2021 RESEARCH HIGHLIGHTS

Fertility and Conception Theme

- **Dr Tiffany Tan, Dr Kylie Dunning** and team developed an innovative approach that overcomes the need for a cell biopsy in embryo testing. Their approach uses light to take a non-invasive 'molecular photo', and is able to detect whether an embryo has the expected number of chromosomes. The work is in collaboration with Fertility SA and colleagues at The University of New South Wales.
- **Prof Paul Thomas** has partnered with the CSIRO and Centre for Invasive Species Solutions to test two breakthrough genetic biocontrol technologies to prevent future mice plagues. The 'X-shredder' approach, eliminates sperm carrying the X chromosome, producing more male than female offspring. The 'female infertility' approach initially spreads a genetic modification through the population, and once saturated, all the females generated will be infertile.
- **A/Prof Wendy Ingman** and team demonstrated that gene expression profiles in mammary cancers are affected by ovarian cycle stage, suggesting breast cancer tests that use gene expression profiling in women could be affected by the menstrual cycle. This has implications for breast cancer treatment decision-making in young women with breast cancer.
- Comparison of three commercial human embryo culture mediums used in assisted reproduction technology found differences in the type and concentration of fatty acids in the human serum albumin component. **Dr Nicole McPherson** and collaborators found this influenced blastocyst development and successful implantation rates in a pre-clinical model.
- **Dr Caremella Ricciardelli** and team demonstrated that CAR-T cells targeting nfP2X7 receptor are effective at killing ovarian cancer cells both in vitro and in vivo. This study demonstrates that nfP2X7-CAR-T cells have potential to be developed as a novel immunotherapy for ovarian cancer.
- Paternal experiences and exposures before conception can influence fetal development and offspring phenotype. The composition of seminal plasma contributes to paternal programming effects. **Drs John Schjenken, Nicole McPherson and Prof Sarah Robertson** demonstrated in mice that an obesogenic high fat diet alters the composition of seminal vesicle fluid and impairs seminal plasma capacity to elicit a favorable immune response in females at conception.
- Pouch of Douglas obliteration is a severe consequence of inflammation in the pelvis, often seen in patients with endometriosis. **Dr Gabriel Maicas, Professors Gustavo Carneiro, Louise Hull** and team developed an accurate deep learning model for the prediction of the dynamic transvaginal ultrasound-based sliding sign classification.
- **Prof Ray Rodgers, Rafiatu Azumah, Menghe Liu** and collaborators are the first to show that candidate genes for polycystic ovary syndrome (PCOS) can be regulated by TGFbeta in the fetal ovary. They found that TGFβ signalling molecules are dynamically expressed during fetal ovary development and that TGFbeta 1 inhibits 7 of the 25 PCOS candidate genes in fetal ovarian fibroblasts in vitro. This suggests that TGFβ signalling could be part of the aetiology of PCOS or at least the aetiology of polycystic ovaries.
- **A/Prof Alice Rumbold** and colleagues found that health care providers perceive infertility to be a major and unrecognised health issue for Aboriginal and Torres Strait Islander people. This highlights the need for better evidence to quantify the impact of infertility and develop culturally-responsive care approaches to manage infertility in Aboriginal and Torres Strait Islander communities.
- **Suliman Yagoub, Dr Kylie Dunning** and team are the first to use a novel micrometre-scale device for oocyte microinjection. The device avoids the use of a holding pipette and only utilises the injection pipette.

- **Drs Megan Lim and Kylie Dunning** demonstrated the dysregulation of bisphosphoglycerate mutase during oocyte maturation in the presence of haemoglobin protein, now published in the Journal of Assisted Reproduction and Genetics. They also reviewed the role of hypoxia in ovarian health in an invited review paper for *Reproduction*.

- **Prof Frank Grutzner** and team co-led a project that delivered the first error free platypus and first draft echidna genome. This research revealed new insights into monotreme biology and mammalian evolution. The resources provided with these genomes will enable research in mammalian evolution, monotreme biology and conservation.

- **A/Prof Wendy Ingman** and team demonstrated that immune cells called macrophages respond to a specific signal to modulate the risk of mammary cancer in mice. This is significant as its possible to reduce the risk of cancer by inhibiting this signalling process.

- By interrogating data from Repromed, **Dr Nicole McPherson** found that in-vitro fertilisation increases the chance of a live birth 2 to 3 fold when compared to intracytoplasmic sperm injection technique when used in women aged 35 years or older and when the male is fertile.

- **Dr Nicole McPherson** found that increased sperm reactive oxygen species (ROS) concentrations associated with advanced paternal age can be reduced with the addition of the antioxidant agent idebenone in vitro, and that this intervention is associated with improved fertilisation rates, embryo quality and implantation rates after IVF in mice.

- Seminal fluid factors modulate the female immune response at conception, but whether sperm affect this response has not been clear. **Drs John Schjenken, Dexter Chan and Prof Sarah Robertson** showed that sperm assist in promoting female immune tolerance by eliciting uterine cytokine expression through TLR4-dependent signaling. The findings indicate a biological role for sperm beyond oocyte fertilization, in modulating immune mechanisms that promote female receptivity to pregnancy.

- Maternal immune adaptation to accommodate pregnancy depends on sufficient availability of regulatory T (Treg) cells to enable embryo implantation, but the mechanisms by which these are generated aren't fully defined. **Drs Dexter Chan, John Schjenken and Prof Sarah Robertson** utilised a genetic approach to demonstrate that TLR4 is essential for an inflammation-like response in the pre-implantation uterus that induces generation of Treg cells to support robust pregnancy tolerance and ensure optimal fetal growth and survival.

- Intravenous infusion of Intralipid is an adjunct therapy in assisted reproduction treatment (ART) when immune-associated infertility is suspected. **Drs Kerrie Foyle, David Sharkey and Lachlan Moldenhauer** investigated the effect of Intralipid infusion on regulatory T cells (Treg cells), effector T cells, and plasma cytokines in peripheral blood of women undertaking IVF, and found no change in these T cell subsets. Their results do not support Intralipid as a candidate intervention to attenuate the Treg cell response in women undergoing ART.

- **Prof Ray Rodgers and Menghu Liu** developed a system to successfully culture the two somatic cell types of the fetal ovary leading to their characterisation and comparison to adult counterparts. The team demonstrated that the two fetal cell types are morphologically different, have distinct gene expression profiles and differ from the adult cell types they ultimately differentiate into. This progresses the understanding of cell lineage development in the fetal ovary.

- A new unique high throughput screening method for identification of new non-hormonal contraceptives has been developed by **Prof Darryl Russell** and team. It has led to the discovery of previously unknown molecular pathways essential for ovulation and unique candidate drugs with promise as new contraceptives.

Pregnancy and Birth Theme

- **Prof Jodie Dodd** and colleagues led an international individual participant data meta-analysis of child follow-up at 3-5 years from antenatal dietary intervention studies for pregnant women with overweight or obesity. They found that antenatal diet and lifestyle interventions do not reduce the risk of child obesity.

- There is no standard dietary approach that effectively mitigates risk for gestational diabetes. **Drs Jessica Grieger and Nahal Habibi** are using mathematical modelling to identify links between nutritional and metabolic markers that will characterise personalised diets in a clinical trial. Study outcomes will lead to a scalable approach in real-world clinical practice, to improve maternal and offspring health, and the development of nutritional recommendations for women at high risk of gestational diabetes.

- Using data obtained from mothers in the ENDIA study, **Drs Madeline Hall and Rebecca Thomson, Prof Jenny Couper** and colleagues showed that maternal mental health in pregnancy is not different for women living with type 1 diabetes versus women without the condition. This study, the largest of its kind, was published in the most influential journal in the field, Diabetes Care.

- In 2021 **Prof Bill Hague, Corey Markus, Dr Suzette Coat** and collaborators completed the Australian arm of the Bile Acid Harmonisation Study (BACH). This study aims to harmonise the clinical serum bile acid assay results across chemical pathology labs providing the clinical testing for sites collaborating in the TURRIFIC study.

- **Dr Hassen Mohammed, Prof Helen Marshall AM** and collaborators demonstrated the safety of maternal pertussis vaccination with no increased risk of adverse pregnancy and birth outcomes. These findings support recommendations for pertussis vaccination during pregnancy to prevent morbidity and mortality associated with early-infant pertussis disease.

- **E/Prof Alastair MacLennan, Prof Jozef Gecz** and colleagues have discovered in the white blood cells of children with cerebral palsy, a 'molecular signature' in the expression of genes which may have commercial potential for an early screening test for newborns, and perhaps in the first trimester of pregnancy. This would allow early treatments and precision medicine while there is brain plasticity to help repair damage.

- Macrophages are commonly thought to contribute to the pathophysiology of preterm labour by amplifying inflammation, but a protective role has not previously been considered. **Prof Sarah Robertson and A/Prof Nardhy Gomez-Lopez** showed that anti-inflammatory M2 macrophages exert a critical regulatory role in sustaining late gestation, and are implicated as a determinant of susceptibility to spontaneous preterm birth and fetal inflammatory injury.

- **Prof Jodie Dodd** and team completed follow-up of children at 8-10 years of women who participated in the LIMIT trial, an antenatal intervention for women with overweight or obesity. We have followed the children at multiple time points, with outcomes including BMI, adiposity, diet, physical activity, sleep, screen time, and cardiovascular stiffness.

- The Obstetric Medicine group continued its work recruiting participants for the Treatment of Booking Gestational Diabetes study (TOBOGM). This study led by **Prof Bill Hague** is asking whether all women who meet the diagnostic criteria for GDM, but in early pregnancy rather than at 26-30 weeks, need to monitor their blood glucose, and if needed, have treatment. Might there be harms from over treatment?

- Research published in 2021 assessing influenza vaccine responses during pregnancy suggested that obesity did not impair vaccine immunogenicity, however it also highlighted that women vaccinated against influenza in their first trimester had lower seropositivity rates. These findings by **Michelle Clake, Prof Helen Marshall AM** and team need to be explored in larger studies to ensure that any impact of gestation at vaccination on subsequent vaccine protection for mothers and infants is better understood.

Early Origins of Health Theme

- **Professors Vivienne Moore, Michael Davies** and colleagues investigated the number of births in South Australia that occurred after dispensing of clomiphene citrate, a medication for infertility. In South Australia, 1.6% of pregnancies of at least 20 weeks' gestation were conceived proximal to clomiphene dispensing. Of these, 5.7% were multiple pregnancies. This takes the proportion of women who achieved an ongoing pregnancy with medical assistance from 4.4%, based on reports from assisted reproductive technology clinics, to 6% in total.

- Despite the dogma that Complement Receptor Immunoglobulin (CRIg) is only expressed by tissue fixed macrophages, **Prof Antonio Ferrante, Dr Annabelle Smal**l and team have shown that a form of this receptor is expressed by neutrophils in the blood stream only when the cells are activated, arming the neutrophil in an inflammation scenario and promoting the cells phagocytosis and killing of bacteria. Unlike other complement receptors CRIg is naturally active and the neutrophil only expresses and deploys the receptor at time of infection. While the findings place new perspectives in infection and immunity, such measurements of CRIg expression may have a potential of a biomarker for inflammation.

- **Prof Leonie Heilbronn and Lijun Zhao** showed for the first time that restricted eating improved glucose metabolism and 24 hour rhythms in hormones and metabolites, and restored rhythm in 450 genes in adipose tissue in men with obesity.

- **Dr Clare Vane Eyk, Prof Jozef Gecz and E/Prof Alastair MacLennan** applied multi-tiered omics investigations including RNA sequencing, epiphenotyping and whole genome sequencing to DNA samples from their cerebral palsy biobank of 526 families. They discovered that one third of children with cerebral palsy have a genetic aetiology, with around half of these children potentially benefiting from a change in management based on their genetic diagnosis.

- **A/Prof Cheryl Shoubridge** and team demonstrated that treatment in early postnatal life with neurosteriods for those with genetic causes of intellectual disability and intractable seizures leads to a reduced frequency and severity of seizures, but without improvement in behavioural deficits. By identifying the molecular pathways that contribute to overlapping phenotypes of intellectual disability, seizures and neuropsychiatric disorders, they are now seeking to uncover drug targets for future treatment interventions.

- Low guanine content sequences associate with false positive CHH methylation calls in the mitochodrial genome. This finding by **Dr Takeshi Okada and Prof Jus St. John** has major implications for assessing DNA methyation calls in mammalian mitochondrial genomes.

- Building on their 2018 Nature paper, **Dr Fatwa Adikusuma, Prof Paul Thomas** and team developed a novel CRISPR gene editing tool called a Nuclease Prime Editor (NPE). They demonstrated that NPE can be used to generate specific genetic changes in cultured cells and mice with very high efficiency. This new technology will facilitate rapid generation and analysis of preclinical cell and animal disease models to investigate disease mechanisms and perform drug-screening trials. NPE also has potential to be developed as therapeutic to repair a wide array of disease-causing mutations.

- The Cerebral Palsy group have found that approximately one third of children with cerebral palsy have a genetic basis. For the remaining cases, Dr Mark Corbett, Prof Jozef Gecz and E/Prof Alastair MacLennan are seeking to understand if it is possible genetics may still be a factor if certain combinations of genetic variants that are more common in the general population confer a high risk for CP, i.e. polygenic risk.

Child and Adolescent Health Theme

- **Ms Sophie Kezior, A/Prof Alice Rumbold** and colleagues found that high school secondary student highly value the inclusion of topics about consent in relationships and sexual health education in secondary school, drawing on results of an annual survey involving 29,533 secondary school students aged 12–16 years.

- **Prof Jozef Gecz and Drs Raman Sharma and Mark Corbett** identified a deep intronic variant in TIMMDC1 gene that codes for an essential subunit of mitochondrial complex I in two children with severe peripheral neuropathy. Complete loss of TIMMDC1 mRNA, protein and mitochondrial function in patient fibroblasts was restored by splice-switching antisense oligonucleotide treatment, opening opportunity for treating such patients.

- **Dr Hassen Mohammed, Prof Helen Marshall AM** and team conducted a rapid review to identify effective strategies for improving the uptake of the influenza vaccination programs in Australia. They found update could be improved by interventions that increase community

demand and access to influenza vaccine and overcome practice-related barriers; and reinforce the critical role healthcare providers play in driving influenza vaccination uptake.

- **Dr Lachlan Jolly** created the first patient-derived stem cell model of Sanfilippo Syndrome Type A, and revealed that alterations in brain development may proceed the overt neurodegenerative characteristics of the syndrome.

- Little is known about patterns of contact with child protection services among culturally and linguistically diverse (CALD) populations in Australia. **Razlyn Abdul Rahim and Prof John Lynch** found that up to age 7, CALD children had lower risk of contact across all levels of contact with the child protection system in South Australia, from notification to out of home care placement. CALD and non-CALD groups did not markedly differ by the type of maltreatment, source of notification, or on background socioeconomic factors.

- Evaluation of the South Australian meningococcal B vaccine program showed high vaccine effectiveness against meningoccoal B disease for infants and adolescents and a significant reduction in meningococcal disease in both age groups. The evaluation led by **Prof Helen Marshall AM** also showed evidence of a significant and moderate vaccine effectiveness against gonorrhoea in 15-17 year olds in South Australia.

- **A/Prof David Parsons and Drs Martin Donnelley and Alexandra McCarron** further developed novel gene delivery methods that demonstrated substantial enhancement of lentiviral vector mediated airway gene transfer in rats. These new techniques resulted in the filing of a patent application in October 2021, and new funding from the Cystic Fibrosis Foundation.

- **Dr Zohra Lassi, A/Prof Alice Rumbold** and collaborators led a series of reviews that quantified the impact of the COVID-19 pandemic on child and adolescent mental health, and identified the maternal characteristics that predict adverse perinatal outcomes following COVID-19 infection in pregnancy, informing clinical guidelines around management of COVID-19 in pregnancy.

- **Prof Jenny Couper** and team established the Women's and Children's Hospital as a recruitment centre for a world-first clinical trial investigating whether a daily Baricitinib tablet could help someone with newly diagnosed T1D to continue to produce insulin for longer and improve their body’s control of blood glucose levels.

- A significant number of babies demonstrate low protein kinase C zeta (PKCζ) levels in cord blood T cells (CBTC), associated with reduced ability to transition from a neonatal Th2 to a mature Th1 cytokine bias and a higher risk of developing allergy, compared to neonates with higher PKCζ levels. **Prof Antonio Ferrante, Khalida Perveen** and team have now demonstrated that PKCζ signalling, actually regulates their differentiation from a Th2 to a Th1 cytokine phenotype. This finding together with our published data demonstrate a key role for PKCζ, a protein which can be modulated by nutritional supplements, in the development of neonatal T cells, towards either a Th1 or Th2 cytokine bias.

- Autosomal-dominant Cryopyrin-associated periodic syndromes (CAPS) are a group of autoimmune inflammatory conditions characterised by the constitutive activation of the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome and excessive interleukin (IL)-1β production. Variants in the NLRP3 gene can cause CAPS, but those that are novel or poorly described require evidence to prove they are disease-causing. **Dr Alex Quach, Prof Antonio Ferrante** and team have implemented a rapid bioluminescent capsase-1 activity assay into a novel NLRP3 inflammasome assay workflow to show gain-of-function activity in primary peripheral blood mononuclear cells of family members carrying a suspect NLRP3 variant.

- Using a combination of whole genome sequencing and RNA sequencing **Prof Jozef Gecz and Drs Raman Sharma and Mark Corbett** identified three different classes of splice-altering genetic variants which led to genetic diagnosis in families with X-linked intellectual disability syndromes. The work highlights strategies that can be used more broadly to increase the rate of genetic diagnosis in unresolved families.