology. Spheroids of control and PODXL-KO Kuramochi cells were compared to determine the effect of PODXL on spheroid morphology and hardiness.

Results: All HGSC tissues showed positive PODXL staining with varying intensity. Tissues with high levels of PODXL showed strong apical staining in cluster-like patterns. PODXL was expressed by many ovarian cancer cell lines, and ICC showed PODXL localisation on the surface of cancer spheroids. Spheroids formed with PODXL-KO Kuramochi cells were less compact, more fragile and broke apart more easily under external force than the control. Among ascites-derived primary cancer cells (n=6 patients) examined so far, 2 showed low whereas 4 displayed moderate-high levels of PODXL expression; when spheroids were generated from these cells, those with high PODXL expression were more compact than those with low PODXL.

Conclusions: PODXL may promote the formation of compact and hardy spheroids in HGSC for cancer metastasis and thus may represent a potential therapeutic target for HGSC treatment.

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Delineating cell-cell communications in testicular germ cell tumours

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The incidence of testicular germ cell tumours (TGCTs), the most common tumour in men aged 19-44, is increasing. Precursor germ cell neoplasia *in situ* (GCNIS) cells arise during fetal life, eventually forming seminoma or non-seminoma tumours. What controls their progression is not understood, though both genetic and environmental factors contribute. This study combines spatial whole transcriptomics on four individual tumour samples with hanging-drop cultures to identify signalling mechanisms, cell profiles and cell-cell interactions within the tumour microenvironment. Fresh testis tissue from orchidectomies were obtained by consent (three non-seminoma and one seminoma, including tumour-free regions). Tissues were 4% PFA-fixed and paraffin-embedded or snap-frozen. Nanostring GeoMx Spatial Whole Transcriptome analysis was performed on fixed sections, with 95 regions of interest (ROI) selected following immunodetection of CD45, PanCK, and a nuclear stain. ROIs included normo- and hypospermatogenic tubules, interstitium, immune cell infiltrates, GCNIS, non-seminoma and seminoma. Following data normalisation, we mapped the expression profile of signalling pathway components and their downstream targets, identifying transcripts robustly linked with specific phenotypes, including activin/TGFβ signalling pathway components (*ACVR1B*, *TGFRB3*). Additional tissue from the non-seminomas were cut (1-2 mm^3 pieces) for 48-hour hanging drop cultures in 30µL media (0.1% BSA/DMEM:F12/ITS/Pen-Strep) containing 5 or 50 ng/mL activin A, 10µM SB431542 (activin/Nodal/TGFβ inhibitor), or vehicle. Fragments (n=2-3 per treatment) were analysed by immunohistochemistry (OCT4 for GCNIS, CD68 for macrophages) and transcripts measured by qRT-PCR. Cultured fragment histology was consistent with uncultured tissue, retaining macrophages and GCNIS. Transcripts including *OCT4*, *SOX2*, *SOX17*, *INHBA* and *KIT* were not significantly altered by activin or SB431542 exposure; variation within treatment groups highlighted non-seminoma heterogeneity. However, *MMP9* increased ~30-fold, and *LEFTY2* decreased following culture. Identification of activin A target genes is ongoing in conjunction with continued sample collection. These approaches will enable identification of cell communication networks within TGCTs and mechanisms governing tumour phenotypes.

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Treatment with placental EVs increased the levels of death-associated miRNAs in ovarian cancer cells

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Introduction: It is now well-recognised that phagocytosis of placental EVs impacts the function of the recipient cells by releasing of their cargos, including proteins and miRNAs. We have previously reported that phagocytosis of placental EVs inhibited the growth of SKOV-3 ovarian cancer cells. However, the mechanism underlying this inhibitory effect is unknown. recent miRNA sequencing data showed that some miRNAs contained in placental EVs including miRNA-519a-5p and miRNA-143-3p, are associated with the promotion of ovarian cancer cell death. In this study, we further investigated whether there is a change in these miRNAs in SKOV-3 after exposure to placental EVs.

Methods: Placental EVs were collected from first trimester placentae (n=4) and SKOV-3 cells were treated with placental micro- or nano-EVs for 24 hours in triplicate. The levels of miRNA-519a-5p, miRNA-512-3p, and miRNA-143-3p were measured in SKOV-3 cells. In addition, mRNA levels of G3BP1 and BCL2L2 which are target genes of miRNA-519a-5p were also measured.

Results: The levels of miRNA-519a-5p, miRNA-512-3p, and miRNA-143-3p were significantly increased, and the mRNA levels of G3BP1 and BCL2L2 were decreased in SKOV-3 after exposure to placental EVs. Transfection of SKOV-3 cells with a mimic of miRNA-519a-5p or miRNA-143-3p significantly reduced cell viability. However, the levels of miRNA-519a-5p returned to baseline in the daughter cells after passaging the SKOV-3 cells. When the daughter cells were exposed to fresh placental EVs, the levels of miRNA-519a-5p significantly increased as in the parental cells.

Discussion:our data demonstrated that phagocytosis of placental EVs contributed to the death of ovarian cancer cells, possibly through increasing the levels of death associated.

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Expression of PCOS candidate genes in human gonadal, metabolic, and brain tissues in fetuses and adults.

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Polycystic Ovary Syndrome (PCOS) is a heterogeneous disorder, affecting 10% of women of reproductive age, with infertility, obesity and type 2 diabetes as risk factors. The cause of PCOS is not known but there is predisposition to PCOS in adult life that begins during fetal or perinatal life. PCOS also has a genetic predisposition and a number of loci associated with PCOS have been identified. They contain 25 candidate genes. Although the name PCOS suggests a syndrome of the ovary, PCOS has also been associated with the central nervous system and other organ systems in the body due to the symptoms it present. Here, we examined the expression patterns of PCOS candidate genes in gonadal (ovary and testis), metabolic (heart, liver and kidney) and brain (brain and cerebellum) tissues during the first half of human fetal development and postnatally till adulthood using publicly available RNA sequence data. We found that the genes were dynamically expressed across all tissues studied. Some genes were significantly expressed in gonadal tissues, whilst others in the metabolic or brain tissues at different time points prenatally and/or postnatally; suggesting tissue specific roles of these candidate genes. Specifically, some genes (*HMGA2, GATA4,* and *TOX3*) were highly expressed during the early stages of fetal development in most tissues but least during adulthood. Others including *AOPEP/C9orf3, SUOX,* and *SUMO1P1* were expressed postnatally. Interestingly, *HMGA2, RAD50,* and *YAP1* correlated significantly with each other in at least 5 of the 7 fetal tissues studied. Overall these studies suggest that the predisposition to PCOS could arise during fetal development by alteration of the expression of PCOS candidate genes in multiple organs.

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Platform for genome modification in human multipotent cell line

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Intersex conditions are a spectrum of conditions in which gonadal, chromosomal, or anatomical sex is atypical. The underlying molecular basis remains unknown in many forms of DSD. This is due, in part, to the lack of manipulable models that recapitulate human sex determination. Sex is determined by the onset of *SRY* expression around gestational week 6 in the XY embryo. In turn, the transcription factor SRY switches on expression of *SOX9,* another DSD gene. *SOX9* is a critical hub gene that regulates dozens of target genes we seek to identify, as they may contribute to DSD etiology. Towards this, we characterised NT2/D1 cells as an *in vitro* model to model Sertoli cell function in the testis. NT2 cells are a human

multipotent clonal cell line derived from a testicular tumour that model a variety of human developmental processes. Undifferentiated, NT2 model sex determination initiated by SRY or by SOX9. When differentiated, NT2 model neuronal development or smooth muscle development. However, as a multipotent line, NT2/D1 cells are difficult to manipulate e.g. by RNAi. To extend the utility of NT2/D1 cells, we have established an NT2/D1-cas9 cell line. We characterise the integrity of these via a suite of cell phenotyping assays including xCELLigence. We are using NT2/D1-cas9 to knockout candidate SOX9 target genes, and investigating the cellular behaviours affected in DSD: cell adhesion, proliferation and migration.

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GM-CSF during in vitro oocyte maturation reduces reactive oxygen species levels in mouse oocytes

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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 in cumulus-oocyte complexes (COCs) in a mouse model of human IVF. The present study was undertaken to determine if adding GM-CSF during IVM reduces the production of reactive oxygen species (ROS) in GM-CSF-treated oocytes. C57Bl6 x CBA F1 female mice aged 21-23 days were injected with 5IU of eCG and COCs aspirated from large antral follicles 46-48 h post-injection. Ten COCs were cultured per 50µL drop in bicarbonate-buffered α-MEM containing 3 mg/ml BSA, 1 mg/ml Fetuin and 5 mIU/mL FSH plus 0 or 10ng/ml of GM-CSF. 16 hours later matured COCs and denuded oocytes were incubated for 30 minutes in 2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFD-FA; Life Technologies, California). After incubation denuded oocytes/ mature COCs were washed to remove excess stain and then mounted on a glass slide for imaging. Oocyte fluorescence intensity was measured at emission wavelengths of 500 nm and 529nm using The Cell Voyager CV1000 Confocal Scanner (Yokogawa, Japan). Z-stack imaging was done and analysed using Fiji Image J software. The experiments were replicated three times each, denuded oocytes and mature COC, 18-20 oocytes per group. Data were analysed using a univariate general linear model in SPSS. The addition of GM-CSF during IVM had no effect on ROS production in mature COCs, however, there was a significant decrease in ROS production in denuded oocytes (7565.3 + 733 vs 14449.5 + 1246; $P < 0.05$). In conclusion, we have shown that the addition of GM-CSF during IVM decreases ROS production which may contribute to the increase in mitochondrial activity in COCs as reported previously (an increase in mitochondrial activity reduces ROS levels)

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In vitro embryo culture perturbs the metabolite profile and expression of genes involved in redox regulation in preimplantation embryos.

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In vitro cultured embryos have significantly poorer development than their *in vivo* counterparts, developing more slowly and fewer reaching the blastocyst stage. *In vitro* culture of preimplantation embryos alters their metabolism, increases ATP production and reduces pyruvate oxidation. This study used LC/MS to measure metabolite concentrations in pre-implantation cultured embryos compared to fresh embryos. Expression of genes involved in redox signalling pathways were examined by RT-qPCR to determine if any differences in metabolites were correlated to changes in gene expression.

In vitro embryo culture caused changes in metabolite concentrations at all stages of development, with 25-45% of detected metabolites being significantly altered, with the largest alterations occurring post-compaction embryos. Metabolite concentrations affected by *in vitro* culture included common amino acids. Concentrations of essential amino acids were decreased or maintained, with maintenance potentially occurring via autophagy. Non-essential amino acids concentrations were increased *in vitro* presumably due to endogenous upregulation of production resulting from activation of the amino acid response pathway. There were also changes in concentrations of metabolites involved in the pentose phosphate pathway, glucose metabolism and the TCA cycle.

In vitro culture also caused a downregulation of genes critical in redox regulation and GSH production suggesting oxidative distress in *in vitro* cultured embryos.

These results highlight the significant physiological differences between *in vivo and in vitro* developed embryos, indicating that the *in vivo* environment is not fully mimicked *in vitro*. These results highlight the need for improvement in embryo culture media to improve the success of *in vitro* embryo production.

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Addressing preventable infertility through understanding ovary development and improving fertility education

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Infertility is one of the greatest global health challenges we currently face with data suggesting 15% of all reproductive-aged couples are affected by infertility. For many, infertility is a preventable outcome resulting from insufficient knowledge about fertility and behaviours that compromise reproductive health, including age. We urgently need novel preventative solutions to address and improve human fertility. My research program focusses on determining the underlying causes of age-related infertility in women alongside improving reproductive health knowledge in adolescents. I do this by combining my expertise in cutting-edge discovery science with public health promotion and education.

My discovery research has been instrumental in demonstrating that unregulated primordial follicle activation results in premature infertility. Most recently, I have applied single cell RNA sequencing to assemble a complete database of the transcriptomic heterogeneity of the supportive granulosa cells of the ovary during follicle activation. I am currently using this novel dataset to determine the regulatory factors driving granulosa cell differentiation and the functional significance of these factors to ovary and follicle development using biological modelling.

To identify the fertility knowledge gaps in adolescents, this year, my team surveyed 1,466 15–18-year-old Australia-based school students. Our participants represented diverse socio-demographic groups and although 61% confirmed their desire to have children in the future, an alarming 81% stated they did not believe that school could answer their sexual education questions. Concerningly, fertility related questions, not mandated within the national curricula, were the most poorly answered. Moving forward, I am using these findings to develop and evaluate a high efficacy, long-lasting fertility education intervention appropriately aligned to relevant syllabus skills statements and UNESCO international learning objectives.

The outcomes of my research encompass fundamental biological discoveries and applied educational reforms, with the capacity to benefit human reproductive health across the entire translational cycle.

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Investigating steroid hormone regulation of TETs and developing a framework for hydroxymethylation studies in human endometrium

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Cyclic changes in DNA methylation machinery occur in the endometrium, and deregulation of DNA methylation could lead to aberrant gene expression, including steroid hormone receptors. Although methylation (5mc), mediated by DNA Methyltransferases (DNMTs) is widely studied in the endometrium, the role of the inter-related process hydroxymethylation (5hmc), mediated by Ten Eleven Translocation enzymes (TETs), is relatively unknown. We have reported temporal changes in endometrial TET expression, and recent studies show deregulation in endometrial pathologies. Measuring levels of 5hmc is challenging as bisulfite conversion, often used to study 5mc, does not discriminate between the two DNA modifications. A robust method to detect/study 5hmc in the endometrium is needed. Here we report further analysis of TET regulation by steroid hormones in endometrial stromal cells and evolving work on developing a framework for measuring 5hmc.

Endometrial stromal cells (HESCs) were primed *in vitro* with 24h estrogen (E) followed by combined estrogen-progesterone (EP) for 24h, 48h and 72h. DNA from HESCs and normal proliferative/secretory endometrial tissues underwent both bisulfite sequencing (BS-seq) and oxidative bisulfite sequencing (oxBS-seq). The methylation/hydroxymethylation status of the CpG island 105 of estrogen receptor alpha *(ERα)* was assessed using Ion Torrent Next-Generation Sequencing or Sanger Sequencing. Real-time PCR was used to assess gene expression.

In HESCs, *TET1* transcription increased and *TET3* decreased following 48h and 72h combined treatment, respectively. No significant changes were observed in *TET2* expression. 24h and 48h EP treatment upregulated *ERα* expression. Consistent methylation levels were observed at CpG105 in both HESCs and tissue samples. Neither sequencing method used to date has detected any hydroxymethylation. BS-seq and oxBS-seq remain inefficient, low DNA concentrations prevent successful sequencing in some cases. Further optimisation using restriction enzymes is in progress. Data implies that CpG site-specific differential methylation/hydroxymethylation might regulate ERa expression.

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Post-natal imprinting of *TH-INS* in the tammar wallaby

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Genomic imprinting is an epigenetic phenomenon resulting in parent-of-origin-specific gene expression. Many imprinted genes play an important role in mammalian reproduction by regulating growth and development. Imprinting has traditionally been studied in the placenta and fetus, but imprints can also alter post-natal nutrient transfer through effects in the mammary gland and brain [1]. The insulin gene, *INS*, is an important growth factor, and in the tammar wallaby, shows

tissue-specific paternal expression both pre-natally, in the yolk sac placenta (like eutherians), and post-natally, in the pouch young (PY) liver and adult mammary gland [1, 2, 3]. Previously, a chimeric transcript was identified containing exons from *INS* and the nearby tyrosine hydroxylase (*TH*) gene [3]. The start site of the *TH-INS* transcript showed an approximately equal presence of methylated and unmethylated DNA [3], but it remained to be confirmed whether this was a differentially methylated region (DMR).

To determine the parental origins of methylation at this site, genomic DNA from tammar PY and matched mothers was genotyped. Of 24 PY, two had single nucleotide polymorphisms for which the maternal allele could be determined. Sanger sequencing of cloned bisulfite PCR products was then used to analyse allele-specific methylation in PY liver samples. A total of twenty clones were sequenced which showed an approximately even representation of maternal and paternal alleles. The majority of sequencing reads showed the five CpG sites at this locus to be methylated on both the maternal and paternal copies.

This result suggests that the *TH-INS* start site is not a DMR. Perhaps parent-of-origin-specific gene expression from this region is regulated by a distal imprint control region [4], or some other aspect of DNA organisation. Addressing the function of post-natal imprinting will be an important step in the field of reproductive biology.

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Characterising immune function in sheep that are permanently infected with the Pestivirus border disease virus that causes Hairy Shaker disease

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Hairy shaker disease (HSD) is caused by the Pestivirus border disease virus (BDV). Pestiviruses (which also include the bovine viral diarrhoea virus; BVDV) are unusual in that when they infect a pregnant female during a specified time during pregnancy, they also infect the fetus, which then becomes permanently infected with the virus. These persistently infected (PI) progeny become a constantly shedding reservoir of the virus that infects other naive animals. Currently, it is not understood how the virus evades the host immune defences. To better understand the changes to the immune system caused by this persistent infection we have looked at expression of immune related genes in PI animals and examined differences in their response to vaccinations and parasite infections. Serum and thymus tissue were collected from 3-month-old PI animals (n=8) and non-infected animals (n=10). RNA was isolated from thymus tissue and gene expression analysis was performed using Nanostring (n=6 each group). Antigen specific IgG was measured in the serum by ELISA. Decreased expression of the gene DQB was observed ($p<0.05$) in PI animals compared to control animals. The DQB genes are part of the MHC class II genes that are involved in the presentation of peptides to T cells. Lower antibodies to toxoplasma antigens were observed ($p<0.05$) in PI animals. The data is consistent with differences in the immune system of PI animals compared to controls and that the immune system in PI animals responds differently to some antigens. Understanding how the virus changes the immune system to establish a PI animal may allow development of new treatments for these viruses.

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Human Seminal Fluid Extracellular Vesicles Induce Pro-Inflammatory Cytokine Responses in Female Cervical Cells *In Vitro*

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Seminal fluid interacts with epithelial cells lining the female reproductive tract to induce pro-inflammatory cytokines and initiate immune adaption for pregnancy. Factors in seminal fluid including transforming growth factor (TGF)β family members have been identified as key signalling agents, but do not fully account for the female response. Seminal fluid extracellular vesicles (SFEVs) likely have signalling potential given their suggested roles in fertility and immune regulation; however, their signalling capacity is yet to be characterised in humans. To assess the impact of human SFEVs on female reproductive tract immune responses, we used a well-established human ectocervical epithelial (Ect1) cell *in-vitro* culture model. Seminal fluid was collected from normozoospermic donors and SFEVs were isolated in accordance with the Minimal

Information for Studies of Extracellular Vesicles guidelines. Ect1:SFEV interactions were assessed by immunofluorescence using biotin-labelled SFEVs (n=5/group). Cytokine gene expression profiles were assayed by qPCR (n=8/group). SFEVs were observed to dock with Ect1 cells and deposit biotinylated protein cargo within 5-minutes post-incubation, with SFEV cargo subsequently detected in Ect1 cells throughout an 8-hour incubation. Following incubation with SFEVs, Ect1 cells exhibited changes in gene expression of several pro-inflammatory cytokines previously documented to be induced by seminal fluid, but not regulated by TGFβ. These included *IL1A* (*p*≤0.01, 2.6-fold), *IL6* (*p*≤0.01, 2.8-fold), *CXCL2* (p≤0.01, 2.3-fold) and *CCL20* (p≤0.05, 5.6-fold), all of which were significantly induced in Ect1 cells following exposure to SFEVs compared to untreated cells. In contrast, genes whose regulation has not been attributed to seminal fluid, such as *GUSB* and *HPRT*, were not regulated by SFEVs. This study provides evidence that SFEVs communicate with female cervical cells, modifying the female reproductive tract immune environment. Our current studies are exploiting transcriptomics to delineate the full breadth of gene expression changes and identify SFEV mediators that may influence the female immune response at conception.

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Comparative analysis of the origins of activin A and B in the mouse epididymis

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Activin A, encoded by *Inhba*, and activin B, encoded by *Inhbb*, regulate epididymal development and inflammatory responses. Their expression is highest in the caput epididymis and lowest in the cauda. Activin A and B have been localised by immunohistochemistry primarily to the epithelial principal cells and interstitial macrophages. Efferent duct ligation had no significant effect on *Inhba* expression, but *Inhbb* was reduced by more than 50%, indicating that activin B, but not activin A, is regulated by lumicrine factors from the testis. In order to clarify these differential responses, the precise cellular sources of activins were investigated in 25 and 56 day old C57/Bl6 mice using *in-situ* hybridisation (ISH), and RT-qPCR analysis of the proximal (segments 1-3), and distal caput (segments 4-5) epididymal fragments. Both activins were more abundantly expressed in the proximal caput than the distal caput. Proximal *Inhba* levels were about twice the levels in the distal caput, while *Inhbb* was almost undetectable in the distal caput. Unexpectedly, *Inhba* was localised by ISH to peritubular and interstitial immune cells, with minimal expression by principal cells. Highest expression was in the efferent ducts and segment 2. *Inhbb* expression was localized to epithelial, especially principal, cells, and was mostly confined to segment 1 and the efferent ducts. The expression pattern was the same at both ages. These data indicate that activin A is produced mostly by peri-epithelial and interstitial cells, while activin B is produced predominantly in the epithelium. Accordingly, lumicrine factors regulate *Inhbb* in segment 1, but have marginal effects on *Inhba*, which is expressed more widely in the caput. These largely segregated production sites suggest slightly different roles in epididymal function. Activin A origins in the interstitium may signify a more important role in immunoregulation, while activin B may be more involved in epithelial function and sperm maturation.

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Assessment of the impact of PFAS chemicals on mouse spermatozoa

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PFAS chemicals are ubiquitous in the global environment due to their widespread use in consumer and industrial products and thus form an emerging risk factor for male reproductive health. In recognition that male reproductive health and overall health are inextricably linked, we explored the effects of PFAS exposure on basic sperm parameters using a mouse model featuring *in vitro* sperm exposure protocols and a 3-month *in vivo* exposure regimen. To assess the direct impact of PFAS, cauda epididymal spermatozoa were exposed to a cocktail of nine PFAS chemicals formulated to mimic that of environmental samples (low-dose) as well as an additional cocktail featuring a ten-fold elevation in the concentration of each PFAS (high-dose). Among the suite of functional endpoints assessed, we recorded a significant reduction in progressive motility and straightness immediately after resuspension of sperm in the high-dose PFAS cocktail ($p<0.05$, n=3); a response that was accompanied by a subsequent reduction in straight line velocity at 1 h ($p<0.05$). Spermatozoa exposed to a low dose of PFAS for 1 h demonstrated a reduction in the straight-line distance they travelled ($p<0.01$). In assessing the impact of *in vivo* PFAS exposure, we recorded a reduction in the rate of daily sperm production (p=0.0192, n=8), as well as pronounced changes in the miRNA profile of mature spermatozoa. Specifically, among 2057 identified miRNAs, 137 (7.6%) were significantly increased in the sperm of PFAS treated mice, whereas 56 (2.7%) were suppressed (+/- 1.5-fold, $p<0.05$ in comparison to controls). The differentially regulated miRNAs included several previously linked with organismal injury and abnormalities (e.g. miR-130b-5p, miR-150-3p and miR-190a-5p), reproductive system disease (miR-130b-5p and miR-190a-5p), cell death and survival (miR-486-5p) and cellular development (miR-130b-5p, miR-486-5p and

miR-574-3p). This study presents new evidence that PFAS chemicals impact sperm quality and begins to elucidate the mechanisms generating that impact.

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TYRO3 controls Sertoli cell proliferation and germ cell survival in the developing testis

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Aims: SOX9 is a key transcription factor that regulates many cellular processes such as cell proliferation and cell migration during testicular Sertoli cell differentiation and maintenance. We speculated that SOX9 divides its labour among many target genes. In this study the aim is to investigate the role of a candidate SOX9 target gene we identified called, *Tyro3*, in testis development.

Methods: Expression and localisation of SOX9 and TYRO3 in a human embryonic carcinoma Sertoli-like cell line, NT2/D1 cells was determined by RT-PCR and immunofluorescence. Cell adhesion and proliferation were performed on an xCELLigence. Mouse XY mouse gonads were dissected from embryos at E11.5 and cultured *ex vivo* for three days treated with either vehicle (DMSO) or with TYRO3 inhibitor, BMS777607, to determine the role of TYRO3 in mouse gonad development.

Results: We established that SOX9 is required for cell adhesion and proliferation. To determine whether either or both of these processes are mediated via TYRO3, we incubated NT2 cells in the presence of the TYRO3 inhibitor. Increasing concentrations of the inhibitor led to a dose-dependent reduction in cell proliferation but not cell adhesion. TYRO3 is expressed specifically in the Sertoli cells in mouse gonads. In cultured. Incubation with TYRO3 inhibitor, mouse embryonic gonads did not change testis morphology compared to vehicle control. However, the germ cell marker MVH is lost in TYRO3 inhibitor-treated gonad comparing to the DMSO-treated gonad. Cell apoptosis shows no difference between control and TYRO3 inhibitor gonads.

Conclusion: Our study suggests that a key role of SOX9 is through TYRO3 to mediate germ cell survival in the developing testis. Future rescue experiments are aimed at determining whether the role of TYRO3 is the exclusive mediator of SOX9 mediated Sertoli cell proliferation and germ cell survival.

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Elucidation of the protein composition of mouse seminal vesicle fluid

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The seminal vesicles are an integral male reproductive tract accessory gland whose secretions constitute a major proportion of seminal plasma. Fundamentally, these secretions are responsible for supporting gamete function and promoting reproductive success. Analysis of seminal vesicle fluid composition by proteomics has proven challenging, largely due to the combined features of a protein signature that is dominated by a small subset of highly abundant proteins and the difficulty of solubilising this viscous fluid. As such, publicly available proteomic datasets have only reported a total of 85 mouse seminal vesicle fluid proteins, although compelling evidence suggests greater fluid complexity. To address this limitation, we have established a new proteomics-based method involving the sequential solubilisation of mouse seminal vesicle fluid in guanidine hydrochloride, acetone precipitation and subsequent analysis using label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS). This approach facilitated the identification of 126 proteins, a significant improvement (1.48× increase) compared to the previously curated mouse seminal vesicle fluid proteome. Consistent with established roles for these secretions, reproductive and immune functions associated with the regulation of sperm survival and function, as well as modulation of the female immune environment were significantly enriched within our dataset. Notably, 83 of the 126 proteins identified in our dataset were previously undetected in this fluid. These proteins include voltage dependent anion channel 3 (VDAC3), members of the serine protease inhibitor, Kazal-type (SPINK8 and SPINK11) and prostate and testis-expressed (PATE8 and PATE9) families, which may act as novel seminal vesicle fluid mediators that influence sperm function and fertilisation capacity. These new insights into the composition of seminal vesicle fluid are relevant to understanding how events during the peri-conception period affect reproductive outcomes. Overall, the knowledge gained through our study and future applications of this methodology may assist in improving reproductive health and developing novel infertility treatments.

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Utilisation of optical tweezers to probe the microenvironment of the developing oocyte

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Mechanobiological properties can be indicative of cellular health and function [1]. For the oocyte, mechanobiological information provided via the cumulus matrix may impact developmental potential. Here, we propose a means to accurately measure these properties *in situ* using optical tweezers – a technique that uses tightly focused light to trap micron-sized particles in 3D [2]. In trapping microscopic dielectric spheres, we can generate a local probe to measure viscoelasticity of the cumulus-oocyte complex extracellular matrix (ECM). Here, we aim to corroborate viscoelastic profiles of cumulus ECM with subsequent pre- and post-natal outcomes in mice.

To examine whether the viscoelasticity of cumulus ECM correlates with oocyte developmental potential, we established a model of decreasing oocyte quality using three methods of oocyte maturation; (1) *in vivo*, (2) *in vitro,* and (3) compromised *in vitro* . The ECM was isolated following oocyte maturation using hyaluronidase and then mixed with 1 µm silica beads. Viscoelasticity was quantified by trapping microbeads and using equipartition and power spectrum density analysis. Viscoelastic measurements of the ECM will be correlated with oocyte developmental potential (cumulus expansion, fertilisation rate, development to the blastocyst-stage, foetal viability and placenta development).

Cumulus expansion was significantly lower in the compromised *in vitro* group when compared to standard *in vitro* maturation ($P < 0.05$). Fertilisation rate did not differ between the three maturation methods ($P > 0.05$), however, fewer embryos developed to the blastocyst stage in the standard *in vitro* and compromised *in vitro* maturation groups when compared to the *in vivo* matured group. Viscoelasticity measurements of the ECM will be correlated with developmental outcomes.

Using this model, we will explore the application of optical tweezers to further understand how the microenvironment of the oocyte during maturation impacts developmental potential.

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Relationship between cumulus cell-bound BMP15 and GDF9 in fertility patients

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Bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are important oocyte-specific paracrine factors which are captured by cumulus cells (CCs) and regulate their function, oocyte quality and ovulation rate. This study aimed to investigate if BMP15/GDF9 bound to CCs can be characterized and quantified, and are associated with IVF outcomes in infertile women. Western blots and novel BMP15 and GDF9 ELISAs were validated and applied to discarded CCs. In study 1, pooled CCs were collected from individual patients (n=200) aged 29-47 years old undergoing superovulation and ICSI. BMP15 and GDF9 levels expressed per CC DNA were retrospectively correlated with clinical data. Western blots showed a number of forms of BMP15/GDF9 on CCs, including high molecular weight precursor forms. Total BMP15 and GDF9 were more closely correlated with total CC DNA than oocyte number, and were highly correlated with each other ($r=0.9$, $p<0.001$). BMP15/CC DNA and GDF9/CC DNA were significantly ($p<0.05$) positively correlated with the number of oocytes/patient. BMP15/CC DNA was negatively associated with maternal age. The BMP15:GDF9 ratio was unrelated to oocyte number or age. In study 2, CCs were collected from 181 individual oocytes from 26 patients and BMP15 and GDF9 levels were correlated with embryo development and pregnancy outcomes following single embryo transfer. BMP15/CC DNA and GDF9/CC DNA were not related to oocyte and embryo outcomes (%GV, %MII, %2PN, %day 3 embryos, %day 5 blastocysts or pregnancy success). However, GDF9/CCDNA and BMP15/CCDNA were highly correlated ($r=0.91$, $p<0.0001$) with a significant difference in the BMP15:GDF9 ratio between oocytes generating low- and high-grade blastocysts. This study reports the application of BMP15 and GDF9 ELISAs to human CCs, thus opening the opportunity for their measurement in infertility patients, as a potential novel avenue for a non-invasive diagnostic test of oocyte function and infertility pathologies.

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An essential novel role for PRC2 during mouse ovarian folliculogenesis

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The development of unique cell types in multicellular organisms is achieved through careful coordination of gene expression, involving signalling, transcription factors and epigenetic modifications. Epigenetic modifications involve heritable chemical alterations to DNA or histone proteins (together termed chromatin) that regulate how chromatin is packaged within the nucleus, with substantial influence on cell identity and function. 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. Our characterisation of the core PRC2 subunits, EED, EZH2 and SUZ12, in the mouse ovary revealed that PRC2 is enriched in granulosa and theca cells of developing ovarian follicles. To investigate the function of PRC2 during folliculogenesis, we generated a mouse model with conditional deletion of *Eed* in somatic cells of the ovary. Deletion of *Eed* resulted in the arrest of follicles at the secondary stage, evidenced by abnormal expression of the cell cycle inhibitor $p27^{kip1}$ and decreased expression of the proliferation marker PCNA in granulosa cells. Furthermore, *Eed*-null ovaries contained fewer steroidogenic cells marked by the steroidogenic enzymes HSD3B and CYP11A1, suggesting that steroid production is impacted in these females. Limited analysis of adult ovaries revealed severely compromised morphology, loss of growing follicles and sub-fertility following *Eed* deletion. These findings provide the first evidence that EED is a novel 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.

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Lysosomal inhibition of mouse oocytes as a model of age-related oocyte quality decline

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Oocyte quality is imperative in determining embryo health and subsequent pregnancy success. Advancing maternal age is a key factor that contributes to the decline of oocyte quality. In this study we aimed to model this decline using an *in vitro* acute treatment regimen targeting the autophagy pathway; a protein degradation pathway necessary for maintaining cell health. We have previously used advanced quantitative image analysis techniques to demonstrate that autophagy is impaired in aged mouse oocytes. These aged mouse oocytes harbour reduced numbers of key pathway components including autophagosomes (LC3B, $P = 0.01$) and lysosomes (LAMP1, $P = 0.01$), alongside an accumulation of amphisomes (LC3B and EEA1, $P < 0.01$) compared to young oocytes. To model these changes, we exposed pre-ovulatory oocytes collected from 4-6 week old C57BL/6 x CBA F1 mice with a lysosomal inhibitor, chloroquine (200 μM), for 6 hours. Oocytes were either assessed immediately after treatment at the GV stage for autophagy pathway markers (LC3B, EEA1, and LAMP1), or incubated overnight and matured *in vitro* to assess functional impacts on meiosis and equivalent pathway markers at subsequent meiotic stages. Chloroquine treatment of young oocytes led to a significant accumulation of amphisomes (staining for LC3B and EEA1) compared with controls ($P < 0.05$), thus mimicking the response seen in aged oocytes. Preliminary assessment of lysosomal inhibition on GV oocytes prior to *in vitro* maturation revealed a marked reduction in polar body extrusion rates, a phenotype indicative of impaired meiosis. Together, these data implicate lysosomal dysfunction as a potential key player in oocyte quality control. These findings highlight the enhancement of autophagy as a prospective target pathway to improve oocyte quality, particularly in an *in vitro* setting.

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Investigation of ticagrelor as a potential therapeutic to delay preterm birth using a pipeline of *in vitro, ex vivo* and *in vivo* models of preterm birth

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Preterm birth is still the leading cause of death in infants; hence there is an urgent need to find new drugs to delay preterm delivery. One approach to therapeutic development is repurposing existing drugs. Ticagrelor is an antiplatelet agent used for cardiovascular events but we have previously found that it can relax vascular smooth muscle. We hypothesised that ticagrelor may have similar relaxant effects on the myometrium (outer uterine muscular layer). Here, we assessed the effect of ticagrelor on myometrial contractility and key inflammatory markers (central to preterm labour pathophysiology) in our pipeline of human and mouse models of preterm birth.

Human myometrial tissue (non-labouring; collected at caesarean-section) was used in tissue bath experiments (DMT myograph) to measure the effect of ticagrelor on spontaneous contractions. To assess ticagrelor's prolonged effects on contraction, human myometrial cells were embedded in collagen gel and treated with pro-inflammatory mediators, tumour necrosis factor (TNF) and lipopolysaccharide (LPS), +/- ticagrelor for 48 hours. TNF and LPS were used to evoke an inflammatory response in cultured myometrial cells co-treated with ticagrelor; altered mRNA expression of pro-inflammatory cytokines was measured via qPCR. Preterm birth was induced in mice using LPS on gestational day 16.5 to determine whether ticagrelor delayed delivery.

Ticagrelor did not reduce *ex vivo* myometrial contractility (n=3) or TNF/LPS-induced contraction in myometrial collagen contraction assays (n=3) when compared with vehicle control treatment. Ticagrelor did not reduce mRNA expression of pro-inflammatory cytokines interleukin (IL)-1B, IL-6 and CXCL8 in cultured myometrial cells (n=3). Preliminary findings showed that ticagrelor could not delay LPS-induced preterm birth in mice (20mg/kg, n=3; 40mg/kg, n=2).

Our pipeline of drug assessment rigorously evaluated ticagrelor. Collectively these data demonstrate ticagrelor does not reduce human myometrial contractions or inflammation, or prevent preterm delivery in mice. This suggests ticagrelor is not a promising preterm birth therapeutic candidate.

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Observation of Merino ewe body condition score on lamb live weight and survival to weaning

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Maintaining pregnant ewes with a body condition score (BCS) of 3.0-3.3 during late gestation is critical as it can strongly influence peri- and post-partum lamb viability, growth and survival (Hinch and Brien 2014; Kenyon *et al.* 2014). This study determined whether the BCS of 96 twin-bearing Merino ewes at approximately d123 of gestation would influence live weight (LW) and survival of their lambs at birth, marking and weaning. The effects of ewe BCS on lamb survival was determined using a chi-square test and an ANOVA was conducted to determine the impact of BCS on lamb LW. Ewe BCS group had no effect on lamb LW at birth (P = 0.322). At marking, lambs born to ewes in the BCS ≤2.5 group tended to be lighter than lambs born to BCS 3.0 and 3.5 ewes (P = 0.071), with these lambs being lighter at weaning than lambs born to BCS 3.0 and 3.5 group ewes (P = 0.044). Twin lamb cumulative survival rates were unaffected by ewe BCS group (P > 0.05). This study demonstrated that twin-bearing ewes with a BCS of ≤2.5 reared lighter lambs at weaning than BCS 3.0 and 3.5 group ewes. Ewe BCS and fat levels are positively related to milk production (Gibb and Treacher 1982). It is, therefore, suggested that ewes with a BCS of ≤2.5 had less body fat to mobilise which may have negatively impacted their lactation performance and consequently the growth of their offspring. As such, it is not surprising that they weaned lighter lambs; however, it is interesting that this did not impact lamb survival from birth to weaning. In conclusion, these findings indicate that farmers should ensure that twin-bearing ewes maintain a BCS of above 2.5 during late gestation in order to maximise lamb growth and safeguard survival to weaning.

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Retinitis pigmentosa GTPase regulator Interacting protein 1 (RPGRIP1L) is dysregulated in placenta in fetal growth restriction

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Fetal growth restriction occurs in 10% of pregnancies and is the most common risk factor for stillbirth. Retinitis Pigmentosa GTPase Regulator Interacting protein 1 like (RPGRIP1L) is essential for placental vascular development, with a mouse knockout model displaying impaired placental development (1). We explore RPGRIP1L in human placenta from FGR pregnancies and perform functional studies examining its role in placenta subjected to FGR stress conditions; hypoxia and low glucose.

RPGRIP1L was significantly increased ($p<0.005$, n=11, n=17 control) in placenta from term FGR pregnancies. Hypoxia (1% Oxygen) increased RPGRIP1L in term trophoblasts ($p<0.0025$) and decreased RPGRIP1L in isolated first trimester cytotrophoblasts ($p=0.0079$). No changes were observed with low glucose. Immunohistochemistry demonstrated expression of RPGRIP1L within extravillous trophoblasts, synctiotrophoblast and in the endothelium.

RPGRIP1L is known to be involved in the Sonic Hedgehog pathway which governs placental differentiation, proliferation, migration and invasion. We explored the expression of Smoothened (SMO), Suppressor of fused homolog (SUFU) and Sonic hedgehog (SHH) upstream of RPGRIP1L, and Glioma-associated oncogene (GLI); GLI1/2/3 genes downstream of RPGRIP1L. Hypoxia increased SMO in the first trimester ($p=0.0079$) and decreased SMO at term ($p=0.008$). SMO induces GLI target gene expression. Thus, as expected, we observed decreases and increases in GLI target gene expression in first trimester (GLI1; $p=0.0079$) and term cytotrophoblasts respectively.

RPGRP1L is upregulated in FGR placentas. Our in-vitro hypoxia experiments indicate RPGRIP1L is differentially regulated at different trophoblast gestations and is associated with changes within the sonic hedgehog pathway. This suggests that RPGRIP1L and the Sonic Hedgehog pathway may be involved in placenta development and insufficiency in first and term trophoblasts.

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IL-1 receptor antagonist rytvela protects against GBS-induced preterm delivery in mice.

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Premature birth is a common and critical health issue in fetal-maternal medicine with long-term consequences especially for early preterm neonates. The pathophysiology of preterm labour is poorly understood and the causal factors uncertain, but inflammatory mechanisms are clearly implicated. Toll-like receptors (TLRs) are critical upstream gate-keepers controlling the inflammatory activation that precedes preterm delivery and pro-inflammatory cytokine interleukin-1 beta (IL-1b) has been identified as a major upstream product following the activation of the TLR pathway. Group B Streptococcus (GBS) or *Streptococcus agalactiae* is a gram-positive bacteria and a TLR2/TLR8 ligand, is considered to be a leading cause in neonatal sepsis following preterm birth. Previously we have shown that inhibition of IL-1 signaling using rytvela, a non-competitive allosteric peptide inhibitor of IL-1 receptor (IL-1R) signaling, can reduce GBS-induced preterm birth in mice by 50% and improves neonatal survival rates. This project seeks to investigate whether inhibition of IL-1 signaling using the IL-1R antagonist rytvela may prevent the parturition cascade caused by GBS-induced inflammation. Pregnant C57Bl/6 mice were administered intrauterine heat-killed GBS ($5x10^9$ IU/100 µl) or PBS, with or without co-administration of rytvela (ip), on gestational day (GD) 16.5 and were killed 4 hours later for RT-PCR analysis of inflammatory cytokines in gestational tissues adjacent to the GBS injection site (n=2 samples from 6-8 dams per group). Administration of rytvela was found to suppress GBS-induced expression of *Il1b*, *Ifng*, *Ptgs2, Il10* and *Il18* expression in the uterus. These results demonstrate that intervention with rytvela to suppress the IL-1-induced inflammatory cascade can mitigate GBS-induced preterm delivery by preventing premature activation of uterine activation proteins and subsequent onset of labour in mice. The data support continued investigation of the IL-1 pathway as a potential target for new prevention or treatment options in women at risk of infection-associated preterm delivery.

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Plasticity in gastric satiety signals during pregnancy and the impact of diet-induced obesity

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Maternal obesity increases the risk of poor pregnancy outcomes and adulthood disease in the offspring. Gastric vagal afferents (GVAs) sense food-related mechanical stimuli and signal to the central nervous system to integrate control of meal termination. Both pregnancy and diet-induced obesity are independently associated with dampened mechanosensitivity of GVAs and increased food intake. Whether an obesogenic diet impacts pregnancy-related adaptations in GVA satiety is unknown. Therefore, we aimed to determine how food intake and GVA function change in response to pregnancy in a mouse model of diet-induced obesity.

Three-week-old female Glu Venus-expressing mice were fed a standard laboratory diet (SLD) or high-fat, high-sugar diet (HFHSD) for 12 weeks, then half of each group were mated to generate late pregnant (d17.5; SLD N=7, HFHSD N=9) or non-pregnant (SLD N=8, HFHSD N= 10) groups. Individual body weight and food intake was automatically monitored for a week prior to humane killing and tissue collection at 0700h for the *in vitro* single fibre GVA recording preparation.

Diet did not affect weight of SLD and HFHSD mice during pregnancy (P=0.541). On the final day of study (d16.5-17.5), food intake and meal size in the light phase were higher in pregnant than non-pregnant mice (each P<0.001). Food intake (P=0.026), meal size (P<0.001) and meal duration (P=0.029) were each lower in HFHS than SLD mice during the same period. In SLD mice, the response of tension sensitive GVAs to stretch tended to be lower in pregnancy (P=0.051), but this response did not differ between HFHSD pregnant and non-pregnant mice.

In conclusion, reduced mechanosensitivity of GVAs during pregnancy would partly permit increased food intake during pregnancy. Our data so far suggests that a HFHSD appears to prevent adaptation of the GVA signalling during pregnancy.

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Investigating the action of the antiplatelet agent prasugrel: offering a novel potential treatment for preeclampsia

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Preeclampsia is a serious pregnancy complication, but treatment strategies are extremely limited. Systemic vasoconstriction is a key feature of preeclampsia. Previously, we identified that the new generation antiplatelet agent prasugrel could prevent vasoconstriction of human maternal systemic arteries. In this study, we aimed to examine the mechanisms of prasugrel action, in particular whether prasugrel works through the nitric oxide pathway (important in regulation of vascular tone).

Pregnant CBA X C57BL/6 (F1) mice received 50mg/kg/day L-NAME (nitric oxide synthase inhibitor; modelling preeclampsia), and 10mg/kg prasugrel or control (vehicle; DMSO) injections from D7.5-D17.5 gestation (n=10/group). Maternal blood pressure was measured at D14.5 and D17.5. Maternal blood was collected (D17.5) to assess circulating sFLT-1, CRP, and ET-1. Fetal and placental size and morphology were assessed (D17.5). Human omental fat biopsies were collected from pregnant individuals at term Caesarean section (n=3). Omental arteries were dissected, pre-constricted with ET-1 and then treated with 0.2-100μM prasugrel or vehicle to assess vasodilation (wire myography). Endothelium-independent effects were examined by pre-incubating arteries with 300uM L-NAME or removing the endothelium.

Prasugrel significantly reduced maternal blood pressure at D14.5 and D17.5, and reduced circulating sFLT-1 and CRP, but not ET-1. Fetuses of prasugrel-treated dams had increased crown-to-rump length, with no change in fetal weight or litter size. Prasugrel administration did not alter placental weight, but significantly expanded the placental blood space. Preliminary vascular assessment demonstrated prasugrel induced vasodilation of human omental arteries pre-constricted with ET-1. Pre-incubation with L-NAME and denuding the endothelium had no effect on prasugrel-induced vasodilation.

This study demonstrates that prasugrel mitigates multiple aspects of the pathogenesis that underpins preeclampsia, irrespective of nitric oxide levels or endothelial function. Together, these data further support the novel use of prasugrel in treatment of preeclampsia, an urgent unmet need in obstetric medicine.

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The role of mitochondria in sensing and responding to oxygen levels in the human placenta

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Objectives

The ability of a tissue to sense and response to oxygen is linked to the metabolic and mitochondrial signalling within. This phenomenon has been deemed mitochondrial oxygen sensing. Oxygen sensing has been well characterised in arterial chemoreceptors and the carotid body to be dependent on mitochondrial subunit composition. However, the role of mitochondria in the response to varying oxygen level in the placenta remains unclear. A mechanism that is of particular important to understanding gestational complications such as fetal growth restriction (FGR), where poor perfusion and hypoxia are common. This study aimed to determine if mitochondrial oxygen sensing mechanisms and subunit composition plays a role in the development of FGR through the inability of the placenta to respond to varying oxygen levels in-utero.

Methods

Fetal growth restricted placentae (n=8) and gestational matched healthy term control (n=14) pregnancies were utilised. Metabolic and hypoxic signalling was assessed at a transcriptional and translational level via PCR and Immunoblotting.

Functional mitochondrial characteristics were measured by Seahorse XF.

Results

Hypoxic (ARNT, HIF1a,) and mitochondrial subunit genes (NDUFS2, COX4I1, COX4I2) were higher (p=<0.05) in FGR compared to term control. A finding not conserved at the protein level for mitochondrial subunit NDUSF2 (p=<0.0001). Likewise, metabolic regulator AMPK decreased (p=<0.05) in FGR tissue along with a functional decline in oxidative phosphorylation (p=<0.03) that was not explained by a change in mitochondrial content.

Conclusion

This data demonstrates the potential for mitochondrial subunits to alter the hypoxic response in the placenta in a manner akin to oxygen sensing in other tissues. Notably, FGR placenta saw a functional decline in mitochondrial oxidative phosphorylation when compared to healthy term placentae, a key indicator of mitochondrial dysfunction. Given the importance of mitochondria to oxygen sensing and response to hypoxia these finding may provide a novel mechanism which underpins the pathogenesis of FGR.

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The Hidden Ingredient: Investigating the effects of ubiquitous molybdenum in placental physiology.

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Background: Molybdenum is an essential micronutrient and critical cofactor within proteins, including molybdoenzymes, integral proteins in redox reactions and the oxidative stress response. However, molybdenum remains an understudied micronutrient in placental physiology. Previous work from our lab found dysregulation in the molybdenum metabolism in placentas from complicated pregnancies. Therefore, this study aimed to investigate the effects of molybdenum supplementation on cell growth and mitochondrial function in the HTR-8/SVneo trophoblast cell line. We hypothesised that molybdenum supplementation can modulate essential physiological pathways, including the antioxidant response and cell proliferation.

Methods: The HTR-8/SVneo trophoblast cell line was cultured with ammonium and sodium molybdate salts at a concentration range designed to reflect physiological (10nM-10uM), and supraphysiological (1mM) molybdenum levels. Cell growth curves were generated using an image-based, phase-contrast method (Incucyte software), and metabolism was assessed via mitochondrial respiration using the Seahorse XF Analyzer. Real-time PCR analysis was used to determine the expression of molybdoenzymes and associated signalling pathways.

Results: Cell growth ($p<0.05$) and mitochondrial maximum respiratory capacity ($p<0.01$) were significantly reduced following ammonium molybdate supraphysiological supplementation. Ammonium molybdate and sodium molybdate supplementation did not inhibit or aid cell growth. Similarly, mitochondrial respiration was not altered by ammonium or sodium molybdate supplementation at physiological levels. Superoxide Dismutase 2 expression increased significantly ($p<0.05$) with 5µM treatment ($p<0.05$) of sodium molybdate.

Conclusion: In a model not exposed to stress, and supplementation at physiological levels, sodium molybdate and ammonium molybdate did not have significant impact on growth and mitochondrial function. However, our PCR results indicate potential modulation of the antioxidant cell response following sodium molybdate treatment at a high physiological concentration. This research is integral for evaluating the role of molybdenum in placental physiology in a healthy model; we postulate that molybdenum supplementation may exert a protective effect following oxidative insult as seen in pathological pregnancies.

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Plasma metabolic profiling of Early-onset Preeclampsia and Fetal Growth Restriction

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This study aims to investigate small molecule metabolic profiles of pregnant women diagnosed with early-onset preeclampsia and fetal growth restriction and determine if there are underlying pathogenesis-related metabolic pathways.

120 plasma samples were collected from non-pregnant women (n=39), and healthy (n=25), preeclamptic (n=50), and fetal growth restricted (n=6) pregnancies. Samples from pregnant women were collected at 23–33 weeks' gestation. Metabolomics profiles were determined using Liquid Chromatography Mass Spectrometry (LC-MS). Statistical and pathway analyses were conducted using MetaboAnalyst 5.0. Differential metabolites were selected according to t-test and Variable Importance Projection (VIP) score. VIP score is the rank of metabolites based on their importance in discriminating two groups via Orthogonal Projections to Latent Structures Discriminant Analysis (oPLSDA) model.

The comparison groups of non-pregnant women versus healthy pregnancies and early-onset preeclampsia versus controls respectively had 60 and 68 significantly different metabolites (q value of 0.05 and VIP score ≥ 1). No qualified differential metabolites were found in fetal growth restriction versus controls. The primary changes belonged to amino acids and fatty acids, including cystine, cysteine, methionine, leucine, glutamine, 2-hydroxybutyric acid, oleic acid, and linoleic acid. Principle Component Analysis plots revealed a clear separation among non-pregnant, healthy pregnancies and early-onset preeclampsia groups. Metabolic pathway analysis showed 12 metabolic pathways overrepresented in early-onset

preeclampsia, such as aminoacyl-tRNA biosynthesis, arginine biosynthesis, valine, leucine and isoleucine biosynthesis, alanine and aspartate and glutamate metabolism, D-glutamine and D-glutamate metabolism. Multivariate Receiver Operating Characteristic curve analysis of early-onset preeclampsia achieved an Area Under the Curve value of 0.95 (95% CI 0.87–0.99) with a five-metabolite-combined model using random forest algorithm.

These findings reveal critical metabolites and metabolic pathways in pregnancy, requiring further studies to explore their roles in early-onset preeclampsia.

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Associations between physical activity volume and sedentary behaviour on placental morphology throughout pregnancy

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Introduction

Abnormal placental morphology and blood flow is associated with pregnancy-related complications and poor perinatal outcomes. The acute haemodynamic changes which occur during an exercise bout act as a stimulus for beneficial vascular adaptations. However, the effect of regular physical activity (PA) and conversely the impact of sedentary behaviour (SB) on placental morphology and vascular function is less clear. This study investigated maternal PA and SB and examined the association of these activities with placental growth and function during gestation and at delivery.

Methods

This study included women recruited from the Queensland Family Cohort study (n=358). Women reported PA behaviours at 24-, 28- and 36-weeks of gestation using a modified Active Australia Survey and were categorised into groups of no activity, low, moderate, and high volume. Participants reported average daily sitting time, whereby SB was considered as ≥8 hours of sitting/day. Placental stiffness and thickness and uterine and umbilical cord arterial resistance were measured through ultrasound at each timepoint, and placental dimensions were measured following delivery. Multivariate regression was used to investigate the associations between PA volume or SB on these placental outcomes.

Results

Engaging in any volume of PA during pregnancy did not affect any placental measures during gestation or at delivery. However, SB at 36-weeks was positively associated with placental stiffness (p=0.025), and negatively associated with thickness (p=0.044) and the umbilical artery pulsatility index (p=0.014). Further, SB was associated with altered morphology at term, demonstrated by a negative correlation with placental weight (p=0.002), depth (p=0.009) and surface area (p=0.043).

Conclusion

PA at any volume during the second half of pregnancy is not associated with significant changes to placental morphology. Although, SB during the antenatal period may be an independent risk to reduced placental growth and development which could result in adverse outcomes for the fetus in complicated pregnancies.

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Hyperglycaemia Modulates Insulin-regulated Aminopeptidase in Human Trophoblasts: Implications for Gestational Diabetes

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Gestational diabetes mellitus (GDM) is associated with increased placental thickness, inflammation and fibrosis. The renin-angiotensin system (RAS) is a key regulator of placental development, acting through receptors including insulin-regulated aminopeptidase (IRAP/AT4R). Notably, IRAP is associated with glucose metabolism as it can promote leptin production and is co-regulated with glucose transporter type-4 (GLUT4). Tissue RAS activity is upregulated in non-pregnant diabetics, but nothing is known about placental RAS activity in GDM. We examined the impact of hyperglycaemia on placental trophoblast expression of IRAP, GLUT4 and leptin, and characterised their placental and circulating levels in GDM pregnancies.

Primary trophoblast cells were isolated from placentae of term, uncomplicated pregnancies (n=5) and cultured in normoglycaemic [5mM glucose] or hyperglycaemic [25mM glucose] conditions**.** Placental tissue and matched maternal blood were collected from term, uncomplicated (n=44) and GDM pregnancies (n=39). Expression of IRAP, GLUT4 and leptin mRNA was measured by qPCR. IRAP and leptin protein levels were quantified using immunoblot and ELISA.

Hyperglycaemia increased trophoblast IRAP and GLUT4 mRNA (p=0.021 and p<0.009, respectively) and decreased leptin mRNA (p<0.001). Placental expression of IRAP, GLUT4 and leptin mRNA was similar in uncomplicated and GDM pregnancies, as were levels of IRAP and leptin. Management of GDM by diet or insulin had no effect on mRNA or protein

expression. Circulating IRAP was similar between groups, however GDM was associated with increased circulating leptin (p=0.014), which was positively correlated with pre-pregnancy BMI (rho=0.727, p<0.001).

This study is the first to characterise placental IRAP, GLUT4 and leptin in GDM and establish that their expression in trophoblasts is sensitive to hyperglycaemia. Our data suggest that trophoblast signalling via IRAP may be upregulated in GDM, promoting glucose uptake via GLUT4 and potentiating placental growth. Further cell-specific research in GDM placentae is warranted to elucidate the role of IRAP in mediating placental abnormalities in GDM.

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The change in body surface temperature in twin-bearing Merino ewes prior to and post-parturition

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Lambing supervision is not common practice in Australian lambing systems, therefore other strategies to reduce dystocia rates are required. The use of temperature loggers to measure body surface temperature (BST) of ewes may assist in predicting the onset and duration of parturition in ewes, due to the decrease in body temperature that occurs prior to parturition (Nabenishi and Yamazaki 2017; Abecia *et al.* 2020). The aim of this study was to validate the use of temperature loggers to detect the fall in BST which precedes and follows parturition in twin-bearing Merino ewes.

On approximately day 146 of gestation, temperature loggers (Micro-T 16-bit; Star Oddi, Iceland) were taped onto the skin surface on the inside of the upper front right leg of 27 twin-bearing, Merino ewes. The loggers recorded temperature every 10 minutes. Temperature data was averaged to hourly intervals (24 hours pre- and post-partum), where time point 0 was considered as the birth of the first-born lamb. Data was analysed through a general linear mixed model with ewe identification number fitted as a random factor.

There was a significant drop in BST from 5 – 0 hours pre-partum (36.37°C ± 0.21) compared to 24 hours pre-partum (36.97°C ± 0.21) $P < 0.05$). There was no difference in BST between the birth of the first and second-born lamb regardless birth interval ($P > 0.05$). BST increased post- partum and was significantly higher ($P < 0.05$) 15 – 24 hours post-partum compared to the time of birth for the second-born lamb (37.22°C ± 0.22 versus 36.74°C ± 0.22).

As BST lowered preceding the birth of the first-born lamb but not between first and second-born lamb, further analysis is required to create prediction models and develop innovative technology which could provide producers with real time information regarding ewes lambing.

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Mucin 15 (MUC15) a cell surface associated protein is increased in preeclampsia

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Mucins are a family of proteins involved in the physical protection of epithelium. A particular subtype, MUC15 is highly expressed in placental trophoblast cells. This study aimed to characterise MUC15 in preeclampsia, a disease associated with placental oxidative stress, and investigate its role in isolated (cyto)trophoblast (placental) stem cells.

MUC15 was measured in placentas of patients with early-onset (delivering <34 weeks' gestation) preeclampsia. MUC15 protein was significantly increased (p=0.0003, n=32 vs n=20 controls) whilst mRNA was unaltered (n=61 vs n=18 controls). Circulating MUC15 protein was significantly increased in the serum of patients with preeclampsia (p=0.0156, n=32 vs n=22 controls).

Given that mucins are localised to epithelium, human trophoblast stem cells (hTSCs) (1) were differentiated into the placental epithelial cell layer, syncytiotrophoblast for 96 hours. Downregulation of cytotrophoblast markers (CDH2, TEAD4) and upregulation of syncytial markers (GATA3, SDC1) confirmed syncytialisation. MUC15 mRNA (p=0.0049) and protein secretion (p=0.0059) significantly increased after 48 hours and 96 hours, respectively as cells syncytialised. To investigate the mechanisms of MUC15 secretion, syncytialised hTSCs were treated with brefeldin A and batimastat. Brefeldin A inhibits protein transport from the endoplasmic reticulum to the golgi complex whilst batimastat inhibits matrix metalloproteinases (MMPs), reducing cleavage of some extracellular matrix proteins. Interestingly, treatment with brefeldin A had no effect while batimastat reduced secretion of MUC15 (p=0.0436) from syncytialised hTSCs.

Finally, to assess if oxidative stress alters MUC15 expression, syncytialised hTSCs were exposed to hypoxia (1% O_2) and inflammatory cytokines (TNFα, IL-6). MUC15 mRNA and protein expression remained unchanged. However, preliminary data suggests that knockdown of MUC15 elevates heme-oxygenase-1, a cytoprotective enzyme.

In conclusion, MUC15 protein is increased in early-onset preeclampsia. It is cleaved by MMPs but not induced by hypoxia or inflammation. Increased MUC15 may be a protective mechanism associated with placental dysfunction. Further research will aid in confirming this.

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Characterising changes in small intestinal glucose absorption across the oestrous cycle in mice

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Food intake and body weight change across the rodent oestrous cycle, each showing a nadir at oestrus (Olofsson 2009). We aimed to assess whether the rate of intestinal glucose absorption also changes throughout the oestrous cycle.

Female C57BL/6 mice (8-9 weeks) were humanely killed at defined oestrus, metoestrus, dioestrus and proestrus cycle stages (N=9-10 mice per stage). Jejunal segments (1 cm length) were mounted in Ussing chambers and the change in short-circuit current (ΔI_{SC}) upon mucosal addition of 50 mM glucose measured as transepithelial glucose transport in the presence of 0, 0.1, 0.3 or 1 mM phlorizin (PZ, an inhibitor of sodium-glucose co-transporter-1, SGLT-1).

Transepithelial glucose transport was lower at oestrus compared to proestrus (P=0.013). PZ reduced glucose transport predominantly in the distal jejunum (dose-region interaction: P<0.005), by > 80% at 0.3 mM and ~90% at 1.0 mM. In the absence of PZ, glucose transport was higher in the distal compared to proximal jejunum (P<0.001). In contrast, glucose transport did not differ between regions in the presence of PZ.

Jejunal glucose transport is primarily SGLT-1-mediated in mice, with greater transport capacity in distal compared to proximal jejunum. Importantly, jejunal glucose uptake was lowest at oestrus, demonstrating similar timing as previously reported nadirs in body weight and food intake. This suggests that these processes are coordinated throughout the oestrous cycle. The relative contributions of food intake and hormonal changes to altered glucose absorption remain to be elucidated.

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Corticotrophin Releasing Hormone (CRH) mRNA is detectible in placental extracellular vesicles (EVs) circulating in maternal plasma

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Introduction:

The human placenta releases EVs, including microvesicles and exosomes, into the maternal blood for feto-maternal communication. The concentration of placenta-secreted exosomes in the maternal plasma increases as gestation progresses. Placental CRH mRNA levels rise with gestation and have been reported to increase in complicated pregnancies. Our study aimed to (i) isolate, purify and characterise placenta-secreted EVs in placental explants and maternal plasma, (ii) determine whether placental EVs contain CRH mRNA and (iii) determine whether CRH mRNA containing placentally-derived EVs were detectible in maternal plasma.

Methods:

Placental EVs (microvesicles and exosomes) were isolated from human term placentas via villus washing (N=6) and from explant cultures (N=6) supernatants by differential centrifugation and then purified on a continuous sucrose gradient (0.25-2.5M). EVs were characterised by western blotting using placenta- and exosome-specific markers and the morphology was studied by Transmission Electron Microscopy (TEM). EVs from maternal plasma (gestational weeks 38-41, N=9) were isolated by ultracentrifugation and Fluorescent Activated Cell Sorting (FACS)using fluorescent-labelled PLAP antibody. CRH mRNA was measured in EVs isolated from the placental washes, explants and maternal plasma using real-time PCR.

Results:

Placental (PLAP) and exosomal markers (CD63, TSG, LAMP-2, Calreticulin) were observed in purified exosomes at a density of 1.16g/ml. TEM images showed microvesicles and exosomes with sizes ranging from 200-500 nm and 100-150 nm, respectively. The PCR data showed the presence of CRH mRNA in placenta-derived EVs from both placental washes

and explants. In blood plasma, size-specific placental EVs (100-500 nm) were sorted by FACS using PLAP-Ab. CRH mRNA was detected in EVs obtained from maternal blood plasma.

Conclusion:

EVs released by the human placenta can be isolated and purified by ultracentrifugation and FACS from ex-vivo explant culture media and maternal blood. These placenta-secreted EVs contain CRH mRNA. However, the physiological role of CRH mRNA in maternal plasma remains unknown.

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MULTIOMICS APPROACH TO DIFFERENTIATE PREGNANT AND NON-PREGNANT MARES: IDENTIFICATION OF EARLY PREGNANCY PLASMA BIOMARKERS

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Diagnosis of early pregnancy in mares is an important component of equine breeding practice, as early embryo loss is relatively common and incurs substantial economic loss. This is compounded by a short breeding season, placing pressure to achieve pregnancies early. Furthermore, a precise signal or mechanism for maternal recognition of pregnancy has not yet been elucidated in horses with current detection only being possible at day 14. We have undertaken multiomic analyses to compare the blood plasma profiles of pregnant (7P) and non-pregnant (7NP) mares at day 7 post-ovulation to identify pregnancy-induced biomarkers. We conducted a proteomics study (n=264) in parallel with lipidomics and metabolomics (n=72). Using a batch mode approach, our established bioinformatical pipelines led us to identify a plasma protein profile of 234 proteins and a lipidome composed of ~700 lipid ions. Amongst these profiles we identified 14 proteins and 24 lipids that were significantly up or down-regulated between 7P and 7NP. Proteomics revealed serpinA6, immunoglobulin lambda light chain variable region, alpha 2 macroglobulin and complement C8 gamma chain to be significantly increased in 7P plasma. Immunoglobulin lambda light chain variable region is important in the immunological recognition of pregnancy and in humans altered Free Light Chains (FLC) or FLC ratios during pregnancy has been identified. Further, lipidomic analysis revealed several ceramides to be significantly increased in 7P plasma, suggesting a role for lipid-mediated signaling in early pregnancy. Moreover, pathway analysis implicated ceramides in many reproductive hormone signaling pathways, including progesterone synthesis. These novel findings support the utility of mass spectrometry driven omics platforms for pregnancy biomarker discovery and indicate that systemic physiological changes occur as early as day 7 following fertilisation in the pregnant mare. Overall, this study represents significant progress toward establishing a panel of biomarkers for the accurate detection of early pregnancy in mare.

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Progression of experimental asthma during ovine pregnancy

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Asthma affects >40,000 Australian pregnancies each year and increases the risk of adverse pregnancy outcomes. Good asthma control normalises risks of most adverse pregnancy outcomes, but ~50% of women with asthma experience a loss of asthma control during pregnancy. It is not clear from human studies whether pregnancy itself worsens asthma.

We explored this question using an experimental sheep model of allergic asthma, induced by sensitisation and repeated airway challenge with house dust mite (HDM); controls received saline. Circulating cytokines were measured in age-matched non-pregnant ewes (8 control, 9 asthmatic), singleton-bearing ewes (5 control, 8 asthmatic) and twin-bearing ewes (6 control, 9 asthmatic) before sensitisation, and before and 48 hours after airway challenge, pre-mating and in late pregnancy (gestational day (GD)132, term = GD150). Pre-challenge airway function was studied in the same animals before mating, in early-mid (GD62) and late pregnancy, and in age-matched non-pregnant ewes.

In late pregnancy, plasma IL-4 (*P=0.026*) and IL-6 (*P=0.010*), but not IL-10 (*P>0.9*), increased post airway challenge, and did not differ between control and asthmatic ewes overall. Plasma IL-6 (*P<0.001*) levels were higher in late pregnant ewes than age-matched non-pregnant ewes. The decrease in dynamic compliance from pre-mating to GD132 was greater in asthmatic than control ewes (*P=0.040*), irrespective of litter size. Transpulmonary pressure, a measure of breathing effort, increased similarly across pregnancy in control and asthmatic ewes (*P=0.123*) and more in twin-bearing than non-pregnant ewes (*P=0.020*). At GD132, transpulmonary pressure was higher in asthmatic than control non-pregnant (*P=0.031*), but not pregnant, ewes.

Asthma had no effect on circulating IL-4, IL-6 or IL-10 concentrations. Lung function in experimental asthma worsened similarly in non-pregnant and pregnant ewes. This suggests that variable changes in asthma phenotype during human pregnancy may reflect behaviours such as reducing medication use rather than effects of pregnancy itself.

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The role of progesterone on maternal behaviour and twin-lamb survival in Merino sheep

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High twin-lamb mortality is a significant issue for Merino sheep, as their poor lamb raising ability leads to starvation-mismothering of lambs. Progesterone (P4) is involved in the expression of maternal behaviours yet has a sedative impact on lambs. We investigated the relationship between circulating P4 in pregnant ewes and neonatal lambs and maternal behaviour and lamb survival. Plasma P4 was measured in samples collected from 56 multiparous, indoor housed twin-bearing ewes on day 80, 100, 120 and 140 of pregnancy, and from their lambs 4 and 24 hr post-partum (pp). Retrospective behaviours were analysed using saved infrared CCTV footage, and lamb survival was recorded through to weaning. Pearson's correlation was used to analyse behaviour and P4. P4 levels were categorised into four levels (Low, L.Med, H.Med, High) for the Chi^2 analysis on lamb survival to weaning. Significance was set at $P \leq 0.05$. Ewes with high P4 displayed fewer negative behaviours overall, specifically towards their second-born lamb (R = -0.99; P = 0.001), without showing preference towards their first (R = -0.97; P = 0.006). Ewe P4 was strongly correlated ($P > 0.001$) to lamb P4 at both 4 (R = 0.73) and 24 hr (R = 0.79) pp. For the second-, but not the first-, lamb P4 levels were positively correlated to increased latency to stand and suck after birth ($P > 0.05$), and fewer lambs within the High P4 group survived to day 7 pp ($P < 0.001$) compared to the other P4 levels. Our findings demonstrate a strong correlation between ewe and lamb P4, and while higher ewe P4 may be linked to positive ewe behaviours, high P4 in lambs negatively impacts their survival. This information may be used to identify high risk ewes prior to parturition to help reduce lamb mortality in Merino sheep.

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Melatonin profiles for mid and late-gestation in Merino ewes

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High pre-weaning lamb mortality is a persistent issue for the sheep industry, impairing productivity and profitability, and representing a significant animal welfare concern. Melatonin supplementation during gestation (dG) improves pregnancy outcomes, with increased survival of the second-born lamb born to melatonin supplemented Merino ewes. We investigated (1) how endogenous melatonin profiles change between throughout pregnancy ewes, (2) how season affects melatonin secretion during pregnancy, and (3) different methods of melatonin supplementation to provide a sustained increase in melatonin for 16 hours. During May/June and Sept/Oct in 2018, from dG 80 ± 2 days, mature, multiparous twin-bearing ewes received either no exogenous melatonin (CTL; n = 8), a 2 mg melatonin oral capsule (MEL-FEED; n = 12), or an 18 mg subcutaneous implant (MEL-IMP; n = 7) administered at dG 78 and dG 125. On dG 80 and dG 130, ewes were fitted with jugular angiocaths, and hourly blood samples were collected for 24 hours. Plasma melatonin profiles from seven time points (baseline (1400 h; 0 hr) then 3-hourly for the following 15 hours) were analysed as repeated measure, with season, dG, and collection time as fixed factors. Endogenous melatonin increased significantly above baseline between 2100 h and 0600 h ($P < 0.001$), which was similar for both seasons (P = 0.161). There was no treatment effect on 0-hour melatonin (P = 0.924); however, MEL-IMP ewes had elevated melatonin 7-hours post-baseline compared to CTL ewes (P = 0.022), but did not differ MEL-FEED ewes (P = 0.222). At dG 130, MEL-FEED ewes had significantly elevated melatonin 1- and 4-hours post-capsule compared to dG 80 samples ($P < 0.001$). The implant provided similar night levels of melatonin compared to the capsule, and was less labour intensive, therefore providing a commercially applicable supplementation method for the sheep industry.

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Differential abundance of proteins in extracellular vesicles from antiphospholipid autoantibody treated human placentae

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Antiphospholipid antibodies (aPL) are autoantibodies that cause pregnancy complications including stillbirth, recurrent miscarriage, and preeclampsia by a poorly defined mechanism. These maternal autoantibodies are internalised by the placental syncytiotrophoblast where they induce intracellular changes as well as changes in the extracellular vesicles (EVs) this cell produces. We have previously identified changes in individual proteins in EVs from aPL (eg HMGB-1) that may trigger adverse reactions in the placenta or maternal physiology. Here we took an unbiased approach to quantifying the total changes in the proteome of EVs from aPL-treated placentae.

Placental explants were cultured with monoclonal aPL (n=5) or an isotype-matched control antibody (n=5). Nano-vesicles were harvested by differential centrifugation and further enriched using qEV size-exclusion chromatography. The proteomes of the enriched vesicles were quantified by SWATH. The String Network software was used to analyse the list of proteins obtained from SWATH analysis.

A total of 2583 proteins were quantified in the nano-vesicles of which 28 were more than twice as abundant in the EVs from aPL-treated placentae. These proteins were involved in pathways regulating the rate of production of proteins within cells. In particular, proteins that are a part of the large ribosomal subunit, the proteasome core and spliceosome subunits were more abundant in EVs from aPL-treated placentae. Proteins involved in the transfer of particles between the nucleus and cytoplasm were also more abundant in EVs from aPL-treated placentae.

Antiphospholipid antibodies either significantly upregulated the expression or increased the packaging of these proteins into nano-vesicles. This suggests aPL may affect syncytiotrophoblast cellular function through upregulation of subcellular components involved in the rate of protein synthesis. The increase in proteins that facilitate nuclear to cytoplasmic transfer is also consistent with our previous findings of increases in cytoplasmic and vesicular HMBG-1 which is typically a nuclear protein.

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The first transcriptomic profile of epithelial gland and stromal cells from the equine endometrium

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Early pregnancy loss has major economic implications for horse breeders. Despite many previous studies focusing on the endometrial environment during early pregnancy, there remains no long-term *in vitro* culture system capable of recapitulating the equine endometrium. To advance knowledge in this space, the present study aimed to characterise the transcriptomes of epithelial glands and stromal cells derived from the equine endometrium.

Endometrial tissue samples were collected from two mares immediately post-mortem and dissociated via enzymatic digestion. Cell populations were purified by selective adhesion of the stromal cells. The epithelial cells were pelleted and frozen at -80ºC whilst stromal cells were cultured to confluence (Day 5) before freezing. RNA isolation was performed on all samples using the Total RNA Isolation Mini Kit (Agilent), before sequencing using DNB-Seq technology. Sequencing data were analysed at SAHMRI and bioinformatics analysis was completed using Database for Annotation, Visualisation and Integrated Discovery (DAVID).

A total of 12,702 genes were identified in epithelial glands, while 11,482 were identified in the stromal cell population (>2 cpm per sample). 11,084 (84.6%) genes were identified in both populations, 1,618 (12.4%) were unique to the glands and 398 (3%) were unique to the stroma. A total of 910 genes were observed to be differentially expressed (Fold-change >log(2); FDR<0.05), 790 upregulated in the glands and 120 upregulated in the stroma. DAVID analysis revealed epithelial enriched genes were associated with signalling, ATP-binding immunoglobulins and the immune response, while those enriched in the stroma were associated with extracellular exosomes, cytoplasm, metabolic pathways and glycolysis. Furthermore, genes associated with oxytocin, oestrogen and prostaglandin receptors were all observed to be significantly enriched within the epithelial gland population. These findings establish a foundation for the development of novel *in vitro* models required for the advancement of knowledge surrounding uterine priming and early pregnancy in the mare.

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The effect of elevated folic acid on placental function *in vitro*

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Mandatory folic acid (FA) food fortification was introduced in Australia in September 2009 to prevent neural tube defects. However, FA food fortification, paired with periconceptional FA supplement recommendations, often results in folic acid intake exceeding the upper tolerable limit (~1000 µg/d) but the consequences are not well established. Increasingly, high maternal FA is associated with gestational diabetes mellitus (GDM) but the mechanism is unknown. Thus, we hypothesized that excess FA dysregulates hallmarks of placental cell function including proliferation, migration, invasion and secretion of placental hormones that orchestrate maternal insulin resistance and pancreatic beta cell expansion. To assess the effect of exogenous FA on placental function, BeWo cells and first trimester placental villus explants (6-12 weeks' gestation) were treated with FA at concentrations of 10 nM (deficiency), 40 nM (adequate), 200 nM (elevated) or 2000 nM (highly elevated). Following treatment, we used Real-Time Cell Analyzer (RTCA) xCELLigence analysis to assess proliferation and migration of BeWo cells in response to FA across 72 h in culture. Secretion of placental hormones by both BeWo cells and placental explants was measured using specific ELISA analysis of culture media. Measured hormones were placental growth hormone (GHv), placental lactogen (hPL) and prolactin (PRL). Collectively, this research will provide the first evidence of *in vitro* effects of high dose FA on trophoblast function and hormone secretion in early human placenta. In the current landscape of widespread FA fortification and prenatal supplementation, characterising the effects of excess FA intake on maternal and fetal health via those on the placenta is essential to ensuring it does no harm. Furthermore, this has important implications in understanding the complex factors which contribute to the development of GDM.

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Characterising leucine-rich repeat-containing G protein-coupled receptor 4 in the preeclamptic placenta

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LGR4 is a progenitor cell marker in high surface area tissues including the colon. It functions as a WNT pathway enhancer by binding its ligand R-spondin 1 (RSPO1); enhancing downstream signalling. In the colon, LGR4+ cells direct rapid proliferation ('transit amplification') and differentiation into terminal epithelial cell phenotypes. The placenta generates rapidly during pregnancy to form a complex high surface area organ dedicated to maternal-fetal exchange. The epithelial placental surface (syncytiotrophoblast) forms via underlying cytotrophoblast fusion and undergoes constant turnover. We hypothesise LGR4+ progenitor cells may be present in placenta and dysregulated in placental insufficiency diseases including preeclampsia. This study sought to characterise *LGR4* in preeclampsia and assess roles in isolated human (cyto)trophoblast (placental) stem cells (hTSCs).

LGR4 mRNA was measured in early-onset (<34-week gestation, n=81 vs n=19 controls) and late-onset preeclamptic placentas (≥34-weeks, n=33 vs n=20 controls). It was significantly increased in early-onset preeclamptic placentas only (p=0.0148). *In situ* hybridisation localised *LGR4* to proliferating (*MKI67*+) villous cytotrophoblasts, and ligand *RSPO1* to endothelial cells (*PECAM1*+) (n=3 preeclamptic and n=3 control).

Preeclampsia is associated with placental hypoxia and inflammation. Thus, we tested whether these increased *LGR4* in hTSCs. However, neither pro-inflammatory cytokines interleukin-6, tumour necrosis-α, nor hypoxia significantly altered *LGR4* expression. We assessed changes in *LGR4* with cellular differentiation by differentiating hTSCs into extravillous trophoblast (EVT) or syncytiotrophoblasts for 96h. Differentiation was confirmed by increased *HLA-G* (EVTs) and reduced cadherin-2/increased syndecan-1 (syncytiotrophoblasts). Intriguingly, LGR4 increased with EVT differentiation (p<0.0001) and peaked at 48h of syncytiotrophoblast differentiation (p=0.002). Finally, *LGR4* siRNA-mediated knockdown did not affect hTSC proliferation (MTS assay) nor differentiation into EVTs or syncytiotrophoblasts relative to control siRNA.

Placental LGR4 is elevated in preeclampsia, and hypoxia nor inflammation alter its expression. Localisation to actively proliferating cytotrophoblasts and increases across differentiation allude to a transit amplifier role in placental development and disease.

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Changes in miRNA profiles in placental extracellular vesicles across gestation may contribute to maternal adaptation during pregnancy

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Aims: During pregnancy, the placenta needs to change through gestation adjusting to fetal needs and controlling maternal physiology adaptations to the cardiovascular system. However, the underlying mechanism of maternal adaptation is unclear. Placental extracellular vesicles (EVs) have been well-reported to play an important role in the regulation of maternal adaptation via the RNA, proteins, DNA, and lipids that they carry to maternal cells/organs. In this study, we compared the miRNA profiles in placental EVs derived from first trimester and term placentae, to understand whether the changes of miRNA profiles contribute to, at least in part, maternal physiologic adaptations across gestation.

Methods: placental EVs from first-trimester and term placentae were collected, and the small RNA they contained sequenced. MicroRNAs with differential abundance between the two groups were identified. Literature searches combined with the target gene enrichment analysis identified differentially abundant miRNAs associated with maternal cardiovascular and immune adaptation.

Results: The total number of miRNAs in placental micro-, and nano-EVs derived from the first trimester placentae was significantly higher than that in term placental EVs. Of the miRNAs present in both early gestation and term EVs, 300 (in micro-EVs) or 208 (in nano-EVs) miRNAs were differentially abundant between the two groups. Gene enrichment analysis showed that some of those differentially abundant miRNAs participate in vascular adaptation, including angiogenesis, vasculogenesis, regulation of blood vessel endothelial cell migration and blood vessel morphogenesis. Those differentially abundant miRNAs include miR-15a-5p, miR-193b-3p, miR-27b-3p, miR-200b-3p, miR-3129-5p, miR93-5p and miR-17-5p.

Conclusion: Significant differences in the numbers and the abundance of miRNAs between placental EVs derived from the two different gestational placentae could participate in maternal adaptation during pregnancy.

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Elucidating the optimal level of ROS in sperm for function and fertilisation

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Increased reactive oxygen species (ROS) induces DNA damage and lipid peroxidation in sperm causing decreased function. However, at physiological levels, ROS capacitates sperm allowing successful fertilisation. Currently, little is understood about the optimal ROS concentrations in sperm.

Sperm from 8-10wk old CBAF1 mice (N=10) were incubated in 50nM and 5nM carbonyl-cyanide-m-chlorophenyl-hydrazone (CCCP) to increase and 5μM and 100μM manganese-(III)-tetrakis-(4-benzoic-acid)-porphyrin-chloride (MnTBAP) to reduce ROS concentrations for 1h at 37^{O}C, 5%O_2, 6%CO_2. Computer-assisted sperm analysis was used to determine motility and flow cytometry with fluorescent probes used to measure ROS (MitoSOX Red (superoxide), CellROX Green (intraceullar), lipid peroxidation (BODPIY) and mitochondrial membrane potential (MMP; JC-1). Fertilisation (2-cell development) was analysed 24hrs post-IVF, pronuclear dynamics assessed using PrimoVision timelapse, and ROS production in the pronuclear embryo by MitoSox Red.

All results are presented in Table 1. Sperm superoxide concentrations were modulated in a linear fashion by CCCP and MnTBAP (R^2=0.96), while MnTBAP (100μM) additionally lowered intracellular ROS (p=0.003), MMP (p=0.024) and straight linear velocity (VCL) (p=0.047). Sperm lipid peroxidation was unchanged. MnTBAP (100μM) decreased 2-cell development (p=0.015), while CCCP (5nM) increased time taken for pronuclear fusion and fading (T5, p=0.005) and increased pronuclear ROS concentrations (p=0.001).

Increasing or decreasing ROS concentrations in sperm results in aberrant sperm function and early embryogenesis which, act via different mechanisms. Understanding the interface between physiology and pathology will provide novel insight in to sperm and embryo redox biology.

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Green tea extract increases mitochondrial oxidative stress and induces DNA damage in ram spermatozoa in vitro

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Green tea extract (GTE) is a common component of several popular herbal supplements, including those marketed as 'fertility-boosting' supplements. Previous research has suggested that although GTE can be toxic to liver cells, it increases motility of sperm cells, although there is little research regarding other functional or metabolic changes in sperm. The current study aimed to elucidate the in vitro effects of GTE on ovine spermatozoa exposed to 0.1, 0.5, 1 or 5 mg/mL GTE. Flow cytometry was used to assess viability, acrosome reaction, membrane lipid disorder, mitochondrial superoxide production, intracellular reactive oxygen species (ROS) and DNA fragmentation. At 0.5, 3 and 6 h, all concentrations of GTE tested increased DNA fragmentation, whilst 0.5, 1 and 5 mg/mL promoted mitochondrial superoxide production at all time points. Intracellular ROS production also increased with 0.1, 0.5 and 5 mg/mL GTE. Despite reported antioxidant activity of GTE, the increase in both intracellular and mitochondrial ROS suggests that exposure to GTE increases oxidative stress in sperm. Additionally, the reduction in DNA integrity indicates that GTE may cause irreversible chromatin damage, whether through promoting oxidative stress or via another mechanism. Contrary to popular belief that these products enhance

fertility, this research indicates that they should be used with caution due to possible detriment to sperm, and a lack of stringent safety data.

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Fertility of *Bos indicus* bull sperm in SpermSafe-B following seven days of ambient temperature storage.

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The precise timing required for successful fixed-time artificial insemination (AI) can be a logistical challenge for producers with large, diverse herds of cattle. A novel method of bull sperm storage at ambient temperature has been developed to avoid the damaging freeze/thaw process, with the aim of extending the post-insemination lifespan of sperm in the female reproductive tract. Previously, this novel method of ambient temperature sperm storage in SpermSafe-B has shown promising results when fixed-time AI using 7-day stored sperm was performed on 2-year-old virgin *Bos taurus* heifers (n=18; 14 of 18 pregnant). To assess the fertility of *Bos indicus* sperm in this medium, a herd of 12 heifers (9 *Bos indicus*, 1 taurine-indicine cross, and 2 full *Bos taurus*) underwent a 12-day oestrus synchronisation protocol and were each inseminated with approximately 25 million 7-day stored *Bos indicus* sperm. At least 67% (8/12) of the heifers are known to have been cycling and all had body condition scores between 2 and 3. At a 35-day ultrasonographic pregnancy diagnosis, six of the 12 heifers (50%) were confirmed pregnant. These findings demonstrate that the fertility of *Bos indicus* sperm can be maintained in SpermSafe-B for 7 days of storage at room temperature and that these stored sperm have the capacity to achieve pregnancy in *Bos indicus* females. Ambient temperature sperm storage using SpermSafe-B could be a viable alternative for *Bos indicus* breeds, as well as *Bos taurus* breeds, when AIs will be performed within 7 days of semen collection. Although viability and motility have been maintained beyond 7 days of storage, there is a significant increase in viable sperm with destabilised membranes between days 10 and 14 (%M540 positive live cells: 19.4±5.70% vs. 47.4±7.64%; $P \leq 0.005$), suggesting 10 days may be the maximum duration of fertility for sperm stored in SpermSafe-B.

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Equine fertility: Predicting the outcome of standardbred artificial inseminations using chilled semen

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The primary objective of this study was to establish whether a combination of pre-insemination stallion sperm parameters can be used to accurately predict pregnancy outcome. This study was based on 46 inseminations of 41 mares, by a cohort of 7 standardbred stallions over a 5-week period at an Australian pacing stud. For the analyses, semen quality was assessed immediately after collection, and again after the semen had been stored at 5 ^{O}C for 24 h in EquiPlus semen extender. Following semen collection and chilling, assessments of ejaculate volume, sperm concentration, and motility were performed using an iSperm® Equine portable device. Additionally, an aliquot of the semen sample was subjected to a migration assay through a 5 µm polycarbonate filter in a Samson™ isolation chamber over a 15 min period. This subpopulation of isolated cells were again assessed for concentration and motility. Furthermore, the ability of the isolated spermatozoa to reduce the membrane impermeant tetrazolium salt WST-1 was evaluated. This information, combined with data that described the stallion and mare ages were used as predictors of pregnancy outcome, as confirmed by rectal ultrasound performed 14 days post ovulation. The predictive criteria used to predict pregnancy via multivariate discriminate analyses were optimized for each individual stallion, resulting in the ability to predict pregnancy outcome following insemination with an overall accuracy of 87.9% if analysed pre-chilling, and 95% if analysed post-chilling. The application of such an approach to semen analysis would be of considerable value to the equine ART industry in managing the reproductive performance of its stallions.

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Testicular hyperthermia causes aberrant alternative splicing in round spermatids.

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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 3-4^{O}C 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.

Integral to the formation of functional spermatozoa is the widespread occurrence of ‘alternative splicing’, a process wherein a single pre-mRNA transcript is spliced into multiple distinct mRNA products. Evidence in other model systems demonstrates hypothermia can lead to sever disruption of alternative splicing [87].

To determine if testicular hyperthermia cause aberrant alternative splicing, Next Generation RNA sequencing was performed on round spermatids isolated from male C57 mice (N=5) subject to testicular heat stress (42°C, 30min) versus controls (30°C, 30min). Using MAJIQ and Voila software packages, 395 differential LSV (Local Splice Variation) events were identified (Δψ ≥0.1, 0.9 confidence). Notably, many of the changes were found in long-non coding RNA species. Work is continuing to identify which splicing factors are involved.

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Developing a new method to enrich and culture marsupial spermatogonial stem cells

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Marsupial research and conservation are limited by the absence of advanced technologies such as artificial reproductive technologies (ART). We are working on filling this gap by developing stem cell lines for marsupials. Our research focuses on spermatogonial stem cells (SSCs), the undifferentiated progenitors of sperm. SSCs provide a logical and valuable means to develop ART in marsupials. However, the lack of established cell markers for marsupial SSCs prevents the use of traditional enrichment techniques.

In our study, fat-tailed dunnart SSCs were enriched by targeting the highly conserved retinoic acid (RA) synthesis pathway which is vital for the initiation of spermatogonial differentiation. Dunnart testes were digested to single cells and stained with a fluorescent dye (ALDEFLUOR^{TM}) that allowed measurements of aldehyde dehydrogenase (ALDH) activity, a surrogate marker of RA synthesis which is low in SSCs. Cells were subsequently fluorescence-activated cell sorted based on their ALDH activity. Quantitative PCR was used to analyse the expression of stem cell (*POU5F1*, *GFRA1*, *ETV5*) and somatic cell markers (*CYPA11A1*, *NR2F2*, *GATA6*, *SOX9*) within sorted populations. Culture of sorted populations was performed to assess SSC enrichment, viability, and proliferative ability.

Increased SSC markers and reduced somatic cell markers were found within the ALDH low population as predicted. Similarly, cultured populations demonstrated a reduced somatic cell presence within the ALDH low population when compared to the ALDH high population. These results suggest that marsupial SSC enrichment can be achieved though sorting cells by ALDH expression. This provides us with the ability to define culture conditions for SSCs and identify novel markers for their future use in ART, genomic editing, conservation, and research applications.

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The role of ATP6AP2 in male fertility

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ATP6AP2 is a multi-functional protein, involved in a number of cellular pathways including WNT and MAPK signalling, protein sorting and folding, and receptor-mediated endocytosis and recycling. Many of these functions depend on its interaction with the vacuolar H+-ATPase (V-ATPase). Due to its role in signalling pathways, we hypothesised that ATP6AP2 plays a role in gonad development. To test this hypothesis, we deleted Atp6ap2 specifically in gonadal somatic cells during foetal development using the Nr5a1-Cre mouse. Surprisingly, these mice appear to develop normally pre-birth, and were born at the expected Mendelian ratio. However, both males and females were infertile. At three months of age conditional Atp6ap2-deficient XY mice presented with significantly smaller testes caused by the loss of spermatogenic cells. Subsequent analysis demonstrated that the loss of Atp6ap2 in testicular somatic cells resulted in the loss of round spermatids by apoptosis, likely caused by a disruption of cell-cell communication due to defects in the trafficking of junctional proteins. The loss of spermatids led to increased differentiation and proliferation of progenitor cells, ultimately leading to exhaustion of the stem and progenitor pool and hence Sertoli cell-only tubule. Together this data suggests ATP6AP2 as a novel candidate for male infertility in humans.

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The experiences of Endometriosis patients with diagnosis and treatment in New Zealand

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Endometriosis is a chronically painful, invasive, inflammatory (1) disease, with limited treatment options (2), long diagnostic delays (3), and impacts 10% of females in New Zealand (4). The goal of this research was to understand the patient experience and include patient voices in the guidance of research priorities.

Fifty New Zealand endometriosis patients participated in anonymous, asynchronous, text-based group discussions on the VisionsLive platform. The patients ranged in age from 18-48. The patients answered 50 questions, 23 text-based and 27 quantitative, and then took part in online group discussions.

The average delay from symptom onset to a surgically confirmed diagnosis was 8.54 years. The top five reported symptoms within the cohort were pain-based, and the participants discussed the many impacts of this pain on their work and education. The four main diagnostic tools employed on this cohort were sharing their symptom history with a medical practitioner (88%), laparoscopy (82%), abdominal (72%) and transvaginal ultrasounds (68%). The most common emotions patients experienced following receiving a diagnosis of endometriosis were relief (86%), feeling overwhelmed (54%), and anger (32%). The main treatments offered to this cohort were pain relief (96%), laparoscopic surgery (84%) and the combined oral contraceptive pill (80%). Of these three treatments, only laparoscopic surgery was viewed positively by the majority of users, with 67% considering laparoscopy an effective treatment, compared to 46% of pain relief users, and 25% of combined oral contraceptive pill users.

Gathering the voices of patients revealed that long delays to diagnosis and dismissal by medical practitioners frequently manifests as a reaction of relief by patients once diagnosed. Results also showed current treatment options were often considered ineffective, but were routinely offered as the first, or only, options for patients. It is therefore important that both faster routes to diagnosis and more effective treatment options be developed.

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Plasma mass spectrometry-based proteomic biomarker discovery for endometriosis

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Aims

Endometriosis diagnosis requires surgical visualisation of lesions and histopathology. To date, no non-invasive diagnostic test has been validated. Discovery research using sensitive high-throughput mass spectrometry (MS) has the potential to improve identification of clinically-relevant plasma proteins. The aim of this study was to undertake MS-based proteomic biomarker discovery using minimally-invasive plasma specimens from patients with and without endometriosis.

Methods

Blood was collected from patients attending the pelvic pain clinic (Royal Women's Hospital) for laparoscopy (investigation/treatment of suspected endometriosis) (HREC Projects #10-43/16-43). Information was collected from patients surveys (demographics/symptoms), medical records and surgeon/pathology reports. Endometriosis status was confirmed using histopathology. Plasma (EDTA-treated, stored at -80°C) from 60 endometriosis cases and 20 non-endometriosis controls were included in the study. Following trypsin digestion, plasma peptides were analysed using a timsTOf Pro (Bruker Daltonics) mass spectrometer. MaxQuant and Perseus were used for data processing and analysis.

Results

A total of 470 proteins were quantified and identified from plasma samples. Compared to non-endometriosis controls, 26 proteins were significantly altered with endometriosis (FDR <0.05). Nine proteins demonstrated a reduced abundance, while 17 proteins demonstrated a higher fold-change in the endometriosis group compared to controls. We also identified 12 proteins that were absent in controls, but present in endometriosis cases. Comparison between each stage of disease (rASRM Stage 1 to 4) versus controls, demonstrated a change in 11 proteins collectively (FDR <0.05). The proteins of interest belonged to biological processes associated with immunity (complement system), angiogenesis, proliferation and cholesterol metabolism.

Conclusions

High-throughput MS-based proteomic biomarker discovery successfully identified 470 proteins in an endometriosis cohort. Case-control analysis revealed significantly different protein expression profiles in association with endometriosis. Further validation in an independent sample set is warranted. Biomarker discovery using plasma proteomics offers a minimally-invasive approach to endometriosis diagnosis.

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A holistic approach to managing endometriosis: what is the optimal structure and format of multidisciplinary care for endometriosis

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Aim:

Endometriosis is a chronic, benign, inflammatory condition characterised by the presence of endometrial-like tissue outside the uterus, causing pelvic pain and infertility. Given the multi-system nature of the disease and the potential significant impact on quality of life, there has been a long-standing recognition of the need for multidisciplinary care for people with endometriosis.

Endometriosis centres of excellence are few and far between around the world as there is no agreed-upon definition of such a centre. Therefore, this study aims to integrate the evaluation of the efficacy of interdisciplinary centres and the unmet treatment needs of patients to inform future development of endometriosis centres of excellence.

Methods:

PubMed, Medline, Embase and Web of Science were searched for all relevant articles published from 1 January 2010 to 7 July 2022. Medical Subject Headings (MeSH) keywords used included: endometriosis AND multidisciplinary OR multidisciplinary team OR patient care OR interdisciplinary AND clinic* OR centre* OR center*.

Results:

Nineteen studies met inclusion and exclusion criteria and were included. Four studies investigated the perceived efficacy of disciplines in current multidisciplinary centres for managing endometriosis, nine studies explored disciplines that are integral to the provision of good care, and six studies uncovered patients' perspectives on the best multidisciplinary care model for endometriosis management.

Conclusions:

A multidisciplinary centre consisting of gynaecologist, pain specialist, nurses, physiotherapist, psychologist, sex therapist, nutritionist, complementary and alternative medicine, and social worker, may provide improved holistic care to patients with endometriosis. Furthermore, better incorporation of patient-centred care should be weighted in planning and development of multidisciplinary centres so that best-practice in endometriosis care can be implemented to improve patients' satisfaction and quality of life.

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An investigation of the plasma lipidome using a mouse model of endometriosis and pelvic pain

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Aims

Endometriosis can be modelled in mice with features (ectopic lesions) and symptoms (pain) of the disease echoing human aetiology. Furthermore, mouse models of endometriosis have demonstrated altered metabolism (aberrant plasma and peritoneal fluid metabolite and lipid profiles). The aim of this study was to analyse the plasma metabolome/lipidome from sham and endometriosis mice to determine if altered metabolic profiles could be:

- linked to pathways associated with poor cardiometabolic health outcomes, and
- employed as biomarkers to diagnose or monitor disease phenotypes.

Methods

Autologous transplantation of uterine horn fragments (or fat, sham surgery) in female C57BL/6J mice were performed to induce endometriosis (HREC number SAM342). Uterine horn fragments were attached to the small intestinal mesentery and alongside the uterus. Histological assessment to confirm lesion (or sham) morphology was assessed between week 8-10. Plasma from n=6 endometriosis and n=5 sham controls were also collected (stored -80°C) at 8-10 weeks. Plasma was analysed using untargeted high-throughput liquid chromatography electrospray ionisation coupled with tandem MS (LC ESI-MS/MS) using reverse-phase LC and trapped ion mobility spectrometry time-of-flight (TIMS-TOF) (Bruker Daltonics). MetaboScape software was used for spectral data processing and databank searching.

Results

A total of 5651 m/z features were detected from plasma across positive and negative modes. Orthogonal partial least squares discriminant analysis (OPLS-DA) revealed m/z features that differed in the endometriosis group compared to sham controls (1146.52, 677.55, 816.58, 990.66). The metabolite classes of interest were triradylglycerols, glycerophosphates, glycerophosphoglycerols, and ceramides.

Conclusions

This pilot study using high-throughput MS-based lipidomics successfully identified 5651 m/z features in an endometriosis mouse model. Case-control analysis revealed differing metabolic profiles in association with endometriosis. Further validation is warranted, including comparisons to matched tissues (autologous endometrial lesions). Discovery of aberrant metabolic profile biomarkers using plasma offers a minimally-invasive approach to better diagnose disease and increase our understanding of endometriosis pathophysiology.

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Extravillous trophoblast cell exosomes induce vascular smooth muscle cell apoptosis via a mechanism associated with miR-143-3p

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Uterine spiral artery remodeling is one of the key maternal adaptations of pregnancy, allowing delivery of the large volumes of maternal blood required for both placental and fetal growth. Failure of this process is associated with obstetric complications including preeclampsia, fetal growth restriction and miscarriage. Spiral artery remodeling is characterized by loss of the musculoelastic wall which is replaced by fibrinoid and intramural extravillous trophoblast cells (EVT). In recent years attention has focused on the initial stages of spiral artery remodeling which include separation of the vascular smooth muscle cells (VSMCs) and their phenotypic switch to a more synthetic phenotype, facilitating their migration away from the vessel wall. However, less is known about the final fate of the VSMCs. In vitro studies suggested that EVT could induce VSMC apoptosis, though VSMC apoptosis is not seen within the wall of the spiral arteries undergoing remodeling. However, apoptotic VSMCs have been observed amongst those cells which had migrated away from the vessel wall, a process associated with the presence of EVT. In the current study we aimed to further explore the mechanism by which EVT induce VSMC apoptosis. Primary cultures of first trimester EVT were established and EVT exosomes (EVT-exo) isolated from the culture medium (CM). Both EVT-CM and EVT-exo induced VSMC apoptosis, the effect of EVT-CM was abrogated by an exosome blocker. EVT-exo uptake was observed in VSMCs In silico analysis suggested several potential miR species may be involved, of which overexpression of miR-143-3p in VSMCs induced their apoptosis. miR-143-3p overexpression by EVT, also led to overexpression in EVT-exo, which in turn induced higher rates of VSMC apoptosis than seen in controls. These data suggest that miR-143-3p packaged in EVT-exo induces VSMC apoptosis and may be critical for the final stages of spiral artery remodeling and establishment of a successful pregnancy.

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The role of CD147 on the differentiation and vascular remodeling function of human extravillous trophoblast and its association with preeclampsia

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Aims: Preeclampsia is a gestational complication and the top cause of prenatal mortality and morbidity. The etiology of preeclampsia is associated with defective trophoblast differentiation and functions causing abnormal placental development and maternal-fetal exchange defects. CD147 is a key component of several most abundant protein complexes on human trophoblast. This study aims to determine the role of CD147 in placental development and pathogenesis of preeclampsia.

Methods: The roles of CD147 in trophoblast differentiation and functions were studied using the trophoblast stem cells, trophoblast organoid and placenta-targeted nanoparticles gene suppression models. Placental and serum CD147 expression in early pregnant women (11–13+6 weeks of gestation) who subsequently develop preeclampsia in late pregnancy were compared to normotensive controls.

Results: In pregnant mice, placenta-specific suppression of CD147 leads to preeclampsia-like phenotypes, including elevation of systolic blood pressure, proteinuria, increase of serum sFlt-1 level, renal dysfunction, and defective placental development with reduced number of the invasive trophoblast giant cells, which correspond to the extravillous trophoblast in humans. In vitro functional assays showed that CD147 mediates the differentiation and spiral artery remodeling activities of extravillous trophoblast. CD147 exists as a receptor complex on the plasma membrane of human trophoblast and the interaction of CD147/integrin-β1 complex with Wnt/β-catenin signaling mediates the activities of CD147. The CD147 levels in early placenta villi and serum from pregnant women who developed early-onset preeclampsia in late pregnancy were lower than those with normal pregnancy.

Conclusion: Dysregulation of the CD147 complex confers defective placental development. Clinically, the results of this study indicate a possible research direction for the use of CD147 for the early prediction of Preeclampsia.

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Understanding luminal and glandular epithelial remodelling for human endometrial receptivity

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The human endometrium is a dynamic tissue that undergoes molecular and cellular changes in preparation for embryo implantation in every menstrual cycle. The luminal epithelium (LE), the first cell layer of the endometrium that would interact with an implanting embryo, must transform from a non-receptive to a receptive state for the initiation of embryo implantation. The other epithelial sub-type, the glandular epithelium (GE), is located inside the tissue on the glands and would interact with the embryo only after it has entered the endometrium. Our previous studies have indicated that during the establishment of endometrial receptivity, genes in LE are regulated differently from GE. However, research in the field has largely overlooked the difference between LE and GE and they are often regarded as one entity, to date it is not well understood how these two sub-types of epithelial cells differ in gene expression in general and when remodelling for receptivity. In this study, we aimed to establish the global transcriptomic profiles of human endometrial LE and GE across the menstrual cycle. Laser capture microdissection was used to dissect LE and GE from endometrial tissues biopsied in the proliferative (non-receptive) and mid-secretory (receptive) phases of the menstrual cycle, total RNA was isolated from these cells and then analysed by RNAseq. Our data showed that the majority of genes were expressed similarly between LE and GE, however, many genes were transcribed very distinctly between LE and GE cycle-stage dependently as well as independently, and many were regulated very differently between LE and GE for receptivity. This is the first study to comprehensively analyse the global gene expression of LE and GE in the human endometrium, and our data provide new and important insights into the understanding of endometrial epithelial sub-types and their distinct remodelling in the establishment of receptivity.

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The Role of the Renin Angiotensin System in Decidualisation and Throughout Pregnancy

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Transformation of the endometrium in pregnancy, or decidualisation, is key for embryo implantation and protecting the conceptus from the maternal immune system. It also provides initial nutrients to the embryo. All components of the Renin-Angiotensin System (RAS) are known to exist within the decidua at term, with downstream signalling cascades having the potential to sustain the decidua. *In vitro* studies have shown that the rate-limiting enzyme of the RAS, prorenin, is increased during decidualisation [1]. Thus, we aimed to describe the expression of the RAS in the decidua throughout gestation and investigate associations between its expression, its downstream targets, and markers of decidualisation.

Decidual tissue was collected from first trimester (n=12), second trimester (n=4) or term pregnancies in the absence of labour (n=12). qPCR was used to examine mRNA expression of decidualisation markers, RAS components and targets of RAS activation.

Expression of decidualisation markers prolactin and IGFBP-1 were highest in the second trimester ($P=0.03$ and $P<0.01$, respectively). Similarly, expression of angiotensinogen (AGT; $P=0.005$ vs. first trimester), prorenin receptor (ATP6AP2; $P=0.016$ vs first trimester and $P=0.019$ vs. term) and angiotensin converting enzyme (ACE; $P=0.026$ vs term) were also highest in the second trimester.

When looking at downstream targets of the RAS that promote proliferation and vascularisation, both plasminogen activator inhibitor (PAI) and vascular endothelial growth factor (VEGF) show a significant increase over gestation ($p=0.0039$ and $p=0.0034$, respectively). Components of the sphingosine-phosphate pathway, SPHK1 and S1PR1, which have been shown to crosstalk with angiotensin II [2], and have roles in the maternal-fetal immune interface, are higher in the second trimester compared with the first ($P<0.0001$ and $P=0.016$, respectively).

These findings suggest that the RAS and its downstream targets may be contributing to the formation of the decidua or its function at critical stages in pregnancy.

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Transcriptomic profile of the uterus during the acquisition of receptivity to embryo implantation

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A key determinant of successful embryo implantation is uterine receptivity. From a non-receptive state during ovulation, this acquisition of receptivity to implantation involves significant molecular and cellular changes in the endometrial lining of the uterus in preparation for the implanting embryo. However, the exact molecular mechanisms underlying this transition remain to be fully characterised. Here, we aimed to generate a comprehensive transcriptome profile of the mouse uterus in the peri-ovulatory and peri-implantation states and characterise transcriptome changes between the two states. Tissue was collected from non-receptive endometrium of estrous C57Bl/6 female mice, and C57Bl/6 females mated to BALB/c males at day 3.5 post-coitum, the day before embryo implantation in mice. High-throughput RNA-sequencing was used to identify the genes and pathways regulated in the endometrium (n=3-4 biological replicates per group). Differential gene expression analysis revealed 546 upregulated and 30 downregulated genes (logFC>1 and adjusted p-value<0.05) in the endometrium of mated mice compared to estrous mice. With the suite of analytical tools from Ingenuity Pathway Analysis, these differentially expressed genes were predicted to activate pathways and upstream regulators involved in implantation, such as the estrogen signalling pathway and vascular endothelial growth factor A. Furthermore, significant molecular changes in the maternal immune and vascular systems occur in the uterus during the pre-implantation phase, as demonstrated by increased functional terms, including leukocyte migration and angiogenesis. Together, our findings confirm that the uterine transcriptome before achieving receptivity is vastly different from the non-receptive estrous uterus, and the changes observed are in line with substantial cellular alterations occurring before embryo implantation. This dataset will serve as a valuable tool and resource for utilisation in future research on the molecular mechanisms of uterine receptivity.

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Maternal peri-conceptional macronutrient intake programs offspring nutrient targets, metabolism and behaviour

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It is well accepted that *in utero* exposure to maternal diet can program offspring development and susceptibility to disease in later life. While animal diet studies have focused on either maternal under-nutrition (e.g., calorie or protein restriction), or over-feeding of high fat diets, little is known about the effects of maternal dietary macronutrient balance (the proportions of protein, fat, and carbohydrate) in modulating offspring health. An important model called *protein leverage* explains that in many animals, including humans, protein is prioritised over carbohydrates and fats when confined to imbalanced diets. This tight, innate regulation of protein intake, influenced by dynamic protein targets, can result in the overconsumption of fats and carbohydrates when given protein-poor diets, leading to obesity and related co-morbidities. However, the question remains as to *when* and *how* this is programmed in an individual.

We hypothesise that protein targets are determined i*n utero* and through early life programming. Using a mouse model, we investigate how maternal protein to carbohydrate (P:C) balance influences offspring appetite and metabolic health. We show that offspring from dams fed high P:C diets throughout gestation and lactation have greater protein targets and increased body weights in early life, consistent across sexes. We also show that these greater protein targets increase offspring food intake when placed on no-choice diets, resulting in an overall increase in body weight and fat mass. The combination of a high protein maternal diet and a Western diet in adulthood is revealed to further exacerbate this phenotype. The interaction of maternal and adult diets is also shown in outcomes such as plasma FGF21 and cholesterol levels, and overall activity and behaviour. This work highlights novel discoveries linking the intricate interaction of maternal nutrition, early-life programming and nutritional targets on long-lasting offspring consequences, providing fundamental new understanding to the field.

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An investigation of the comparative experiences of individuals with mate kirikōpū/endometriosis and/or huahua hua kūao/polycystic ovary syndrome in Aotearoa New Zealand.

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Mate kirikōpū/endometriosis and huahua hua kūao/polycystic ovary syndrome (PCOS) are common diseases linked with oestrogen-dependence and androgen-excess, respectively. Despite certain hypotheses suggesting that disease risk factors are diametrically opposed, there is a cohort of individuals with comorbid endometriosis and PCOS. We hypothesised that disease comorbidity would alter symptom profiles relative to those with one disease only, and increase negative experiences with diagnosis and management. To obtain preliminary data, an anonymous online survey was distributed via social media (NZ residents, >18 years) that included questions related to diagnosis, symptoms, treatment and management of endometriosis and/or PCOS. A total of 1323 responses (median [range] = 31 years [18-72]) were received within a two-week period (endometriosis only: n=615, 48%; PCOS only: n=459, 35%; both diseases: n=247, 19%). In preliminary analyses, the onset of menarche was significantly reduced in individuals with endometriosis (mean ± SD: 12.2 ± 1.55 years) or both diseases (12.0 ± 1.70 years) relative to PCOS only (12.5 ± 1.70 years; p= <0.0012). Preliminary observations suggest there was a significant difference in age of diagnosis between individuals with endometriosis-only, PCOS-only and comorbid individuals; however, statistical analyses are yet to be done. In addition, early analyses suggest heavy menstrual bleeding may be more common in participants with disease co-morbidity, whilst hirsutism appears less common, than those with one disease. Although analysis is ongoing, the symptom profiles of comorbid individuals varies relative to those with only one disease. Remaining quantitative and qualitative data is still to be examined; however, our preliminary results indicate altered experiences between those with a single versus comorbid disease, highlighting the need for further research into the intersection of these two common diseases.

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Endometriotic chocolate cysts contain viable cells of endometrial origin: implications for endometriosis recurrence and infertility

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Endometriosis is a chronic, estrogen dependent disease affecting 11% of reproductive aged adolescents and women. Ovarian endometrioma, or chocolate cysts, are a common subtype of endometriosis yet, our understanding of their origins and pathogenesis is limited. Endometrioma are filled with a chocolate brown fluid believed to arise from the endometrial tissue lining the cyst wall haemorrhaging. Endometrioma are associated with reduced fertility. Their presence and growth is commonly detected by ultrasound or MRI, and treated by a laparoscopic ovarian cystectomy. However, endometrioma recurrence is common (46% of patients within 3-years), with 40% presenting with more severe endometriosis. The origins, nature, and mechanisms of endometrioma fluid production are unknown, but may contain live cells which contribute to recurrence in the event of cyst rupture. Elucidating the cellular make-up of endometrioma fluid is critical for furthering our understanding of endometrioma, and endometriosis-related infertility.

For this study, cystic fluid was collected from endometriomas via aspiration methods during surgery (n=7 patients) followed by dilution in PBS and serial filtration through 100µm, 40µm, and 10µm filters. Each filtrate was pelleted, washed with PBS, and cultured as monolayers or in 3D Matrigel™ domes. Cellular colonies and organoids appeared within 5-10 days. Immunophenotyping analysis confirmed CD10-positive stromal cells, cytokeratin-positive epithelial cells, and CD68-positive macrophages were present in endometrioma fluid. Other cell types observed included vimentin-negative myofibroblast-like cells, elongated cells with nerve-like projections and cobblestone colonies of endothelial-like cells.

These findings demonstrate endometrioma fluid contains a plethora of viable cells including CD10- and cytokeratin-positive cells that may be of endometrial origin. Future work will include isolating cells from endometrioma fluid and cyst wall to determine cellular origin linkages using single cell transcriptomics. Uncovering the origins of endometrioma and their contents is critically important for enhancing our understanding of endometriosis and its impact on fertility.

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Validation of temperature classification using remote-sensing technology during oestrous synchrony in Merino ewes

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Remote-sensing technology facilitates longitudinal collection of body temperature, to help understand the physiological thresholds of extensively grazed livestock [1]. The objectives of this study were (i) identify ewes with the ability to maintain a low core temperature when exposed to high ambient daytime temperatures, and (ii) validate the selection of ewes based on pre-determined temperature groups prior to summer mating.

Two hundred Merino ewes were selected from Turretfield Research Centre, Rosedale, SA, according to their average daily vaginal temperature measured automatically at 10-minute intervals. A silicon probe housing a temperature logger (Micro-T 16-bit; Star-Oddi, Iceland) [2], was initially deployed once into each ewe during two, 3-day periods when maximum daily ambient temperature was ≥ 32.0 ℃. Low temperature status (LTS; n = 100) and high temperature status (HTS; n = 100) ewes were classed as having an average daytime vaginal temperature of ≤ 38.85 ℃ and ≥ 38.95 ℃, respectively. The temperature loggers were then re-deployed into selected ewes, in conjunction with a progesterone pessary for the synchronisation of oestrus over a 14-day period.

Vaginal temperature was not correlated with ewe live weight (r = - 0.125) but was weakly, negatively correlated (P = 0.003) with ewe body condition score (r = - 0.213). Mean daytime vaginal temperature during oestrus synchronisation was lower (P < 0.005) for LTS (38.76 ℃ ± 0.01) compared with HTS ewes (38.93 ℃ ± 0.01). With reference to ewe selection, 72% were consistently grouped based on mean daytime vaginal temperature between initial deployment and oestrus synchronisation.

Within commercial sheep flocks, core temperature status of ewes prior to the time of mating allows subgroups of individuals with varying thermoregulatory capabilities to be identified. Future research will focus on linking ewe thermoregulation with fertility and fecundity, as well as with pre- and post-natal lamb growth and development.

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Reduction In Smoking in Pregnancy A Key Contributor To Reduction In Preschool Obesity In New Zealand

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In New Zealand one in three preschool children (<5 years of age) are overweight or obese, escalating to 54% in Pasifika children. Despite alarming rates of preschool obesity, remarkably in recent years we have shown this rate has progressively declined across the country for 10 years, which has not been reported in any other country. This has occurred across all ethnicities, socioeconomic classes and regions in NZ. This reduction in preschool obesity began between 2000 and 2010. The key question is what has occurred in NZ at a national level that led to this change. From the early 2000's NZ launched a strong anti-smoking campaign that included progressive tobacco taxation and from 2005 graphic hard-hitting anti-smoking TV advertisements.

New Zealand's anti-smoking campaign is associated with a reduction in smoking in pregnancy which is associated with a reduction in preschool obesity.

The New Zealand Government's 22 national datasets are now all integrated (the Integrated Data Infrastructure). Data from four of these datasets were linked and analysed; national smoking records, the maternity health records, birth records and the Before School Health Check (4-5 year old children). Tobacco smoking data in pregnancy and lactation, birth weight, gestation, ethnicity and birth weight, together with BMI at 4-5 years of age were collected. BMI expressed as BMI SDS. Birth and childhood BMIs were adjusted for maternal BMI and childhood BMI for birth weight.

Data was collected on 312,000 mother and child pairs nationwide. Smoking in pregnancy and lactation is still common in NZ. In Māori women 37% and in Pasifika 18% smoked in pregnancy compared to 9% in Europeans. In addition, smoking is more common in less affluent women. Importantly, there has been a dramatic reduction in smoking in NZ women of childbearing age between 2006-2018, most notably between 2006-2013 when the campaign was most active. Between 2006-2018 there was a major reduction in the percentage of regular smokers in women aged 15-39 years, from 53.5% in 2006 down to 14.8% in 2018. This reduction occurred across all ethnicities, most markedly in Māori and Pasifika women.

Smoking during pregnancy was associated with an increased risk of small for gestational age (birth weight <-2 SDS) newborns with risks for light ($OR_{adjusted}$ 3.2) medium ($OR_{adjusted}$ 4.2) and heavy smoking ($OR_{adjusted}$ 4.7). Evaluation of women who smoked in pregnancy only and postnatally only showed independent effects of SGA with $OR_{adjusted}$ of 2.3 and 2.6 respectively.

Smoking during pregnancy was associated with an increased risk of childhood obesity at 4-5 years of age with risks for light ($OR_{adjusted}$ 1.38) medium ($OR_{adjusted}$ 1.49) and heavy smoking ($OR_{adjusted}$ 1.6). Evaluation of women who smoked in pregnancy only and postnatally only showed independent effects of childhood obesity with $OR_{adjusted}$ of 1.35 and 1.44 respectively.

There has been reduction in smoking during pregnancy and lactation, both associated with a reduction in preschool overweight/obesity in NZ children. We speculate that the reduction in smoking in pregnancy has contributed to the fall in preschool obesity in NZ.

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The DSD clinical coordinator role: Impact on clinical care

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Background:

The Differences in sex development (DSD) clinical coordinator (CC) role began in 2016 to facilitate clinical coordination and psychosocial support of individuals living with DSD and their families at the Royal Children's Hospital, Melbourne. Here, we report on the impact of the role on service delivery and its current vital components.

DSD Multidisciplinary team (MDT):

Formal DSD MDT meeting coordination by the CC has resulted in many benefits. The joint monthly meeting across two paediatric centres in Melbourne has increased clinical participation, which now includes over 35 specialists and further encompasses clinicians from regional Australian and international centres.

Clinical coordination:

The CC supports families through paediatric and adolescent care, transitioning into adulthood, and increasingly in the prenatal setting. Common CC support includes appointment coordination (multiple specialists), translation of health information, and referral for psychosocial services including peer support. The CC has a 410+ patient load and provides psychosocial support for 4-15 patients and their families per day.

Stakeholders:

The CC is a central liaison point for a broad range of stakeholders. This includes providing expert advice for government intersex advisory groups and other organisations, developing relationships with peer support groups, and membership in clinical and academic organisations.

Research and education:

To promote evidence-based care, the CC has led projects and collaborated with research teams, identifying the health gaps and priorities for this community. As a member of a Reproductive Development Research Laboratory, the CC liaises with participants and encourages connections between clinical groups and researchers.

Informed by individuals' lived experiences, the CC has developed online resources available in different formats and has provided DSD education for external organisations such as schoolteachers, book authors, and midwives.

The CC raises awareness of DSD/intersex variations in the community, promoting better outcomes for individuals and their families.

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The mobile food record: The usability and acceptability of image-based dietary assessment in Australian children and adolescents with type 1 diabetes.

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Background: Monitoring dietary intake and accurately estimating the macronutrients of foods is a daily challenge for children and adolescents living with type 1 diabetes (T1D), especially those with low literacy and numeracy levels. Harnessing technology through image-based dietary assessment tools, such as the Mobile Food Record (mFR^{TM}), shows promise to reduce diabetes burden and improve clinical care. However, the mFR^{TM} has not been studied in paediatric T1D.

Aim: This pilot study aimed to assess the usability and acceptability of the mFR^{TM} in children and adolescents with T1D.

Method: 9 to 18 year olds with T1D >1 year were recruited from Perth Children's Hospital. The mFR^{TM} App was downloaded onto personal devices and users captured before- and after-eating images for four consecutive days. Sociodemographic and usability surveys were collected. A trained analyst assessed mFR^{TM} for dietary intake, image clarity and the inclusion of the fiducial marker for portion size estimation.

Results: 25 participants have been recruited to date (60% female), with a mean age of 14.2(SD2.5) years and diabetes duration of 7.8±4.2 years.

A mean of 16±7 images sets were taken over the 4-days, with 86.7±14% of 'before eating' images considered clear. 96.5±7% of image sets contained 'after eating' images. All participants agreed it was easy to remember the fiducial marker in meal images, supported by 97.2±6% of images containing the marker.

Ten participants stated the mFR^{TM} interfered with their daily activities, while only two agreed the mFR^{TM} made them behave differently. Only one participant reported using a shortcut when taking images if they were in a rush, while 96% of participants reported forgetting to use the mFR^{TM} at least once.

Conclusion: The mFR^{TM} App demonstrated usability in the cohort of children and adolescents living with T1D. Future investigations involve evaluating the feasibility of using the mFR^{TM} to tailor clinical care.

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Type 1 Diabetes diluted insulin and automated insulin delivery a success story.

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Aim

To describe the use of dilute insulin to treat Type 1 diabetes (T1D) in infancy using a Hybrid Closed Loop (HCL) system.

Methods

Case report.

Results

An 8 month old boy presented with vomiting, lethargy and polyuria on a background of static weight gain (8.8kg) for 3 months. Investigations demonstrated moderate diabetic ketoacidosis (DKA) and insulin infusion was commenced.

Diabetes antibodies: negative GAD, IA2 and ZnT8 and positive insulin antibodies, 9.9U/mL (<0.4). Neonatal diabetes genetic panel was negative. Consistent with early onset T1D.

Subcutaneous insulin was commenced. A total daily dose of 4units was required. Given small insulin requirements, Medtronic 640G pump and Dexcom G6 sensor were commenced on day 4 using diluted insulin 1:5 (50units Novorapid (0.5mL) + 2mL Normal Saline - 20units/mL).
Review six weeks post pump start highlighted extreme parental anxiety and fatigue. Issues included frequent breast feeding with difficulty quantifying carbohydrate intake, problematic transition to solids and reliance on the diabetes team for frequent insulin dose adjustment. He was admitted for transition to a HCL system and education, using the Medtronic 780G insulin pump and SmartGuard™, acknowledging the algorithm was not licensed for use at this age.

Table 1 shows that transition to HCL, improved glucose management indicator (GMI) and time in range (TIR) with greatest improvement in time spent with glucose >13.9mmol/l. Increased TIR reduces the risk of developing microvascular and macrovascular complications (1). Hypoglycaemia was similar using standard pump and HCL.

Reduction in boluses per day using HCL indicated significant reduction in parental workload. This reduced reliance on regular adjustments. There has been a significant improvement in parent reported quality of life including improved sleep.

Discussion/Conclusion

This case highlights HCL with diluted insulin is safe, effective and can improve glycaemic control, parental quality of life and reduce reliance on the diabetes team.

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Evaluation of evidence-based physical activity and exercise resources for children/teens with type 1 diabetes

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The SCHN (Randwick) diabetes team has developed evidence-based resources to assist children/teens with type 1 diabetes and their families navigate physical activity/exercise when using insulin injections or insulin pump therapy.

As regular physical activity has a range of benefit essential for a healthy body and mind, the SCHN (Randwick) diabetes team strongly encourage children/teens with type 1 diabetes to remain physically active in keeping with Australian guidelines which recommend a minimum of 1 hour per day.

In 2021, the SCHN (Randwick) diabetes team developed evidence-based booklets and activity planners for children/teens using either insulin injections or insulin pump therapy. The team have used these resources when facilitating group education sessions focussing on physical activity/exercise and at other key times education times including at initial diagnosis, as part of routine clinic appointments and when changing insulin delivery method such as transition from multiple daily insulin injections to insulin pump therapy.

Children/teens with type 1 diabetes and their families in the SCHN (Randwick) diabetes service have been asked to evaluate these evidence-based resources via a survey which is being conducted under a quality improvement activity framework (CHARLI #7395).

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Audit of Endocrine Features in paediatric patients with Langerhans Cell Histiocytosis

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Langerhans cell histiocytosis (LCH) is a rare proliferative disorder characterised as an inflammatory myeloid neoplasia (1). Cases may present to a wide range of clinicians including dermatology, ophthalmology, orthopaedics and endocrinology (2). Endocrine manifestations of LCH, particularly central diabetes insipidus (CDI), have been described as early as the 1940s, but primarily through case studies and small cohort analyses (3 – 5). There are limited Australian paediatric data described in recent literature.

Aim: To investigate the incidence of endocrine features in paediatric patients with Langerhans Cell Histiocytosis (LCH).

Methods: A retrospective chart review of electronic medical records (EMR) of patients with LCH managed at a Victorian Paediatric Tertiary Centre. Diagnosis term “Langerhans Cell Histiocytosis” was searched in the EMR and collated with the same search within the institution’s Oncology database. Patients excluded if a biopsy did not suggest LCH or if records were incomplete.

Results: 171 patients were identified and 133 records of patients diagnosed with LCH over the last 30 years were assessed for endocrinopathies from diagnosis to last documented follow up. Mean age at diagnosis was 5 years 8 months. 15.8% of patients (n=21) had confirmed central diabetes insipidus (CDI), whilst 10% of patients (n=13) had documented endocrine features other than CDI. Of these, almost 70% were growth hormone deficient (n=9). Almost half of patients (46%) were pre-pubertal at the time of audit or upon discharge from tertiary services. Of all records assessed, 7.5% of patients had more than one endocrinopathy noted during their follow up for LCH.

Conclusions: Pituitary assessment as a component of managing LCH is imperative especially as features other than CDI suggesting impact on hypothalamic-pituitary axis (for example GHD and gonadotropin deficiency) can be subtle, late and missed. Close follow up of growth and progression through puberty, even if discharged from tertiary care, is essential.

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User experience testing of a healthy lifestyle check IT application for scale of multidisciplinary programs for children and youth affected by weight issues.

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The World Health Organization recommends multicomponent lifestyle weight management services for affected children/youth.(1) However, there is a lack of these services across Australia(2) and globally. Based on a 10-year evidence-base from a healthy lifestyle assessment/intervention program, we developed a healthy lifestyle check (HLC) IT application to individualise care, providing efficient screening of weight-related comorbidities in home-based assessments within such programs. The study aim was to evaluate and implement the HLC IT application within a program, Whānau Pakari.

Beta-testing with health professionals and volunteers was conducted, with feedback refining the application (Phase One). Implementation within Whānau Pakari of the HLC IT application prototype was conducted (Phase Two). Participants included two end-user groups: health professionals administering the HLC (n=2), and the children/youth (and families) referred. Children aged 4-16 years (BMI≥98th percentile, or ≥92nd percentile with pre-existing weight-related comorbidities) and their accompanying adult were eligible. A heuristic evaluation of user experience (UX) was conducted along with post-assessment interviews and survey questions, including System Usability Score (SUS), tracking progress on usability/acceptance. Phase three involved refining the application based on end-user feedback for implementation.

The HLC IT application demonstrated an above average SUS (mean = 88). 11 sessions were conducted to attain data saturation. Qualitative themes identified were session length, input types, input roles, and engagement. Session length was subsequently reduced, inputs were modified for ease of use, input role clarification was achieved, and a more engaging platform was created. The HLC IT application was refined in phase three, ready for further UX testing.

A scalable unit (program and application) has been developed to allow greater access to healthy lifestyle programs. With inclusion of a HLC IT application, programs could be scaled, ensuring weight- related comorbidities are systematically addressed in demedicalised appointments, whilst providing flexibility for communities in terms of the intervention they deliver.

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Prevalence of type 2 diabetes risk factors, including overweight and obesity, among youth attending hospital-based paediatric services in Western Melbourne

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This study aimed to determine the prevalence of risk factors for type 2 diabetes in overweight and obese adolescents attending hospital-based paediatric care in Western Melbourne.

100 overweight and obese adolescents (aged 10-17 years) who attended an outpatient clinic at Western Health between May 2019 and May 2020 were randomly selected for structured telephone interview to assess for presence of other risk factors.

45% of the eligible adolescents were overweight or obese. Of those selected for interview, 77% had an additional risk factor for type 2 diabetes. No association between the number of risk factors and body mass index standard deviation score was found, however there was an association between the number of risk factors for type 2 diabetes and both family history of type 2 diabetes and ethnicity.

This paediatric subpopulation had a high rate of risk factors for type 2 diabetes in addition to overweight and obesity, and are at risk of premature mortality and chronic morbidity should they develop type 2 diabetes. The high rate of at-risk youth identified in our cohort may be indicative of the risk for youth in similar populations.

International variation in diazoxide treatment rates for neonatal hyperinsulinaemic hypoglycaemia

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Diazoxide is used in the treatment of neonatal hyperinsulinaemic hypoglycaemia (HH) as it is an inhibitor of pancreatic insulin production. It also causes smooth muscle relaxation, and has been shown to disrupt intestinal motility in animal models.(1) An independent association has recently been suggested between neonatal diazoxide treatment and the occurrence of necrotising enterocolitis (NEC).(2) We are currently conducting a large multi-site study in order to verify this putative association. To start with, we hypothesise that differential diazoxide treatment rates for HH between hospitals may affect any diazoxide-NEC association, and would therefore require independent consideration within our larger association study.

The aim of this study is to determine the diazoxide treatment prevalence at an Australian centre, and to compare this to published international diazoxide treatment rates.

All infants aged 0-1 year who were exposed to diazoxide over the 15-year period from 1 January 2007 to 31 December 2021 at the Monash Children's Hospital neonatal intensive care unit were included. There were 30 293 infants studied, with 31 receiving diazoxide treatment for HH (0.10%). This treatment rate is similar to most published incidence rates, ranging from 0.04% in Singapore(3) to 0.09% in the United States(4). However, notably these published treatment rates are all significantly lower than the 0.36% diazoxide treatment rate reported in the Canadian study where the diazoxide-NEC association was found.(2)

This data suggests that variation in practice exists among international centres in rates of diazoxide use. This variation may be associated with differences in patient population or clinical practice. We will analyse diazoxide treatment rates within our ongoing multi-centre analysis of diazoxide-NEC association. Given this preliminary data, it would be very important to ascertain any association between high rates of diazoxide use and increased NEC incidence.

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Paediatric Post-Prandial Hyperinsulinaemic Hypoglycaemia

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Objective: Postprandial hyperinsulinaemic hypoglycaemia (PPHH) is a well-recognised complication following gastrointestinal surgery and has been less commonly identified idiopathically in paediatric patients. This study reviewed the characteristics, diagnosis, management and outcomes of paediatric idiopathic and surgical PPHH.

Design: Retrospective chart review of children (≤ 18-year-old) at a single tertiary paediatric hospital diagnosed with PPHH between 2003 and 2018.

Method: PPHH cases were identified from the Endocrinology unit database. Demographics, method of diagnosis, management and outcomes were evaluated. Diagnostic tests included standard/prolonged oral glucose tolerance test (OGTT/POGTT), mixed meal test (MMT) and feed test (FT). Demographic data were summarised using standard descriptive statistics. Continuous and discrete variables were analysed using unpaired t-tests. Statistical significance was defined as P value <0.05.

Results: 34 children, 18 idiopathic and 16 post-gastrointestinal surgery, were diagnosed and managed for PPHH. Idiopathic patients had significantly longer duration of symptoms prior to diagnosis (2.54 vs 0.18 years, p=0.0009). 18% of cases required repeat testing to confirm diagnosis. Feed change was the most common intervention, used in 88%. 79% of cases required management with corn-starch or other medications. Acarbose was the most common medication (n=20) and improved glycaemic control in 80%, followed by diazoxide (n= 10) which improved control in 50%. Patients were followed for an average of 4.0 years (±3.4). 18 (53%) had improved glycaemic control and nine (26%) had no hypoglycaemic episodes on treatment. Resolution occurred in 12% taking between 1.54-5.54 years.

Conclusion- This study is the first and largest to describe and compare paediatric surgical and idiopathic PPHH. PPHH diagnosis was often delayed, particularly in idiopathic PPHH, and repeated testing may be required. Acarbose and diazoxide can be considered as medical agents in those with refractory hypoglycaemia, although use was limited by side-effects. PPHH improved in most children, but resolution was uncommon in our population.

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Residual beta-cell function in the first year after diagnosis in children with type 1 diabetes

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Aims

To compare demographic and presenting factors of the Type 1 Diabetes (T1D) patient cohorts from the New Patient Clinic (NPC) at the Queensland Children's Hospital (QCH) in 2020-2021 and the Mater Hospital Clinic (MCH) in 2010.

To investigate factors that may potentially affect residual beta-cell function as determined by Estimated C-peptide (ECP).

Methods

Clinical data collected up to 12 months post diagnosis were obtained for patient cohorts diagnosed from June 2020 to July 2021 at QCH and MCH in 2010. ECP was calculated from a published algorithm at 3,6- and 12-months post diagnosis in the QCH cohort using the variables HbA1c, age, BMI, sex and total daily insulin dose (1). Subjects were stratified by age groups (0-5 years, 5-10 years and 10-15 years) to investigate rates of DKA and changes in ECP over time. Comparisons between groups were analysed using ANOVA and Welch-Forsyth testing.

Results

Demographic data are shown in Table 1. QCH had a higher proportion of DKA (38.6% vs 33.3%) and moderate-severe DKA (21.6% vs 14.1%) at presentation. Children <5 years of age presented with a higher proportion of DKA (44.4%) and severe DKA (22%) compared to older children (21.9% in 5-10 year-olds, 6.3% severe and 38.2% in 10–15-year-olds, 11.8% severe). Younger age at diagnosis correlated with a decreased incidence of partial remission, as defined by ECP, and younger children also had a steeper decline in ECP in the first 12 months after diagnosis (Figure 1).

Conclusions

Compared to an historical cohort, the proportion of DKA presentations at diagnosis is higher.

Younger children (0-5 years) have a higher incidence of DKA and severe DKA than older children.

In younger patients, beta-cell function is lower after diagnosis and declines more rapidly in the first year compared to older children, indicating more aggressive disease.

Table 1. Demographic details of Cohorts

		QCH Cohort 2020/21	Mater Cohort 2010/11
Number of patients		n=103	n=92
Sex distribution		52.3% female	44.6% female
Mean age at diagnosis (years) ± SD		9.97 ± 3.91	8.78 ± 4.04
Proportion less than 5 years old		8.1% (n=9)	14.7% (n=15)
Presented in DKA (HCO_3<15mmol/L)		38.6%	31.5%
Presented in moderate to severe DKA (HCO_3<10mmol/L)		21.59%	14.1%
0-5 years	Presented in DKA Severe DKA	44.4% 22%	
5-10 years	Presented in DKA Severe DKA	21.9% 6.25%	
10-15 years	Presented in DKA Severe DKA	38.2% 11.76%	

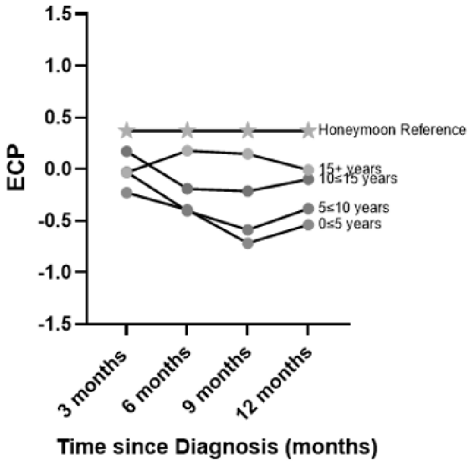

Figure 1. ECP over time by age group.

Data are represented as the mean ECP for each age group. 'Honeymoon' or partial remission is defined by an ECP of 0.37 (corresponding to a stimulated peak C-peptide of 0.3pmol/L)

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Duration of failure to thrive and development of obesity in children with Prader-Willi syndrome

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Prader-Willi syndrome (PWS) is a genetic disorder affecting almost 1 in 16000 live births.(1) It often presents with hypotonia and failure to thrive (FTT) as an infant, with later progression to hyperphagia and obesity.(2) The aim of this study was to examine the association between duration of FTT and age of onset and severity of overweight/obesity in children with PWS. It was hypothesised that longer duration of FTT would be associated with more severe and earlier onset overweight/obesity.

A retrospective pilot study was conducted, examining auxological data from hospital records of children with a genetic diagnosis of PWS attending Paediatric Endocrinology clinics at Sydney Children's Hospital and Royal North Shore Hospital. Height and weight standard deviation (SD) were calculated using CDC 2000 LMS method, with BMI SD included above 24 months age, using the same method. Recovery from FTT was defined as weight SD change by greater than +0.67.(3) Age of BMI SD +1.04 and +1.64 were considered age of overweight and obesity, respectively. Pearson correlation was used to investigate the association between duration of FTT and onset and severity of overweight/obesity. Cases were excluded if data was incomplete.

Auxological data from 29 children with PWS were extracted. Five cases were excluded from analysis due to incomplete data. A Pearson correlation coefficient was computed to assess the linear relationship with between duration of FTT and age of onset of overweight/obesity and found no correlation, r=-0.080, p=0.776 (n=16). There was a non-significant positive correlation between duration of FTT and severity of overweight/obesity, r=0.381, p= 0.066 (n=24).

The current pilot study identified a statistically non-significant trend of longer duration of FTT being associated with more severe degree of overweight/obesity. We can hypothesise that optimising nutritional treatment may have benefits in reducing future obesity risk in this population. Larger studies are required.

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Anti-GAD beyond Beta-Cells: A case of Paediatric Stiff-Person Syndrome in a person with known Type 1 Diabetes Mellitus

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Anti-Glutamic Acid Decarboxylase (GAD) antibodies are associated with not only Type 1 Diabetes Mellitus (T1DM) but also the much rarer Stiff-Person Syndrome.(1,2,3) We present a case of a young man with strongly-positive anti-GAD antibody positive T1DM, who later also developed Stiff-Person Syndrome.

This patient was diagnosed with T1DM (anti-GAD Ab 527 U/mL (<10), anti-IA2 Ab 32 U/mL (<10), anti-ZnT8 Ab <15 U/mL (<15)) at 11 years old. At 14 years old, he developed back pain and back muscle spasm which could be induced by touch and woke him from sleep. He had an abnormal posture and gait with a rounded back and "crab-like gait" maintaining external rotation of the right hip and leg, with toes pointing outward by 40˚, persistent right hip flexion and eversion of the left foot, with loss of lumbar lordosis. He also had visual disturbance. He had normal nerve conduction studies with a normal MRI brain but evidence of intravertebral disc protrusion on MRI spine, with L4/L5 moderate to severe spinal canal stenosis. Following neurological review he was given a preliminary diagnosis of Atypical Stiff-Person Syndrome, and underwent CSF studies. His initial lumbar puncture was traumatic but was positive for raised CSF anti-GAD antibody levels. He is due to undergo spinal stenosis surgery and a repeat lumbar puncture.

This is a rare but important differential diagnosis to consider for back pain and abnormal gait in GAD-positive T1DM. We discuss the diagnosis of Stiff-Person Syndrome and review available treatment modalities including benzodiazepines, baclofen, botulinum toxin, IVIG, rituximab, and haematopoietic stem-cell transplant.

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Children and adolescents with Type 1 Diabetes in New Zealand: an online survey of workforce and outcomes

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Background: Type 1 diabetes (T1D) is one of the most common chronic diseases in children and adolescents in New Zealand and the incidence of the disease continues to rise, both in New Zealand[1] and worldwide[2]. Unfortunately, glycaemic control in New Zealand patients with T1D has generally been poorer than other international cohorts, and there is considerable inequity faced by Māori and the socially deprived[3]. Optimal management requires multidisciplinary input from many health-care professionals (HCP)[4]. However, a previous HCP survey of tertiary centres in Australasia demonstrated that the T1D workforce for 2016 was under-resourced and failed to meet the resource allocation proposed[5]. Five years on, it is important to assess the current paediatric T1D HCP workforce and outcomes in New Zealand.

Aim: To survey the model of care and workforce that manage children with T1D in New Zealand and link to outcomes previously gathered and provide further scope regarding the upcoming reform of district health boards.

Method: A representative for each tertiary diabetes services in New Zealand operating for the 2021 calendar year was asked to participate in an online survey. Respondents were asked the amount of FTE their district health board (DHB) provided for caring for children and adolescents with T1D. Results were compared to previously reported data on regional glycaemic outcomes.

Results: Data collection is still ongoing and will be complete by the November scientific meeting.

Conclusions: Tertiary and regional centres in New Zealand have previously been under-resourced. This study will determine whether the resource allocation for the T1D workforce is appropriate and relate these findings to regional glycaemic outcomes.

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A case of parathyroid adenoma masquerading as familial hypocalciuric hypercalcaemia.

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Hypercalcaemia is a common clinical scenario in Paediatric Endocrinology. We report a case of a delayed diagnosis of parathyroid adenoma in the presence of vitamin D and dietary calcium insufficiency.

A 12-year-old boy, a refugee from Sudan, was referred for evaluation of an incidental finding of asymptomatic hypercalcemia. He was previously treated for tuberculosis. He also had lactose intolerance with significantly restricted dietary calcium intake. There was no family history of hypercalcaemia or endocrine neoplasia. He had normal growth with dark skin complexion and a normal systemic examination.

His initial investigations showed significant hypercalcaemia (corrected calcium 3.27 mmol/l), normal phosphate level with elevated PTH. He had moderate Vitamin D deficiency with high alkaline phosphatase (ALP). There was no radiological evidence of rickets nor subperiosteal bone resorption. The two main differential diagnoses at this stage were primary hyperparathyroidism (PHPT) or familial hypocalciuric hypercalcaemia (FHH) with concomitant vitamin D deficiency. There was no nephrocalcinosis nor evidence of parathyroid adenoma on ultrasound. His initial 24 hour urine calcium excretion showed hypocalciuria raising the possibility of familial hypocalciuric hypercalcaemia. Parental calcium status was unknown as the biological parents were deceased.

He was lost to follow up but represented with persistent hypercalcaemia with further elevation of PTH although he remained asymptomatic. His vitamin D level normalized with treatment but his ALP and PTH remained elevated. His subsequent urine calcium: creatinine ratio showed hypercalciuria and neck ultrasound revealed a left inferior parathyroid adenoma. A focused

parathyroidectomy confirmed benign parathyroid adenoma. His ALP normalized after parathyroidectomy. A genetic panel was negative for MEN1, RET, and CDC73 mutations.

This case illustrates how early biochemical manifestations of parathyroid adenoma could be masked by concomitant Vitamin D and dietary calcium deficiency highlighting the importance of follow up and further review to establish the correct diagnosis.

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Efficacy and safety of once-weekly somatrogon in pediatric subjects with growth hormone deficiency: lack of impact of anti-drug antibodies

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Somatrogon, a long-acting recombinant human growth hormone (GH) is approved in several countries as a once-weekly treatment for children with GH deficiency (GHD). In this phase 3 study, children with GHD received somatrogon or Genotropin. We evaluated the impact of testing positive for anti-drug antibodies to somatrogon (ADA+) on the efficacy and safety of somatrogon.

In the 12-month main study, subjects were randomized to once-weekly somatrogon (0.66 mg/kg/week) or once-daily Genotropin (0.24 mg/kg/week). After the main study, subjects could enroll in an open label extension (OLE), where all subjects received somatrogon (0.66 mg/kg/week). Samples to assess ADAs were collected quarterly during the main study and every 6 months during the OLE. Samples were initially assessed for anti-somatrogon ADAs; ADA+ samples were further assessed for anti-hGH and anti-CTP ADAs. Neutralizing ADAs (NAb) to somatrogon and hGH were also identified. Subjects with ≥1 ADA+ result were compared with subjects with no ADA+ samples (ADA−).

By Month 12, 84/109 (77.1%) somatrogon-treated subjects had ≥1 ADA+ result; most were anti-hGH+. Two of the 84 subjects tested NAb+ for somatrogon. At OLE Month 12, of the 212 subjects (108 subjects had received Genotropin in the main study), 114 (53.8%) had ≥1 ADA+ result; most ADA+ samples were anti-hGH. No additional subjects tested NAb+ for somatrogon while 3 subjects tested NAb+ for hGH. ADA status had no effect on growth parameters (**Table**). Being ADA+ was associated with numerically higher mean IGF-1 SDS values and greater changes from baseline but there was considerable overlap between ADA+ and ADA− subjects (**Table**). The presence of ADAs to somatrogon did not affect the incidence of treatment-emergent adverse events (AEs), serious AEs, or AEs of special interest; there was no association between the incidence of AEs and ADA titer.

ADA+ did not affect the efficacy or safety of somatrogon.

Clinicaltrials.gov:NCT02968004

Table. Growth and ADA results at OLE Month 12

	Soma/Soma		Geno/Soma	
Mean (SD)	**ADA+ (n=91)**	**ADA− (n=18)**	**ADA+ (n=38)**	**ADA− (n=69)**
Annual height velocity, cm/year	8.10 (1.86)	7.47 (1.38)	8.44 (1.94)	8.14 (1.83)
Change in height SDS	1.29 (0.61)	1.19 (0.56)	1.57 (0.74)	1.17 (0.58)
IGF-1 SDS	1.28 (1.29)	0.49 (1.14)	1.48 (1.07)	1.20 (1.21)

Soma/soma refers to subjects who received somatrogon throughout the main study and the OLE. Geno/soma refers to subjects who received Genotropin during the main study and somatrogon during the OLE. ADA, anti-drug antibodies; SDS, standard deviation scores.

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Efficacy and safety of once-weekly somatrogon vs once-daily Genotropin in Asian subjects: post-hoc subgroup analysis of the pivotal phase 3 study

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Somatrogon, a long-acting recombinant human growth hormone (GH), is approved in several countries as a once-weekly treatment for children with pediatric GH deficiency (GHD). A global phase 3 study in pediatric subjects with GHD compared the efficacy and safety of once-weekly somatrogon with once-daily Genotropin. This post hoc subgroup analysis evaluated the efficacy and safety of once-weekly somatrogon vs once-daily Genotropin in Asian subjects.

The open-label phase 3 study randomized 224 subjects 1:1 to receive either once-weekly somatrogon (0.66 mg/kg/week) or once-daily Genotropin (0.24 mg/kg/week) for 12 months. Randomization was stratified by geographic region, peak GH level, and age. The primary endpoint was height velocity (HV) at month 12. This subgroup analysis included 45 Asian subjects from Australia, Great Britain, India, New Zealand, South Korea, Spain, Taiwan, and the USA.

Both treatment subgroups (somatrogon: n=24; Genotropin: n=21) had similar demographic and baseline characteristics. Least squares mean HV at month 12 in the somatrogon and Genotropin group was 10.94 cm/year and 9.56 cm/year, respectively, with a treatment difference of 1.38 (confidence interval [CI]: -0.14-2.89) favoring somatrogon. The lower bound of the two-sided 95% CI of the treatment difference (somatrogon-Genotropin) was −0.14 for this subgroup. This was similar to that for the overall study population (−0.24), in which the efficacy of once-weekly somatrogon was demonstrated to be non-inferior to once-daily Genotropin. The efficacy outcomes of this post hoc analysis are presented below (**Table**). Adverse events were reported in 83.3% of subjects in the somatrogon group and 76.2% of subjects in the Genotropin group. Serious adverse events were recorded for one and two subjects in the somatrogon and Genotropin groups, respectively.

The efficacy and safety results from this analysis of Asian subjects are similar to the overall study population, in which non-inferiority of once-weekly somatrogon to once-daily Genotropin was statistically demonstrated.

Clinicaltrials.gov:NCT02968004

Table: Efficacy outcomes in Asian patients from the pivotal phase 3 study comparing once-weekly somatrogon with once-daily Genotropin

Mean (SD)	Somatrogon (n=24)	Genotropin (n=21)
Height velocity (cm/year)		
6 months	11.23 (3.71)	8.31 (2.10)
12 months	10.73 (3.21)	9.12 (1.91)
Height SDS		
Baseline	-3.84 (1.90)	-4.05 (2.15)
6 months	-3.19 (1.71)	-3.66 (1.91)
12 months	-2.70 (1.52)	-3.21 (1.76)
IGF-1 SDS		
Baseline	-2.14 (1.16)	-2.05 (0.93)
6 months	-0.01 (1.67)	-1.13 (1.14)
12 months	0.50 (1.30)	-1.06 (1.18)

IGF-1: insulin-like growth factor-1; SDS: standard deviation score

Efficacy of once-weekly somatrogon vs once-daily Genotropin in subjects stratified by peak stimulated growth hormone values (≤3, >3 to ≤6.7, and >6.7 ng/mL): post hoc analysis of the pivotal phase 3 trial

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Somatrogon, a long-acting recombinant human growth hormone (GH), is approved in several countries as a once-weekly treatment for children with GH deficiency (GHD). The peak stimulated GH cut-off value for diagnosis of GHD varies according to country-specific guidelines. This post hoc subgroup analysis of the pivotal phase 3 study evaluated efficacy outcomes for subjects stratified by their baseline peak GH values: ≤3, >3 to ≤6.7, and >6.7 ng/mL.

The phase 3 study randomized 224 subjects (peak stimulated GH<10 ng/mL) 1:1 to receive either once-weekly somatrogon (0.66mg/kg/week) or once-daily Genotropin (0.24mg/kg/week) for 12 months. Randomization was stratified by peak GH level, age, and geographic region; the same stratification was used in this post-hoc analysis. Subjects were subdivided into 1 of 3 subgroups based on their peak GH values and then further stratified. The primary endpoint was height velocity (HV) at month 12.

There were 43, 92, and 89 subjects in the ≤3, >3–≤6.7, and >6.7 ng/mL groups, respectively. The least-squares mean HV at month 12 was similar between somatrogon- and Genotropin-treated subjects across all 3 peak GH subgroups (**Table**). This finding was consistent with the overall study population (peak GH <10 ng/mL), wherein mean HV at month 12 was 10.10 and 9.78 cm/year for somatrogon- and Genotropin-treated subjects, respectively, with a treatment difference of 0.33 favoring somatrogon. Efficacy results are presented below (**Table**). The increase in IGF-1 SDS from baseline to month 12 was higher in somatrogon-treated subjects compared with Genotropin-treated subjects across all 3 subgroups. Mean bone maturation at 12 months was similar between somatrogon- and Genotropin-treated subjects across all 3 subgroups.

This post-hoc analysis showed that somatrogon- and Genotropin-treated subjects had similar growth parameters. These findings are consistent with those from the overall study population, in which non-inferiority of once-weekly somatrogon to once-daily Genotropin was statistically demonstrated.

Clinicaltrials.gov:NCT02968004

Table: Efficacy outcomes of once-weekly somatrogon vs once-daily Genotropin in subjects stratified by peak stimulated GH values

Efficacy endpoint (LS means estimate)	Somatrogon	Genotropin	Treatment mean difference (95% CI)
Annual HV at 12 months (cm/year)			
≤3 ng/mL[a]	12.72	11.33	1.39 (-0.35, 3.13)
>3 to ≤6.7 ng/mL[b]	8.42	8.74	-0.31 (-1.12, 0.49)
>6.7 ng/mL[c]	9.53	9.22	0.32 (-0.57, 1.20)
Annual HV at 6 months (cm/year)			
≤3 ng/mL[a]	13.58	12.33	1.25 (-1.05, 3.56)
>3 to ≤6.7 ng/mL[b]	9.21	9.01	0.21 (-0.81, 1.22)
>6.7 ng/mL[c]	9.49	8.87	0.62 (-0.26, 1.51)
Change in height SDS at 12 months			
≤3 ng/mL[a]	1.54	1.33	0.22 (-0.17, 0.60)
>3 to ≤6.7 ng/mL[b]	0.61	0.66	-0.06 (-0.20. -0.09)
>6.7 ng/mL[c]	0.75	0.71	0.04 (-0.12, 0.19)
Change in height SDS at 6 months			
≤3 ng/mL[a]	0.90	0.77	0.13 (-0.14, 0.40)
>3 to ≤6.7 ng/mL[b]	0.40	0.37	0.03 (-0.06, 0.13)
>6.7 ng/mL[c]	0.41	0.35	0.06 (-0.02, 0.14)

CI: confidence interval; HV: height velocity; GH: growth hormone; LS: least squares; SDS: standard deviation score

[a] N=22 for somatrogon; N=21 for Genotropin;

[b] N=45 for somatrogon; N=47 for Genotropin

[c] N=42 for somatrogon; N=47 for Genotropin

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Persistent, diazoxide-unresponsive hyperinsulinaemic hypoglycaemia in a child with Trisomy 21

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Hyperinsulinaemic hypoglycaemia (HI) is associated with genetic syndromes and a rare feature in aneuploidies (45,XO; mosaic Trisomy 13). The Exeter Genomics Laboratory reported higher-than-expected referrals of individuals with HI and Trisomy 21. (1) The clinical phenotype (n=11) was variable with high burden of non-genetic HI risk factors. There are single reports of Trisomy 21 with insulinoma or Beckwith-Wiedemann syndrome (BWS).

Aim

To describe a case of persistent, diazoxide-unresponsive HI in a child with Trisomy 21.

Method

Single retrospective case report.

Results

A female with antenatal diagnosis of Trisomy 21 was delivered at 34^{+4} weeks gestation due to intrauterine growth retardation. She had postnatal CMV infection, congenital hypothyroidism and a perimembranous ventriculoseptal defect (repaired at 3 months). She was dependent on gastrostomy feeds (continuous/bolus) until surgical repair of a long-gap oesphageal atresia (gastric tube with pyloroplasty) at 6 months. After a complicated peri-operative course, she progressed very slowly from gastrostomy/jejunostomy to oral feeds.

The child presented with HI at 17 months: plasma glucose 1.9mmol/L, insulin 11mU/L, low beta-hydroxy butyrate and elevated glucose infusion rate. She was unresponsive to oral Diazoxide (15mg/kg/day) and immediate-release subcutaneous Octreotide (25mcg/kg/day).

Targeted next-generation sequencing of 20 HI genes did not identify any pathogenic variants. BWS genetics was negative. 18F-DOPA PET/CT scan revealed diffuse uptake.

Safe fasting tolerance after weaning of continuous intravenous dextrose and gastrostomy feeds has been achieved with escalating monthly Lanreotide (60mg-2 months; 90mg–3 months).

Discussion

This adds to limited reports of the co-existence of HI with Trisomy 21. Other cases were perinatal, transient and not requiring diazoxide or diazoxide-responsive. Early dependence on gastrostomy and continuous/frequent feeds may have masked HI. Post-operative reduced stomach capacity and rapid gastric emptying may have contributed intermittently to ‘dumping’. Any future pancreatectomy will be complicated by past gastroesophageal surgery.

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Characteristics at presentation of neonates with Congenital Adrenal Hyperplasia secondary to 21-hydroxylase deficiency identified on a second-tier steroid profile newborn screening programme

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Aim

To describe the biochemical, genetic, and clinical characteristics of a cohort of patients with congenital adrenal hyperplasia (CAH) secondary to 21-hydroxylase deficiency identified on a second-tier steroid profile newborn screening (NBS) programme.

Methods

A retrospective medical record review of neonates identified by a state-wide NBS programme between August 2020 to April 2022 was undertaken. Data collected included sex, gestational age, birth weight, timing of notification, CYP21A2 gene analysis, and clinical assessment and biochemistry at initial presentation.

Results

There were 18 NBS notifications however 2 extreme premature neonates died before confirmatory testing. The mean age at notification of 2nd tier results was 11 days (range 3-16). Mean age of clinical investigation for CAH was 7 days (range 1-15) with 11 as a direct result of NBS notification (7 males, including 2 sets of twins), 2 due to known 21-hydroxylase deficiency in a sibling, and 3 due to clinical concerns regarding ambiguous genitalia.

Equal males and females were identified, with the majority of females having minimal virilisation (6/8 Prader score of 2, 2/8 Prader score ≥ 3). Most neonates (12/16) were asymptomatic at initial assessment, with 1 male presenting in adrenal crisis, and 3 being described as lethargic (2 males, 1 female). Six neonates (4 males) had evidence of salt-wasting on initial presentation. One premature female (28 weeks gestation) had evidence of salt-wasting as early as day 3.

Fourteen neonates had mutations in CYP21A2 consistent with classical CAH while one had a genotype consistent with non-classical CAH, and has not commenced treatment. The remaining baby has not had CYP21A2 gene analysis but has a salt-wasting phenotype.

Table: Characteristics of neonates identified on 2nd-tier steroid profile newborn screening programme for congenital adrenal hyperplasia secondary to 21-hydroxylase deficiency

	Laboratory data from newborn screening programme					Clinical, biochemical, and genetic data on initial clinical review									
	Sex	Gestation (days)	Birth Weight (kg)	Age at first sample (days)	Age at initial notification (2nd tier)	Reason for clinical review	Clinical status	Prader score	Age (days)	Sodium (mmol/L) [135-145]	Potassium (mmol/L) [3.5-6.2]	Glucose (mmol/L) [3.0-7.8]	Bicarbonate (mmol/L) [16-29]	CAH Genotype	Age at treatment initiation (days)
1	M	266	3.46	3	10	Sibling with CAH	Adrenal crisis	-	9	121	8.4	4.4	18	SV/SW	9
2	F	288	3.51	2	3	Ambiguous genitalia	Well	3-4	3	140	4.2	6.3	22	SW	3
3	F	273	3.70	3	7	NBS notification	Lethargic	2	8	127	5.5	7.8	19	SV/SW	8
4	F	199	1.04	2	8	NBS notification	Respiratory Distress	2	3	122	5.6	5.3	21	SV/SW	8
5	F	266	2.84	2	11	Ambiguous genitalia	Well	5	1	136	4.6	2.8	18	SV/SW	5
6	F	273	3.89	4	12	Ambiguous genitalia	Well	2	1	141	4.7	2.3	20	SV/SW	3
7	F	273	3.83	3	16	Ambiguous genitalia	Well	2	1	138	5.0	6.6	24	N/A	5
8	M	280	3.80	2	8	NBS notification	Well	-	6	136	5.7	3.9	25	SV/SW	6
9	M	259	2.33	2	6	NBS notification	Well	-	7	139	5.2	3.5	24	NC	Not commenced
10	M	245	2.95	2	14	NBS notification	Lethargic	-	15	129	5.9	3.8	21	SV/SW	15
11	M	245	2.92							122	5.8	5.6	20		
12	M	266	3.56	2	8	NBS notification	Well	-	9	122	8.1	5.9	18	SW	9
13	F	273	3.37	3	13	NBS notification	Well	2	13	136	6.3	3.5	25	SV/SW	14
14	F	266	3.79	3	12	NBS notification	Well	2	6	135	6.1	3.4	22	SV/SW	6
15	M	266	2.61	3	15	NBS notification	Well	-	7	135	6.2	5.4	29	SW	7
16	M	266	2.55							135	6.0	5.7	23		

Conclusion

Given that typical clinical features of CAH may not be evident in the first week of life, especially in premature infants, early notification of NBS results are essential to avoid presentation with adrenal crisis.

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Neonatal severe hyperparathyroidism presenting with life-threatening hypercacaemia in a remote hospital treated successfully with medical and surgical management

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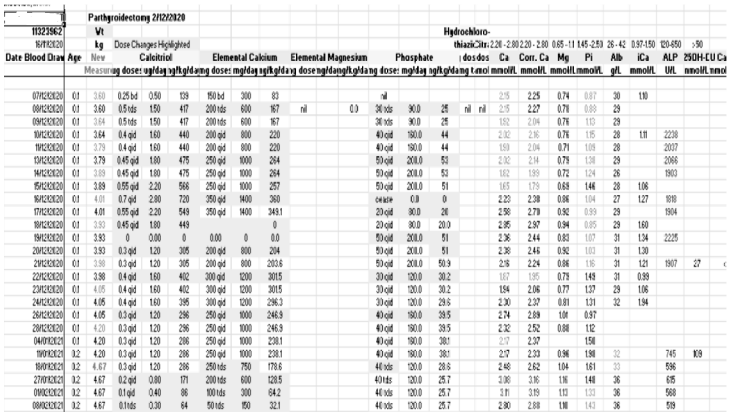

		Parthyroidectomy 2/12/2020																												
11223962		Wt														Hydrochloro-														
16/12/2020		kg	Dose Changes Highlighted													thiazideCitrate	2.26 - 2.80	2.20 - 2.80	0.65 - 1.1	1.45 - 2.53	2.6 - 4.2	0.97-1.50	120-850	>50						
Date Blood Draw	Age	New	Calcitriol			Elemental Calcium			Elemental Magnesium			Phosphate				dosdos	Ca	Corr. Ca	Mg	Pi	Alb	iCa	ALP	25OH-LC	Ca					
		Measurm	g dose	ng/dag	ng/kg/d	amg dose	mg/dag	ng/kg/d	amg dose	ng/dang	g/kg/da	ng dose	mg/dag	ng/kg/d	amg	t.xnol	mmol/L	mmol/L	mmol/L	mmol/L	g/L	mmol/L	U/L	nmol/L	nmol/L					
07/12/2020	0.1	3.60	0.25 bd	0.50	139	150 bd	300	83				nil					2.15	2.25	0.74	0.87	30	1.10								
08/12/2020	0.1	3.60	0.5 tds	1.50	417	200 tds	600	167	nil		0.0	36 tds	90.0	25	nil	nil	2.15	2.27	0.78	0.88	29									
09/12/2020	0.1	3.64	0.5 tds	1.50	417	200 tds	600	167				36 tds	90.0	25			1.92	2.04	0.76	1.13	29									
10/12/2020	0.1	3.64	0.4 qid	1.60	440	200 qid	800	220				40 qid	160.0	44			2.32	2.16	0.76	1.15	28	1.11	2238							
11/12/2020	0.1	3.79	0.4 qid	1.60	440	200 qid	800	220				40 qid	160.0	44			1.59	2.04	0.71	1.09	28		2027							
13/12/2020	0.1	3.79	0.45 qid	1.80	475	250 qid	1000	264				50 qid	200.0	53			2.32	2.14	0.79	1.38	29		2066							
14/12/2020	0.1	3.89	0.45 qid	1.80	475	250 qid	1000	264				50 qid	200.0	53			1.82	1.99	0.72	1.24	26		1903							
15/12/2020	0.1	3.89	0.55 qid	2.20	566	250 qid	1000	257				50 qid	200.0	51			1.65	1.79	0.69	1.46	28	1.06								
16/12/2020	0.1	4.01	0.7 qid	2.80	720	350 qid	1400	360				on site	0.0	0			2.23	2.38	0.86	1.04	27	1.27	1918							
17/12/2020	0.1	4.01	0.55 qid	2.20	549	350 qid	1400	349.1				20 qid	80.0	20			2.56	2.79	0.92	0.99	29		1904							
18/12/2020	0.1	3.93	0.45 qid	1.80	449			0				20 qid	80.0	20.3			2.85	2.97	0.94	0.85	29	1.60								
19/12/2020	0.1	3.93	0	0.00	0	0.00	0	0.0				50 qid	200.0	51			2.36	2.44	0.83	1.07	31	1.34	2225							
20/12/2020	0.1	3.93	0.3 qid	1.20	305	200 qid	800	204				50 qid	200.0	51			2.36	2.46	0.82	1.03	31	1.30								
21/12/2020	0.1	3.98	0.3 qid	1.20	305	200 qid	800	200.6				50 qid	200.0	50.9			2.18	2.24	0.86	1.16	31	1.25	1907	27						
22/12/2020	0.1	3.98	0.4 qid	1.60	402	300 qid	1200	301.5				30 qid	120.0	30.2			1.67	1.95	0.79	1.49	31	0.99								
23/12/2020	0.1	4.05	0.4 qid	1.60	402	300 qid	1200	301.5				30 qid	120.0	30.2			1.84	2.06	0.77	1.37	29	1.06								
24/12/2020	0.1	4.05	0.4 qid	1.60	395	300 qid	1200	296.3				30 qid	120.0	29.6			2.30	2.37	0.81	1.31	32	1.04								
25/12/2020	0.1	4.05	0.3 qid	1.20	296	250 qid	1000	246.9				40 qid	160.0	39.5			2.74	2.89	1.01	0.97										
26/12/2020	0.1	4.20	0.3 qid	1.20	296	250 qid	1000	246.9				40 qid	160.0	39.5			2.32	2.52	0.88	1.12										
04/01/2021	0.1	4.20	0.3 qid	1.20	286	250 qid	1000	238.1				40 qid	160.0	38.1			2.17	2.37		1.58										
19/01/2021	0.2	4.20	0.3 qid	1.20	286	250 qid	1000	238.1				40 qid	160.0	38.1			2.17	2.33	0.96	1.98	32		745	109						
19/01/2021	0.2	4.67	0.3 qid	1.20	286	250 tds	750	178.6				40 tds	120.0	28.5			2.46	2.62	1.04	1.61	33		596							
27/01/2021	0.2	4.67	0.2 qid	0.80	171	200 tds	600	128.5				40 tds	120.0	25.7			3.06	3.16	1.16	1.48	36		615							
01/02/2021	0.2	4.67	0.1 qid	0.40	86	100 tds	300	64.2				40 tds	120.0	25.7			3.11	3.19	1.13	1.33	36		568							
08/02/2021	0.2	4.67	0.1 tds	0.30	64	50 tds	150	32.1				40 tds	120.0	25.7			2.90	2.88	1.16	1.43	36		519							

Neonatal severe hyperparathyroidism is a rare disorder that presents with potentially life-threatening hypercalcaemia. This case report aims to describe the management complexities of this condition. A Day 3, full-term male presented to a remote hospital in New South Wales with tachypnoea and hypoxia and was found to be severely hypercalcaemic, with a calcium of 4.32mmol/L (1.89-2.8) and an elevated PTH of 112pmol/L (1.6-6.9). X-rays showed marked osteopaenia, coarsened thoracic cage trabeculae, long bone bowing and rib fractures. With telephone guidance from the paediatric endocrinologist, the general paediatrician commenced IV hyperhydration, frusemide 2mg/kg BD, prednisone 2mg/kg/day and pamidronate 1mg/kg. Following NETS transfer to a tertiary hospital, subcutaneous calcitonin 2u/kg q6hourly and oral cinacalcet (an allosteric calcium sensing receptor activator) 2mg/kg/day were administered as pre-arranged by the ICU team. Calcium levels responded from an initial peak of 5.4mmol/L to 2.1mmol/L on day 4 of treatment, but rebounded to 3.9mmol/L on cessation of IV fluids and reintroduction of calcium-containing feeds. Total parathyroidectomy was performed on day 16 of life. Histopathology showed parathyroid hyperplasia, weight 31-52mg, similar to adult size. Genetic testing revealed compound heterozygosity for two likely pathogenic variants involving the calcium sensing receptor (c. 190A>G; p. (Asn64Asp) on chr3: 122257085 and c. 101T>C; p.(Leu34Pro) on chr3:122254290). Postoperative hypocalcaemia from hungry bones was anticipated, therefore calcitriol 284ng/kg/day and oral calcium 150mg BD were commenced. ALP peaked at 2238U/L (120-550). Calcitriol dose peaked at 720ng/kg/day and calcium dose at 360mg/kg/day, but fell after 50 days post-surgery to 296ng/kg/day and 246mg/kg/day, respectively (See image 1). Maintenance dosing of these medications has continued at 20-40ng/kg/day for calcitriol and 5-10mg/kg/day for calcium. The child's development is normal and there has been clinical resolution of long bone bowing. This case demonstrates successful emergency medical and surgical management of this life-threatening disease.

Three Year Follow Up of Two Siblings Treated with IGF-1 for Growth Hormone Insensitivity Syndrome due to a Novel Mutation of Intron 4 of the Growth Hormone Receptor

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Growth hormone insensitivity syndrome (GHIS) results from absent/attenuated growth hormone response on its receptor (GHR). It causes severe post-natal growth failure, hypoglycaemia, and obesity, high circulating growth hormone and low insulin-like growth factor 1 (IGF-1). We present two female siblings (S1 and S2) with GHIS due to the same novel mutation of the GHR and their response to IGF-1 over the first 3 years of treatment. S1 had normal size at birth (weight 3.46kg (77th

centile), length 45cm (2^{nd} centile)), but developed severe early growth failure, and was referred to endocrinology at 6 months of age. She had high circulating GH (67.5mIU/L, RI 6-30) and low IGF-1 (< 2.0 nmolL, RI 7.0-43.3). IGF-1 generation testing for suspected GHIS was consistent with the diagnosis (Table 1). Genomic sequencing detected a novel mutation of intron 4 of the GHR (GHR c.266+68T>G) with a predicted frameshift and early protein termination. S2 also developed post-natal growth failure due to the same GHR mutation. Both girls were commenced on IGF-1 treatment (Mecasermin 150mcg/kg/day) as a once daily s.c. injection (Laron, 2014). Blood and interstitial glucose monitoring were instituted. Growth velocities of S1 and S2 improved from 5.8cm/year (3^{rd} centile) to 9.3cm/year (>97^{th} centile) and 3cm/year (<3^{rd} centile) to 7.7cm/year (>50^{th} centile), respectively. The parents reported an improvement in energy, strength and appetite. Over the 3 years of treatment to date there have been ongoing improvements in height z scores from -6.6 to -4.0 for S1 and from -6.3 to -4.7 for S2. Despite dietary precautions, weight has increased disproportionately consistent with the GHIS phenotype, with current BMIs of 22.6kg/m^2 (98^{th} centile) for S1 and 16.5kg/m^2 (76^{th} centile) for S2. Hypoglycaemia has not increased on IGF-1 treatment. These cases highlight the important benefits of IGF-1 and difficulties encountered in the management of children with GHIS.

Table 1. Results of IGF-1 generation testing. GH given at 0.25mcg/day for 3 days and IGF-1 measured at baseline and on day 4.

	Baseline	Day 4
GH (mIU/L)	148	-
IGF-1 (nmol/L)	<2	<2
IGF-BP3 (mg/L)	-	<0.5

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An unusual case of 46,XY DSD with an overgrowth syndrome

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Differences of Sex Development (DSD) are a common congenital condition with an estimated frequency of 1%. However, 50% of cases remain undiagnosed genetically.

Aims: To report a patient with a 46,XY DSD and an overgrowth syndrome of unknown genetic etiology.

Methods: Clinical phenotype was examined and hormonal profile was evaluated. DNA was extracted for whole exome sequencing.

Results: A 13-year-old Mexican patient with a 46,XY DSD and an overgrowth syndrome. Atypical genitalia was diagnosed since birth characterised by micropenis, subglandular hypospadias, bilateral inguinal cryptorchidism, and hypoplastic scrotum. Müllerian ducts were absent by ultrasonography. Hormonal evaluation at 9 years of age revealed hypergonadotrophic hypogonadism. When the patient was ten months old, tall stature and a developmental delay were detected followed by accelerated growth velocity and advanced bone age. The patient also presented with camptodactyly of the third, fourth, and fifth finger, submucous cleft palate, speech delay and mild intellectual disability. Facial dysmorphic features include broad forehead, hypertelorism, down slanting palpebral fissures, wide nasal bridge, long philtrum and large ears. Brain magnetic resonance imaging showed periventricular hyperintensities and enlargement of the subarachnoid space.

Among overgrowth syndromes, facial dysmorphisms and intellectual disability are commonly shared while camptodactyly and advanced bone age are unique features of Weaver/Weaver-like syndrome. However, the co-occurrence of Weaver like syndromes with atypical genitalia including micropenis and hypospadias has not been reported yet. WES analysis revealed no pathogenic or likely pathogenic variants among 82 known and candidate DSD target genes, as well as PRC2 complex genes (*EZH2, EED, SUZ12*).

Conclusion: The patient with a 46,XY DSD and an overgrowth syndrome remains genetically undiagnosed by this initial evaluation. Therefore, differential diagnoses should be considered, and a further molecular/genetic analyses are underway.

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Novel Y chromosome-PML nuclear body disrupted in ATR-X syndrome model

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ATRX mutations cause X-linked α-thalassemia/mental retardation syndrome (ATR-X) characterized by intellectual disability, developmental delay, facial dysmorphism, seizures, and genital abnormalities. To study genital abnormalities, we deleted *Atrx* from mouse testicular Sertoli cells; knockout mice develop small testes with discontinuous unbranched tubules and apoptosis of Sertoli cells during fetal life.

Aims: To investigate the mechanism underlying the ATR-X syndrome gonadal phenotype.

Methods: Mouse embryonic gonads from wildtype and *Atrx* knockout E16.5 embryos were processed sectioned and Immunofluorescence (IF) or Immuno-FISH performed followed by quantification using ImageJ.

Results: PML nuclear bodies (PML-NBs) are spherical structures in the nucleus with 1 micron in diameter; they vary in protein composition and have been implicated in gene expression, chromatin assembly, telomere lengthening and DNA repair. Wildtype Sertoli cell nuclei showed unexpectedly two types of PML-NBs that contain ATRX, its binding partner DAXX and heterochromatin protein HP1a. Firstly, classic PML-NBs and secondly a single novel “GATA4 PML-NB” that strongly expressed the somatic cell marker GATA4. Immuno-FISH showed that the GATA4 PML-NBs localize on the short arm of the Y chromosome. In knockout mice, ATRX-deficient PML-NBs showed loss of DAXX and HP1a, suggesting a failure in heterochromatin formation. In addition, ~30% of ATRX-deficient Sertoli cells showed GATA4 PML-NBs that were 2-3 times larger, and 84% of them (vs 4.1% in control) showed DNA damage (gH2Ax staining) either within the GATA4 PML-NBs or widespread throughout the nucleus of apoptotic cells.

Conclusion: A novel PML-NB, bearing GATA4 and the short arm of the Y chromosome, was identified in Sertoli cells. Within these PML-NBs, ATRX mediates chromatin condensation of the Y chromosome, which is required for Sertoli cell survival, and testis development**.**

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Novel FGF9 variant reveals a role for dimerization in sex determination

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46,XY gonadal dysgenesis (GD) is a Disorder/Difference of Sex Development (DSD) that can present neonatally as ambiguous genitalia or at puberty as a phenotypic female lacking secondary sexual characteristics and primary amenorrhea. Only 43% of 46,XY DSD cases receive a molecular diagnosis. In mice, Fibroblast growth factor 9 (FGF9) is an important component of the male sex-determining pathway. While pathogenic *FGF9* variants that cause skeletal defects in humans show gonadal alterations in mouse models, a role for *FGF9* in human testicular development is not known. Here, we describe an *FGF9* missense variant, NM_002010.2:c.583G>A:p.(Asp195Asn), in a female patient with isolated 46,XY GD. Using biochemical and cell-based approaches, we demonstrate that the D195N variant disrupts FGF9 protein homodimerisation and FGF9-heparin-binding, reducing Sertoli cell proliferation. XY $Fgf9^{D195N/D195N}$ mice showed a mild gonadal phenotype (disrupted testis cords), while XY $Fgf9^{D195N/-}$ mice showed male-to-female sex reversal (ovotestis). The incomplete penetrance and variable expressivity of the D195N variant in mice suggested an oligogenic basis for the patient's DSD. Further detailed analysis of the patient and parental exome revealed variants in genes expressed in human embryonic Sertoli cells at the time of sex determination, which may contribute to the gonadal anomalies. Our findings therefore suggest that disruption to FGF9 signaling may, in combination with other variants, compromise human testis determination and lead to 46,XY DSD.

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Sirolimus as a Novel Agent for the Treatment of Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED) in Children

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Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED) is a rare, monogenic disease caused by mutations in the Autoimmune Regulator (AIRE) gene, resulting in loss of central and peripheral immune self-tolerance. It is clinically defined by the presence of two of the classic triad; chronic mucocutaneous candidiasis (CMC), hypoparathyroidism and adrenal insufficiency, or one of these in a sibling of an APECED patient (1). We present two patients with markedly differing presentations of APECED at age 2years and 4years, who have both been successfully managed with the immunomodulatory agent Sirolimus. It was commenced in one patient due to critical illness with brainstem encephalitis, and in the other due to growth failure and rapidly progressive onset of autoimmune endocrinopathies. Their markedly differing features and clinical courses illustrate the heterogenous nature of APECED and the inadequacy of the current diagnostic criteria. By broadening the criteria to include some of the more common features outside of the classic triad, the median age at diagnosis can be brought forward and significant morbidity and mortality associated with life threatening endocrine crises can be mitigated(2).

Whilst Sirolimus has been widely used as an immunoregulatory agent in many conditions (eg.transplant medicine), it has not been recognised as a preferred agent in APECED(3). Both patients have exhibited significant improvement in symptom control, well-being and stability of their clinical course within months of commencement of therapy. As yet, neither of our cases have experienced adverse side effects from the Sirolimus, likely in part by aiming for trough levels well below that used in other autoimmune conditions.

We propose that the diagnostic criteria for APECED should be broadened so as to avoid diagnostic delay, and that Sirolimus should be considered early in the disease course to achieve clinical stability, optimise wellbeing, and potentially modulate the rate and character of disease progression.

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Ketotic Hyperinsulinemic Hypoglycemia in a Patient with Acute Lymphoblastic Leukemia on 6-Mercaptopurine

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6-MP is the backbone of treatment of children diagnosed with acute lymphoblastic leukaemia. Severe hypoglycemia induced by 6-mercaptopurine (6-MP) is a very rare occurrence. Hyperinsulinism is observed in several case reports on literature review. A 10-year-old boy presented with severe symptomatic hypoglycemia that was eventually found to be associated with 6-mercaptopurine treatment. Fasting sample revealed elevated serum insulin, C-peptide, cortisol with ketosis. His liver transaminases were raised. He was able to fast longer, with marked reduction of serum insulin, C-peptide and liver transaminases after stopping 6-MP upon serial fasting tests. No hypoglycemia symptoms reported following reduction of 6-MP and split dosing. This case has verified the evidence of the mechanism by which 6-MP causes hypoglycemia in a small number of individuals through hyperinsulinaemia. This is reversible after stopping 6-MP and adjusting its dosing.

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Denosumab for Williams syndrome associated hypercalcaemia

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Aim

To describe denosumab use for hypercalcaemia associated with Williams syndrome (WS) and renal impairment.

Methods

Case report.

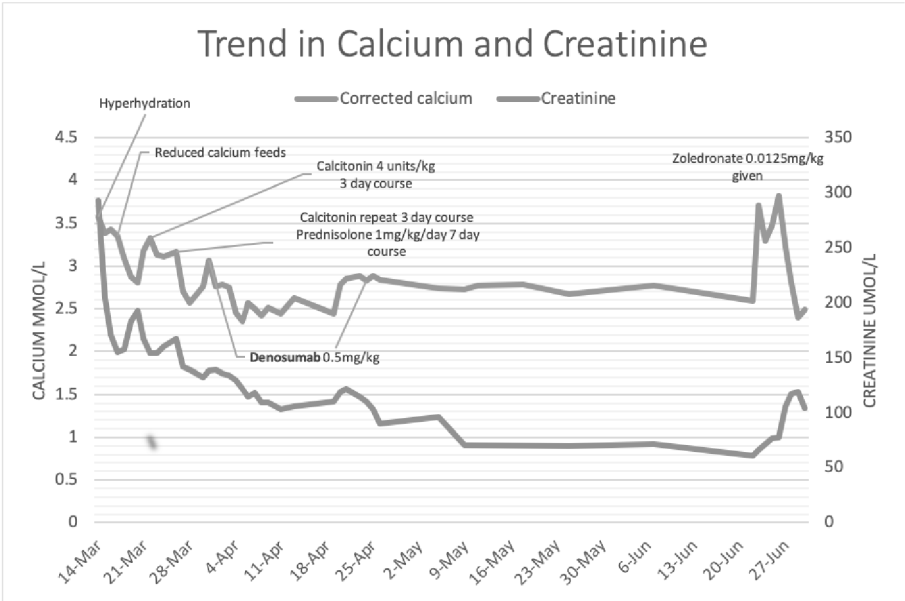
Results

A 20-month-old boy (AB) presented to his local hospital with a respiratory tract infection. He had known WS, diagnosed after identification of severe supravalvular aortic stenosis aged 13 months.

At presentation, AB had severe hypercalcaemia and renal impairment (Table)

Investigations	Results	Reference range
Corrected calcium	**3.58**	2.2-2.7 mmol/L
Creatinine	**293**	<36 umol/L
PTH	**0.8**	2.0-9.3 pmol/L
25-Hydroxy-Vitamin D	90	50-150 nmol/L
1,25 Dihydroxy Vitamin D	**25**	48-190 pmol/L
Urine calcium:creatinine ratio	**4.8**	-
Calculated eGFR	**20**	>90 ml/min/1.73m^2
Renal ultrasound	Bilateral renal nephrocalcinosis involving the renal pyramids	

The impression was that of chronic idiopathic hypercalcaemia, consistent with his diagnosis of WS. AB was commenced on intravenous hyperhydration, mindful of impaired renal function. Treatments trialled (Figure) included reduced calcium feeds, calcitonin, and prednisolone. While there was improvement in serum calcium levels, none of these treatments achieved normocalcaemia.



AB's hypercalcaemia rapidly improved following administration of 0.5mg/kg denosumab. This was repeated after 4 weeks due to rising serum calcium. Eight weeks later his eGFR had improved to 40ml/min/1.73m[1], however he subsequently experienced rebound hypercalcaemia. This was treated effectively with zoledronate given his improved renal function.

Mild to moderate idiopathic hypercalcaemia is well described in WS.[2] Bisphosphonates have been successfully used to treat WS associated hypercalcaemia, however are not recommended with comorbid renal impairment due to risk of acute tubular necrosis[3].

Denosumab is a monoclonal antibody with affinity for nuclear factor-kappa ligand (RANKL), mimicking the inhibitory effect of OPG[4]. It is used in hypercalcaemia management - primarily in adults with malignancy or hyperparathyroidism[5,6]. As denosumab is not renally cleared, it is considered safe in patients with renal impairment. There is a paucity of data in its use in paediatric hypercalcaemia, and its duration of action is unclear. Concerns around rebound hypercalcaemia and bone and tooth development persist.

Conclusion

Denosumab is a novel treatment for paediatric hypercalcaemia when bisphosphonates are contraindicated. It has not to our knowledge been previously described in WS.

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Hyponatraemia in diabetic ketoacidosis (DKA)

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Aim

To describe an unusual presentation of autoimmune adrenal insufficiency

Methods

Case report.

Results

An 8 year old presented to the ED with kussmaul breathing, fevers and vomiting. He was a previously well child, and had a significant family history for maternal T1D. An initial diagnosis of SARS-CoV-2 infection was made, however blood gas demonstrated hyperglycaemia, ketosis, a high anion gap metabolic acidosis and moderate-severe hyponatraemia consistent with severe DKA.

The patient was given a preliminary bolus of 10ml/kg of 0.9% saline, with subsequent intravenous fluids of 150ml/hour (calculated as maintenance and 7.5% dehydration replacement over 48 hours). As per protocol he was also commenced on an insulin infusion.

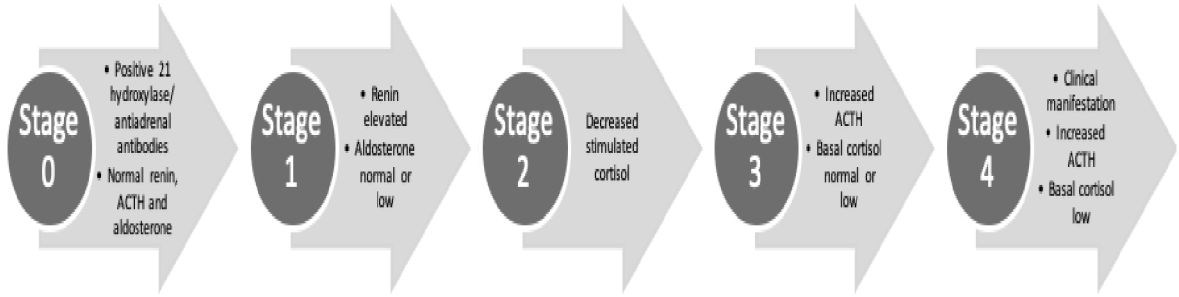
Over the following 48 hours his hyperglycaemia and acidosis corrected and he was transitioned to subcutaneous insulin. Subsequently, his hyponatraemia, which had almost normalised, began to worsen and measurement of urine sodium suggested renal loss of sodium.

Further investigations revealed an elevated renin and low aldosterone with normal ACTH and cortisol. Adrenal antibodies titres were significantly elevated. He was commenced on mineralocorticoid replacement and diagnosed with an early presentation of autoimmune adrenal insufficiency (ADI). Table 1 summarises his clinical course and investigations.

Table 1: Relevant diagnostic investigations performed during clinical course	**Reference Interval**	**At presentation**	**+ 18 hours**	**+ 50 hours**	**+96 hours**
pH	7.35-7.45	7.02	7.29		
Bicarbonate (mmol/L)	17-30	6	10	4	26
PCO2 (mmHg)	35-45	22	21		
Sodium (mmol/L)	133-144	119	128	133	131
Corrected sodium (mmol/L)[c]		127	131	135	131
Potassium (mmol/L)	3.6-5.3	5.7	4	5.7	5.6
Chloride (mmol/L)	97-110	99	108	98	97
Glucose (mmol/L)	3.0-7.8	24.3	11.9	10.7	5.3
Anion gap (mmol/L)	Apr-13	14	10	7	8
Cortisol (nmol/L)	60-570	1130	729		
ACTH (ng/L)	Oct-50		16		
Urinary sodium concentration (mmol/L)				295	
Aldosterone (pmol/L)	100-1500			48	295
Renin (mass) (mU/L)	3-40 (Ambulatory)			593	2641
Aldosterone/Renin ratio	(<55)			<1	<1
Comment		Hypovolemic, critically unwell	Early morning samples. On intravenous insulin and 0.9% saline (with dextrose and KCL)	Transitioned to subcutaneous insulin injections, and oral salt supplementation.	Following cessation of sodium supplementation for 24 hours

ADI is characterised by development of anti-adrenal or 21-hydroxylase antibodies directed against the adrenal cortex. It has been associated with autoimmune polyglandular syndrome (APS) and in our patient, a diagnosis of APS 2 has been made.

ADI demonstrates a classic natural history of 5 stages, in which there is progressively increasing adrenal dysfunction (Figure) (1). Our patient was diagnosed as likely being in Stage 1 ADI, with multiple risk factors to continue progression including male gender and a diagnosis of APS 2 (2).



Conclusion

This case demonstrates the importance of considering other cause of electrolyte disturbance in patients with DKA.

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Neonatal hypothyroidism: factors associated with the diagnosis of transient vs permanent hypothyroidism and management at a tertiary Australian centre

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Aim

Congenital hypothyroidism, when identified in hospitalised neonates, may be either transient (TCH) or permanent (PCH). We aimed to describe the investigation and management of these infants and identify associated variables.

Methods

Data were extracted from handover documents and electronic medical records on infants with suspected hypothyroidism between 1/7/2019 and 30/6/2021.

Results

Of 124 infants (57% male) who met inclusion criteria, 43.3% were premature (<37 weeks gestation). There was a high rate of infants with common neonatal comorbidities including jaundice (61.9%), respiratory distress (64.1%) and hypoglycaemia (37.5%). 45.1% had cardiac anomalies and 18% had a syndrome such as trisomy 21, 22q11, or other genetic abnormality.

Median TSH at referral was 10.5mIU/L [IQR 3.8-24] at a median age of 28.5 days. In those who had presumed TCH, thyroid function tests (TFTs) demonstrated both a significantly lower TSH (7.32mIU/L [3.3-12.8] vs 83.8mIU/L in PCH [9.8-270] (p=0.01)) and higher FT4 (12.4pmol/L (10.15-16.75) vs 8.6pmol/L (8.1-13) vs (p=0.017)). 80% of nuclear medicine scans performed were abnormal, demonstrating either dyshormonogenesis or dysgenesis, compared to only 29.3% of thyroid ultrasounds revealing abnormalities.

The working diagnosis was TCH in 73/117 (62.4%), and PCH in 25/117 (21.4%). NBS TSH levels were significantly higher in those with presumed PCH. 36% of those with presumed PCH were not identified through the NBS and were diagnosed through formal TFTs for clinical indications in the hospitalised infants.

Levothyroxine, at an average dose of 8.7mcg/kg/day, was commenced in 65.8% of infants. At last review only 11/77 infants had ceased therapy. Duration of this therapy varied widely between 10 days to 15 months.

Conclusion

TFT abnormalities are common in unwell infants. While most were presumed to have TCH, the majority were nevertheless commenced on thyroxine treatment. Further prospective studies are required to develop predictors to differentiate between

transient and permanent CH and guide treatment.

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Physiology of prolactin in infancy and childhood

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Prolactin is a polypeptide hormone with a short half-life and multiple physiological effects. Low prolactin in infancy is associated with neonatal respiratory distress and infant irritability, and changes in paediatric prolactin are linked with gonadal axis activation and growth hormone excretion. Despite these many associations, our understanding of the normal developmental physiology of prolactin in children is incomplete.

The aim of this study is to identify the normal physiology of prolactin secretion in infancy and childhood via meta-analysis of published paediatric data.

A literature search using the search terms ‘p(a)ediatric’, ‘prolactin’, and ‘reference interval’,was completed via Pubmed and Ovid Medline databases. Additional MeSH and keyword phrase utilisation ensured inclusion of all appropriate studies. Prolactin data for children aged 0-18 years were converted to mIU/L. Data from different analysers cannot be directly compared, therefore we overlaid the median trend of each analyser to assess temporal physiological variation. A weighted average was calculated to determine a schematic nomogram for paediatric prolactin.

Fourteen studies of paediatric prolactin from prematurity to 18 years were found, and 7915 measurements included. The median line derived for each available age partition, and the fold increase in the median from baseline was calculated. Analysis of overlay data shows the prolactin nadir is in mid childhood (5-7 years) with the highest peak occurring soon after birth in both pre-term (12 fold) and term (14 fold) newborns, down trending in the first year of life to eventually reach the pre-pubertal nadir, followed by a slight pubertal increase (1.6 fold). There was no sex difference.

This meta-analysis demonstrates the physiology of paediatric prolactin production. Investigation of the cause and purpose of the prolactin neonatal peak is planned through analysis of a large dataset at our centre, with review of the clinical course of infants found to have outlying low prolactin.

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Models of care for children newly diagnosed with Type 1 Diabetes

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Newly diagnosed children with juvenile Type 1 Diabetes (T1D) typically stay in hospital, which incurs healthcare costs and can be disruptive to families. A range of alternative models of care have been trialled to reduce costs and improve health outcomes and experiences for children and families. This research aims to explore implemented alternative models of care and their effectiveness.

We conducted a literature search of four databases, for peer reviewed studies focused on new diagnosis T1D models of care published from 2010 to 2021 written in English.

A total of eight studies met study inclusion. Model components included hybrid short hospital stays followed by home based, or outpatient clinic visits, for education and monitoring, and early integration of technology, specifically CGM (4T model). Outcomes included HbA1c levels, readmissions, cost, and model preference.

For home based or hybrid short hospital stays, four studies reported no significant differences in HbA1c or readmission rates between patients treated with in- and out- patient care. Cost reductions were observed when patients spent less time in hospital, along with reductions in health service usage. Patients and families preferred out-patient models of care to in-hospital care. Nurses looking after children with newly diagnosed T1D also preferred home-based care models, feeling that it improved relationships with families and opportunities for more effective education. A model based on the “four Ts” (teamwork, targets, technology and tight control) resulted in lower levels of HbA1c compared to a historic cohort.

Home-based or hybrid short-stay models proved cost efficient, did not reduce health education or outcomes, and were acceptable to healthcare professionals, patients and families. Additionally, technology with education support improves health outcomes. With only eight studies identified, there is a need for further research, especially on the effectiveness, cost-effectiveness and implementation determinants of each model of care.

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Transition models of care for Type 1 Diabetes: a scoping review

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Managing the care regimen for Type 1 Diabetes (T1D) is challenging for emerging adults, as they take on greater responsibility for self-management. A diverse range of models of care have been implemented to improve safety and quality of care during transition between paediatric and adult services. However, evidence about acceptability and effectiveness of these is limited.

Our aim was to scope transition models and their components, examine the health and psychosocial outcomes, and to identify determinants associated with the implementation of person-centred models of transition care.

We searched Medline, CINAHL, EMBASE and Scopus. Peer reviewed empirical studies that focused on T1D models of care published from 2010 to 2021 in English, reporting experimental, qualitative, mixed methods, and observational studies were included.

Fourteen studies reported on health and psychosocial outcomes, and engagement with healthcare. Three key models of care emerged: structured transition education programs (6), multidisciplinary team (MDT) transition support (5) and telehealth/virtual care as an integral component of a broader model (3). Compared with usual practice, three of the six structured transition education programs led to improved maintenance of glycaemic control, improved psychological well-being, and improved engagement with health services. Four MDT transition care models reported improved health outcomes, and improved engagement with health services, however, three studies reported no benefit. Reduced diabetes related stress and increased patient satisfaction were reported by two studies, but three reported no benefit. Telehealth and virtual group appointments improved adherence to self-management and reduced diabetes distress but no change in health outcomes. No study reported on implementation determinants or applied an implementation framework to guide their research.

Although some benefits are reported, the results were mixed. We identified a gap in evidence about model acceptability, adoption, and appropriateness as experienced by clinicians delivering care and this should be a priority for future research.

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Dominant Osteogenesis Imperfecta Caused by a Heterozygous *SP7* Variant

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Mutations in *SP7* (osterix) have been identified as a rare cause of recessive osteogenesis imperfecta ('OI type XII') (1-3) and in two cases of dominant disease with bone fragility, high bone density and high bone turnover (4, 5). We present the first description of siblings with OI due to a heterozygous mutation in *SP7*.

The phenotype was characterized by fragility fractures (primarily of the long bone diaphyses), poor healing, scoliosis, and dental malocclusion. Both siblings had very low cortical volumetric bone mineral density on peripheral quantitative computed tomography of the radius (z-scores -6.6 and -6.7 at the diaphysis), porous cortices, and thin cortices at the radial metaphysis. Histomorphometry demonstrated thin cortices and low bone turnover with reduced osteoblast function. Both siblings were heterozygous for a missense variant in a highly conserved zinc finger domain of *SP7* (c.1019A>C; p.Glu340Ala) on DNA sequencing. Co-transfection of plasmids carrying the *SP7* mutation with *DLX5* and a luciferase reporter demonstrated that this variant impacted gene function (reduced transcription co-activation compared to wild-type SP7).

The low cortical density and cortical porosity seen in our patients are consistent with previous reports of recessive OI due to *SP7* mutations (1). However, the low bone turnover in our patients contrasts the high turnover state seen in patients with OI due to recessive *SP7* mutations and in the cases with dominant *SP7* mutations with high bone density (1, 4, 5).

This report indicates that dominant variants in *SP7* can give rise to OI. The predominant feature, low cortical density, is common in patients with recessive *SP7* mutations, however other features appear to depend on the specific variant.

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Opioid-induced adrenal insufficiency - an under-recognised phenomenon in children and adolescents

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To report two cases of opioid-induced adrenal insufficiency(OIAI) in children and adolescents with opioid use.

Opioid-induced adrenal insufficiency (OIAI) is a well-documented cause of iatrogenic adrenal insufficiency in adults, arising as a result of hypothalamic-pituitary-adrenal(HPA) axis suppression(1). Here we report two paediatric cases of opioid-induced adrenal insufficiency(OIAI). De-identified clinical and biochemical data pertaining to 2 adolescents known to the authors were extracted from the electronic medical record for this report.

Case 1 had a history comprising acute lymphoblastic leukaemia(ALL) and multi-focal avascular-necrosis(AVN) which necessitated chronic opioid analgesia. Recovery of adrenal function post initial ALL-related prednisolone therapy had been documented one months later. Investigation of incidentally-noted hypoglycaemia revealed low ACTH and cortisol levels(Table1). Central nervous system ALL or alternative disease was suspected; however, excluded with investigation. Testing of other anterior pituitary hormonal axes and causes of hypoglycaemia were normal. Hydrocortisone replacement led to resolution of hypoglycaemia. She remains on opioid therapy due to persistent complex pain despite two doses of zoledronic acid for AVN. While OIAI is presumed responsible, this will only be confirmed when opioid is ceased.

Case 2 presented with slipped-upper-femoral-epiphysis(SUFE) and received opioid therapy for analgesia. Investigation for possible hormonal causes of SUFE revealed low ACTH and cortisol levels(Table1). Thyroid function tests were normal. Opioid was ceased and stress hydrocortisone dosing advice provided. Recovery of the hypothalamic-pituitary-adrenal axis(HPA) was observed on repeat testing two weeks after opioid cessation.

Case 1	17 years old	Acute Lymphoid Leukaemia	Hydromorphone 9mg daily	Unexplained hypoglycaemia - 2.8	(3.6 - 5.4mmol/L)	Free T4 17.9 (10-25 pmol)
		Multi-focal Avascular necrosis	- 4 months duration			Urinary steroid profile: NAD
			Morphine 45-60mg daily			
			- 4 months duration (preceding hydromorphone)			Cortisol (100-455 nmol/L)
						Post cessation of steroids previously - 306nmol/L
						Initial screen - 15 at 0400
						- 42 at 0947
						SynACTHen
						0 30 60 (Minutes)
						Cortisol 25 342 421 (100-455 nmol/L)
						ACTH 0.9 (<20 pmol/L)
Case 2	12 years old	Obesity	Oxycodone 20-40mg daily	Work-up for factors potentially contributing to SUFE		TSH 0.46 (0.50 - 4.50 mIU/L)
		SUFE	- 1 week duration			Free T4 17 (10.0 - 25.0 pmol/L)
						Free T3 4.4 (4.0 - 6.8 pmol/L)
						Initial screen
						Cortisol 23 at 0628 (100-440 nmol/L)
						ACTH 1.7 at 0628 (<20 pmol/L)
						SynACTHen
						0 30 60 (Minutes)
						Cortisol 142 521 577 (100-440 nmol/L)
						ACTH not sent (<20pmol/L)
						Repeat post cessation of opioids
						Cortisol 182 at 0832 (100-440 nmol/L)
						ACTH 5.6 at 0832 (<20 pmol/L)

The prevalence of OIAI in adults on chronic opioids is estimated to range from 8.3% to 29%(2,3) but reports in children and adolescents are limited. Our cases indicate OIAI can arise with both short and longer-term paediatric opioid use. Given its prevalence in adults, OIAI may be under-recognised in the paediatric population. Further research is necessary to identify the frequency and guide optimal management of OIAI in paediatric populations.

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Increase in BMI SDS in youth with type 1 diabetes during the COVID-19 pandemic

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Objective: Overweight and obesity are more prevalent in children and adolescents with type 1 diabetes than in youth without type 1 diabetes. COVID-19 related lockdowns in Australia and New Zealand in 2020 and 2021 meant significant restrictions on activity and face to face school attendance. We compared changes in BMI SDS in children and adolescents < 18 years between the pre-pandemic years 2018-19 and pandemic years 2020-21.

Methods: Clinical and demographic data for children and adolescents < 18 years of age and type 1 diabetes diagnosed after 6 months of age, registered with the Australasian Diabetes Data Network (ADDN) were extracted for 2018-2021. Generalised estimating equations (GEE) were used to analyse trends in BMI SDS over time and account for repeated measures from the same individuals. Multivariable GEE regression models were used to adjust for potential confounding variables.

Results: 67419 visits over 2018-2021 for 24383 individuals aged < 18 years, from 33 sites were included. There was a positive association with BMI SDS over time. All jurisdictions, in comparison with Western Australia, had a positive association with BMI SDS. Total daily dose, CSII use, and female sex were also positively associated with a higher BMI SDS. Age, HbA1c and in an Australia only model, higher socioeconomic status, were negatively associated with BMI SDS in 2018-2020 The same analysis with 2021 data is pending.

Conclusions: Lockdowns have been associated with higher BMI SDS in children and adolescents with type 1 diabetes in Australia and New Zealand. This has implications for public health and social policy in future lockdowns.

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A virilizing ovarian tumour following previous rhabdomyosarcoma masking ovarian failure

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An 11.8-year-old girl presented with recent voice change and increased leg hair. She had a past history of pelvic rhabdomyosarcoma at age 2, with relapse and local metastasis at age 4. Prior to original tumour treatment, oophoropexy was performed, aiming to prevent radiation exposure.

Puberty commenced at age 10, with 6-months of increased leg hair growth plus voice deepening, with a gravelly quality. Examination revealed Tanner stage 2-3 breast tissue bilaterally and stage 4 pubic hair. She was pre-menarchal. Maternal family history of cancer did not suggest a genetic link. Androgen excess was confirmed, with testosterone 10.4 nmol/L (0.1-1.9), FSH 4.7 IU/L (0-10), LH 4.5 IU/L (1-3), oestradiol 107 pmol/L. MRI demonstrated a large (42 x 36 x 42 mm) pelvic mass, abutting the left external iliac vessels, uterine fundus and urinary bladder.

Histopathology was consistent with stage 1 ovarian Sertoli-Leydig cell tumour (SLCT), with resolution of androgen excess (testosterone <0.4nmol/l/). But there was clear evidence of primary ovarian failure with FSH 60.7 IU/L (1-10), LH 32.1 IU/L (1-3.5) and Oestradiol 19 pmol/L, in the prepubertal range. Oestrogen was commenced to continue normal feminization, remaining linear growth and bone mass accrual. Further genetic investigations have not demonstrated a DICER mutation.

This case demonstrates several important points. The enormous androgen levels due to SLCT mask past chemotherapy /radiotherapy related ovarian failure, only becoming evident after tumour removal and cessation of androgen excess. SLCT are rare sex-cord ovarian tumours, accounting for 1% of all childhood cancers and 10% of all ovarian tumours in paediatrics (1, 2). Up to 85% have associated virilisation (1-3) with up to 63% reported to be due to a DICER 1 gene mutation (2, 3). Association between the rhabdomyosarcoma, the commonest soft tissue sarcoma of childhood (3) and later SCLT may be linked with DICER mutation (1).

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Extra-articular calcification in an adolescent boy: where history is paramount

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A 15-year-old active boy, presented to a surgeon with a painful left elbow. There was no history of preceding trauma, he had full range of motion and was systemically well. Imaging demonstrated extra articular calcification at the distal humerus. Serum phosphate was elevated 2.53 mmol/L [1.10-1.80] with calcium 2.48 mmol/L [2.10-2.60] and tubular reabsorption of phosphate (TRP) of 94.6% [82-100%]. Endocrine assessment revealed a history of short tooth roots. A diagnosis of hyperphosphatemia due to a mutation in GALNT3 was suspected. Additional investigations revealed calcification of the left hip and nephrocalcinosis on renal ultrasound. Dietary phosphate restriction and phosphate binder (Sevalmer) was commenced.

Over the following months, enlargement of the elbow lesion occurred, with worsening pain, reduced mobility limiting sporting activity, accompanied by deterioration of mental health. Serum phosphate remained elevated 2.22 mmol/L, calcium in normal range 2.31 nmol/L, low FGF23 13.6 ng/L [23.2 – 95.4 ng/L] with TRP of 98.04% [82-100%]. Imaging confirmed significant increase in calcification at both sites. Surgical resection was undertaken, due to marked joint restriction. Other medical interventions being trialled included acetazolamide (carbonic anhydrase inhibitor), IL1 blockade (Anakinra), monoclonal antibody against IL-1β and sodium thiosulfate.

Hyperphosphatemia Familial Tumoral Calcinosis is a rare, disabling disorder characterised by ectopic calcifications, painful swellings and enamel hypoplasia with bulbous tooth roots (1, 2). Periarticular calcification leads to pain and reduced range of movement (1). It is caused by autosomal recessive inheritance of a pathological variant in either the gene encoding FGF23, GALNT3 or KLOTHO (1-3); resultant deficiency of/or resistance to the phosphate regulating hormone fibroblast growth factor 23(1, 3).

The case underlines the importance of careful history, the unusual history of short tooth roots enabled rapid diagnosis. The aim of treatment is to reduce serum and urinary phosphate and to reduce pain; there is no definitive cure (1, 4).

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Management of pancreatic agenesis in an infant: use of diabetes and telehealth technologies to overcome challenges of regional living and complex diabetes management

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This abstract describes the ongoing management of a previously reported infant with PTF1a (encoding pancreas-specific transcription factor 1a) mutation with pancreatic agenesis. She was born at 36 weeks gestation via Caesarean section weighing 1840g (5th percentile). Management of diabetes was complicated by reduced subcutaneous fat for sensor/pump cannulas as well as comorbid gastrointestinal dysmotility (requiring ileostomy from 3.5m to 12m and Total Parental Nutrition from birth until 2.5m) and variable carbohydrate absorption. Management included insulin infusion initially with introduction of insulin pump at 11w and Continuous Glucose Monitoring at 15w. After discharge from hospital at 5m, she was managed with dilute insulin until 7m, and Medtronic 770G Auto mode was commenced at 11m.

This abstract and Table 1 describes the challenges in ongoing management of this case. The use of diabetes technologies including Auto mode has been integral to her care. There have been difficulties related to her co-morbidities and age (including frequent snacking) but these have been overcome with the use of such technologies and frequent meetings with her care teams.

Management of intercurrent illness is challenging, especially in the context of the family living regionally. Sick day letters, parental and local team education, and frequent communication between the tertiary endocrine team, local paediatric services, and local emergency department has been important in ensuring her safety and wellbeing. Use of telehealth is essential in ensuring access to specialist care and importantly involving the family and local paediatric team in her management.

At most recent follow-up at 21 months of age, time in range was 50% with <4% time in hypoglycaemia on the 770G system. Total daily insulin dose was 8 units. Reassuringly, despite frequent hypoglycaemia and hyperglycaemia occurring in the neonatal period, this child is thriving and developmentally normal. There are current plans to convert to 780G system.

Table 1: Challenges in and strategies for managing neonatal diabetes

	CHALLENGES	STRATEGIES
INITIAL MANAGEMENT	• Limited subcutaneous fat • Low weight and insulin requirements • Variable absorption of feeds • Vomiting, diarrhoea and stoma losses (comorbid gut dysmotility requiring stoma)	• Practical care and application of cannulas and sensors including using a 20° angle for subcutaneous cannulas • Dilution of insulin 1:10 until 7m of age • Parental and local team teaching on glucose management • Use and adaptation of diabetes technologies – use of Medtronic 770G Auto mode system from 11m with upgrade to 780G system in August 2022
ONGOING CARE	• Management of intercurrent illness	• Importance of sick day management letters • Education of local healthcare providers and parents • More regular telehealth meetings and glucose monitoring by tertiary team
	• Living in a regional centre	• Telehealth availability • Regular meetings between family and health professionals involved including local team and tertiary diabetes team (weekly then monthly) • Education of parents and local staff
	• Use of a hybrid closed loop system in a young infant	• Adaptation of diabetes technologies to lifestyle, eating patterns and physiology of a young infant with pancreatic agenesis is challenging • A longer active insulin improved time in range (TIR)

Knowledge, safety, and impact of alcohol consumption in young adults with Type One Diabetes: a qualitative study

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Aims To explore the lived experiences of young adults with type one diabetes regarding how their chronic condition has impacted their behaviours around alcohol consumption and the associated risk-taking and glycaemic management.

Methods Fourteen semi-structured interviews were conducted amongst young adults aged between 18 and 25 years inclusive (mean age 21.14 (SD 2.21)) with type one diabetes who consume alcohol. Interviews were transcribed verbatim and thematically analysed to identify common themes regarding their experiences.

Results Three broad themes were elucidated: alcohol consumption increasing potentially harmful glycaemic risk-taking; the adaptation of alcohol consumption & glycaemic strategies; and the sources & quality of knowledge concerning the impact of alcohol on glycaemic management.

Conclusions This study provides new insights into how young adults with type one diabetes are engaging with alcohol consumption, the risks they are taking, and how they are managing their own behaviour to reduce those possible harms. These findings help to expand current understanding on these areas and highlights the importance for education around alcohol consumption to promote safety for these individuals. It also emphasises where current guidelines are falling-short in providing socially realistic solutions for these young people on how they can positively engage with alcohol. These findings can be used to direct the development of better clinical resources for these young adults in future.

Introduction of a gluten-free diet in children with type 1 diabetes and newly diagnosed coeliac disease increases the glycaemic index of the diet but does not adversely impact glycaemic outcomes or quality of life

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AIM

To evaluate the impact of a gluten-free diet (GFD) on BMI, glycaemic outcomes, nutrient intake, and quality of life (QoL) in children (2-18 years) with type 1 diabetes (T1D) and newly diagnosed coeliac disease (CD).

METHODS

This was a prospective cohort trial conducted at three Australian paediatric T1D centres. Prior to CD diagnosis, baseline height, weight, HbA1c, 14-days of continuous glucose data; and a 4-day food diary were collected. Participant and parent QoL were measured using the PedsQL. On diagnosis, participants received standardised GFD education. Baseline measures were repeated at 3-months. Dietary adherence was assessed using the gluten-free compliance questionnaire.

RESULTS

Participants (n=20) had a mean age of 8.2±3.6 years and diabetes duration of 1.4±1.9 years, 19/20 were assessed as adherent to the GFD. Compared to baseline, at 3-months post-GFD there were no significant differences in BMI *Z*-score (0.59 vs 0.58, p=0.893), glycaemic outcomes including HbA1c (53.6mmol/mol vs 53.8mmol/mol, p=0.944); TIR, 3.9-10.0 mmol/L (65% vs 62%, p=0.236); TAR, >10.0 mmol/L (29% vs 31%, p=0.413); TBR, <3.9 mmol/L (6.6% vs 6.9%, p=0.720) and daily intake of carbohydrate (206 vs 197g, p=0.435), fat (56g vs 69g, p=0.475), saturated fat (25g vs 26g, p=0.624) and protein (62g vs 60g, p=0.607). Post-GFD there was a significant decrease in the daily intake of iron (8.8mg vs 6.9mg, p=0.020), folate (618ug vs 389ug, p=0.007) and magnesium (223mg vs 188mg, p=0.028) and a significant increase in the glycaemic index of the diet (55 vs 58, p<0.001). QoL of the participants (59 vs 63, p=0.300) and parents (63 vs 68, p=0.135) was unchanged.

CONCLUSIONS

In children with T1D and newly diagnosed CD, introduction of a GFD does not impact QoL or glycaemic outcomes at 3-months post-diagnosis despite the glycaemic index of the diet being higher. Intensive dietary review is needed to ensure diet quality is maintained.

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Streamlining the review of complex hypospadias referrals at a Differences of Sex Development (DSD) multidisciplinary team meeting

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The Sydney Children's Hospital Network DSD multidisciplinary team (MDT) meetings are a forum to support consistent decision making for children and adolescents with DSD and complex hypospadias (CH) (defined as peno-scrotal hypospadias or any hypospadias with undescended testis, microphallus or bifid scrotum). To minimise inconsistencies in referral patterns and enable a time efficient and streamlined review of patients with CH, a flow chart for diagnostic work up and checklist based peer review process to document the decision making process prior to any surgical intervention was introduced in 2021.

All patients have endocrine investigations, a chromosome microarray and are offered a DSD gene panel as per the flowchart. The checklist incorporates clinical details and 11 items which include documentation of clinical discussions by treating clinicians on aspects of investigations, awareness of surgical sequelae and long term outcomes. The checklist is reviewed by the conveners of the DSD MDT and if found acceptable, filed in the patient's medical records. If not suitable for the checklist, the case is brought to full discussion at the MDT.

Since 2018, 36 cases of CH were reviewed by the MDT, 16 prior to introduction of the checklist by full discussion and 20 since introduction (all checklist). All had minipuberty and/or HCG stimulation hormone testing. Despite variable uptake of genetic testing, variations were found in 11, 5 were pathogenic. 17 have proceeded on to correction of hypospadias surgery and 18 are currently on surgical waiting lists, with one family deciding to defer surgical intervention. The average turnaround time for checklist review was 1 week.

The introduction of a flowchart and a peer reviewed checklist system has streamlined the MDT process for complex hypospadias, whilst maintaining consistent evidence based management, and enabled greater time for patients requiring more complex and ethically challenging discussions by the DSD MDT.

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Efficacy, observer-reported outcomes and safety of once-weekly somapacitan in children with growth hormone deficiency (GHD): 4-year results from the REAL 3 trial

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Aims: Children with GHD are currently treated with daily subcutaneous growth hormone (GH) injections, which can be burdensome. Somapacitan is a long-acting GH derivative in development for once-weekly use in children with GHD.

Methods: REAL 3 (NCT02616562) is a phase 2, multinational, randomised, open-label, controlled trial assessing efficacy and safety of somapacitan versus daily GH (Norditropin®). Prepubertal, GH-naïve children with GHD received 0.04 (n=16), 0.08 (n=15) or 0.16 (n=14) mg/kg/week somapacitan, or 0.034 mg/kg/day daily GH for 1 year. In a 2-year safety extension, all patients on somapacitan (n=45) received 0.16 mg/kg/week; the daily GH group remained unchanged. In a 4-year safety extension, the daily GH group switched to 0.16 mg/kg/week somapacitan (daily GH/somapacitan, n=11), while the somapacitan group remained unchanged (somapacitan/somapacitan, n=39). We present results from year 4, the first year of the 4-year safety extension.

Results: Data are mean (SD). Height velocity (HV) was 7.4 (1.6) cm/year for somapacitan/somapacitan and 6.6 (1.6) cm/year for daily GH/somapacitan, versus 8.3 (1.7) and 7.6 (2.0) cm/year for somapacitan/somapacitan and daily GH, respectively, at year 3. HV SD score (SDS) was 1.55 (1.70) and 0.88 (1.61); change in height SDS from baseline was 2.85 (1.25) and 2.28 (0.97); insulin-like growth factor-I SDS was 1.29 (1.23) and 0.94 (1.60) for somapacitan/somapacitan and

daily GH/somapacitan, respectively. The table shows overall scores of GHD-child treatment burden and GHD-parent treatment burden, reported by parents/guardians.

During year 4, 20 patients (51.3%) receiving somapacitan/somapacitan experienced 84 adverse events (AEs), and eight patients (72.7%) receiving GH/somapacitan experienced 12 AEs. Most AEs were mild/moderate and unrelated to treatment.

Conclusion: Height-related outcomes were similar between, and as expected for, both treatment arms. Somapacitan may lead to improvement in treatment burden versus daily GH. Somapacitan safety profile was consistent with previous reports.

Table: GHD treatment burden measures from years 2–4; mean (SD) scores (lower indicates improvement)

	Daily GH/somapacitan			Somapacitan/somapacitan		
	n=11			n=39/40	n=39	n=37/38
Year	2	3	4*	2	3	4
GHD-CTB†	6.4 (8.2)	15.1 (16.3)	7.1 (9.4)	7.6 (9.4)	7.4 (6.8)	5.8 (7.5)
GHD-PTB‡	8.7 (10.9)	11.3 (16.4)	6.3 (12.2)	9.5 (11.2)	9.6 (12.2)	8.7 (12.1)

†Across physical, emotional wellbeing and interference domains.
‡Across emotional wellbeing and interference domains.
CTB, child treatment burden; GH, growth hormone; GHD, growth hormone deficiency; PTB, parent treatment burden; SD, standard deviation.

Once-weekly somapacitan versus daily growth hormone (GH) in children with GH deficiency (GHD): the randomised phase 3 REAL 4 trial

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Aims: GH replacement therapy usually requires daily subcutaneous injections potentially burdensome for patients and caregivers. Long-acting GH formulations aspire to a less burdensome dosing regimen as safe and efficacious as daily GH, potentially improving adherence and clinical outcomes. Somapacitan, a long-acting reversible albumin-binding GH derivative, is in development for once-weekly subcutaneous administration in children with GHD.

Methods: REAL 4, a randomised, open-labelled, active-controlled, parallel-group phase 3 trial (NCT03811535), investigated efficacy and safety of once-weekly somapacitan versus daily GH (Norditropin®), with a 52-week main phase and 3-year safety extension. Two-hundred GH-treatment-naïve, prepubertal children with GHD (74.5% male) were randomly assigned 2:1 to receive 0.16 mg/kg/week somapacitan (n=132) or 0.034 mg/kg/day daily GH (n=68) for 52 weeks. Here, we present secondary endpoints, including change from baseline to week 52 in height velocity (HV) standard deviation score (SDS), height SDS (HSDS), bone age, and patient-reported outcomes assessing disease and treatment burden.

Results: Non-inferiority in HV at week 52 was confirmed. Estimated changes in HVSDS and HSDS from baseline to week 52 were not statistically different between once-weekly somapacitan (8.05 and 1.25, respectively) and daily GH (8.82 and 1.30, respectively). Mean estimated bone age to chronological age ratio advanced similarly in both groups (somapacitan: 0.06; daily GH: 0.08), with no changes in skeletal proportions. Disease burden was reduced similarly between treatment groups from baseline to week 52. Treatment burden assessments favoured somapacitan over daily GH across all domains. The somapacitan and Norditropin® FlexPro® devices were both considered to be easy or very easy to use (96%) and to learn to use (>90%). Safety was consistent with the daily GH profile.

Conclusion: The efficacy and safety profile of somapacitan was similar to that of daily GH. Both treatments reduced disease burden to a similar degree; treatment burden was consistently lower with somapacitan.

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Real world glycaemic outcomes in Western Australian youth with Type 1 Diabetes starting Control IQ hybrid closed loop technology

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With availability of advanced hybrid closed loop (aHCL) systems in Australia, there is a need to evaluate whether improvements reported in clinical trials (1) are reflected in real-world outcomes (2). The aims of this study were to measure glycaemic outcomes in youth commencing Control IQ (CIQ), and to evaluate the model of care for aHCL initiation.

Youth with Type 1 diabetes (T1D) starting CIQ from April 2022 were included. On-line education, baseline telehealth review with structured follow-up was provided until pre-defined benchmarks for system use were met. 2-week CGM metrics were collected prospectively at baseline (BL) and at 1 and 3 months post-CIQ start. Outcomes included time in range (TIR 3.9-10 mmol/l), time below range (TBR < 3.9 and <3 mmol/l), time above range (TAR 10.1-13.9 and >13.9 mmol/L) and number of reviews required to meet all benchmarks.

37 youth, mean (SD) age 13(2.7) years, diabetes duration 4.8 (3.2) years, HbA1c 7.4 (0.9)% were included. TIR increased from 60.5 (15.4)% at BL to 68.1 (11.1)% and 67.7 (14.2)% at 1 month (p<0.001) and 3 months (p = 0.009) respectively. TBR was unchanged (BL vs 3 months <3.9: 1.7% vs 1.5%, p=0.400, < 3.0: 0.4% vs 0.4%, p=0.844). TAR 10.1-13.9 reduced from 23 (5.9)% at BL to 19.4 (6.4)% and 19.0 (6.5)% at 1 and 3 months respectively (p = 0.004). TAR > 13.9 was 14.4 (11.8)% at baseline, 9.4 (6.9)% at 1 month (p=0.004) and 11.5 (10.5)% at 3 months (p = 0.162). 28/37 (76%) youth met all pre-defined benchmarks at the first post-CIQ review; 8/37 and 1/37 required 2 and 3 reviews respectively.

With structured education and follow-up, glycaemic outcomes in real-world use of CIQ technology approach those seen in clinical trials. Ongoing evaluation will be important to ensure results in early adopters are mirrored in subsequent cohorts.

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Prevalence and incidence of type 1 diabetes in children aged 0-14 years old in New Zealand between 2020-2021

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National data sets on prevalence and incidence are important for understanding population trends and allocating healthcare resources. We aimed to provide a current snapshot of national prevalence and annual incidence rate of type one diabetes (T1D) in children aged 0–14 in New Zealand and identify differences associated with geography and ethnicity.

All paediatric diabetes centres across New Zealand were invited to record anonymised demographic and diabetes data on children under their services for the year 1st Oct 2020 to 30th Sept 2021 with a diagnosis of T1D. The national prevalence

and incidence, stratified by age, ethnicity, and region was calculated using estimated resident population counts from the 2018 census. The effect of ethnicity on prevalence and incidence was assessed using Poisson regression.

There were 1209 children aged 0-14 with T1DM on October 2021. National prevalence was 128/100,000 (95% CI 121 to 135). Compared to Māori or Pacific Peoples, European have twice the prevalence. There was no effect by gender; and as expected, prevalence increased with age. Collectively children of Māori and Pacific ethnicity made up over 25% of the cohort. The one-year incidence of T1D was 22/100,000 (95% CI 19 to 25). Compared to children of Māori ethnicity, European children were 2.5 times more likely to be diagnosed with T1D in that year (adjusted PRR=2.5, 95% CI 1.55 to 4.02). Regional differences in prevalence and incidence were noted and are shown in figure 1.

In this capture of incidence and prevalence in New Zealand, we demonstrate that T1D affects an ethnically diverse population, and important regional differences exist which may impact workforce planning.

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A curious case of a boy with repeated fractures

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A 5 years 3 months old boy born of a third degree consanguineous union sustained multiple fragility fractures involving the forearm and legs since age 3 years. Deformities of the lower limbs due to repeated fractures were present. He had normal hearing, development and dentition and no family history of repeated fractures, deformities, hearing or dental problems. On examination dentition was normal, sclerae white, no birth marks, pallor or organomegaly.

Bone turnover, Vitamin D, PTH, coeliac screen urine Calcium/creatinine, pH and electrolytes were all normal. Skeletal survey demonstrated normal bone density,Z score : AP spine: z+0.1 Total body: +0.7. Long bone fracture sites demonstrated poor callus formation, normal vertebrae, no bone dysplasia.

Clinical exome revealed a homozygous missense pathogenic mutation in Exon 1 of the Bone Morphogenetic Protein 1 c.34G>V (p.Gly12Arg) causing Osteogenesis Imperfecta type 13.The BMP1 gene on chromosome 8p21.3 is a multifunctional protein acting as a procollagen type 1 C-terminal propeptide endopeptidase. Inactivation of BMP1 impairs proteolytic removal of the carboxyl terminal propeptide from procollagen type 1 and the normal assembly of mature collagen type 1 fibrils

Autosomal recessive mutations result in clinical type 3 (moderate to severe) and genetic type 13 OI.

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Cutaneous Skeletal Hypophosphatemia Syndrome : A Case Report

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A 4.25 year old female child ,born of a 3rd degree consanguineous marriage presented with a history of bow legs noticed by the mother since 1 year of ageShe had multiple pigmented skin lesions all over the body and an occipital scalp lesion since birth. There was no significant family history.She was small WT- 11.9 kg (-2.51 SDS) , HT- 85.1cm (-4.41 SDS)Multiple nevi were present all over face , chest , arms & back , plus hairy nevi over back , buttock and thigh. Clinical features of rickets with leg bowing with an Intercondylar distance of 10cm were noted. Examination was otherwise normal. There was no organomegaly

Biochemistry revealed serum Ca: 2.52 mmol/L(2.2-2.6)S. PO4: 1.1 mmol/L(1.12-1.45),S.ALP: 726 U/L, Urine Creatinine – 5.7, Phosphorous – 4.4mmol/l, TRP -93 % with normal pH7.43 PCO2- 28.4mmol'l HCO3- 18.6mmol/l, normal vitamin D. FGF-23 : 6300.8 RU/ml (<150)

MRI Brain demonstrated multiple melanin deposits in bilateral medial hippocampal and pial surfaces of cerebellum and bilateral parasagittal frontal lobes. S/O Neurocutaneous melanosis. Dermatology review melanocytic Naevus Syndrome (Congenital) with Cutis Vulgaris Gyrata

CSHS is a rare disorder, with 30 cases reported . features epidermal or melanocytic nevi and hypophosphatemic rickets with elevated levels of a serum phosphatonin, fibroblast growth factor-23 (FGF23).The epidermal nevus lesion in ENS follows the lines of Blaschko, with neurologic involvement in 50-70% of patients.Hypophosphatemic rickets is a rare manifestation. Skeletal defects manifestations include kyphoscoliosis genu valgum, clinodactyly, skeletal asymmetry,

intoeing gait, microcephaly, limb reduction defects, calcaneovalgus, talipes equinovarus.Ocular abnormalities are seen in 30%.Precocious puberty, aortic coarctation, renal anomalies are rare.

Phosphate and calcitriol supplementation to maintain mineral homeostasis have been used, with a single report of burosumab use to try to control FGF23. Excision or ablation of nevi as treatment for hypophosphatemia is not advised.

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