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Reproduction, Fertility and Development

Reproductive biology research down under: highlights from the Australian and New Zealand Annual Meeting of the Society for Reproductive Biology, 2021

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ABSTRACT

Against the backdrop of a global pandemic, the Society for Reproductive Biology (SRB) 2021 meeting reunited the Australian and New Zealand reproductive research community for the first time since 2019 and was the first virtual SRB meeting. Despite the recent global research disruptions, the conference revealed significant advancements in reproductive research, the importance of which span human health, agriculture, and conservation. A core theme was novel technologies, including the use of medical microrobots for therapeutic and sperm delivery, diagnostic hyperspectral imaging, and hydrogel condoms with potential beyond contraception. The importance of challenging the contraceptive status quo was further highlighted with innovations in gene therapies, non-hormonal female contraceptives, epigenetic semen analysis, and in applying evolutionary theory to suppress pest population reproduction. How best to support pregnancies, particularly in the context of global trends of increasing maternal age, was also discussed, with several promising therapies for improved outcomes in assisted reproductive technology, pre-eclampsia, and pre-term birth prevention. The unique insights gained via non-model species was another key focus and presented research emphasised the importance of studying diverse systems to understand fundamental aspects of reproductive biology and evolution. Finally, the meeting highlighted how to effectively translate reproductive research into policy and industry practice.

Keywords: assisted reproduction, contraception, embryo, medical microrobots, oocyte, reproductive ageing, seminal plasma, Society for Reproductive Biology.

Introduction

With the emergence of a global pandemic, the years 2020 and 2021 brought unforeseen challenges to the global scientific community. One such challenge was the cancellation of scheduled annual meetings of scientific societies due to restrictions on travel and gatherings. The Society for Reproductive Biology (SRB), the premier Australian/New Zealand society for basic and applied research in all aspects of reproductive biology, was not immune to the effects of the pandemic, being forced to cancel its annual 2020 in-person meeting. Scientific events such as annual meetings of societies are significant grounds for the dissemination of research findings and building networks for new collaborations. Scientific meetings are also crucial in the career development of early career researchers, allowing them to present their work and gain critical exposure. Determined to limit such detrimental effects on the reproductive biology community, SRB hosted a virtual meeting in 2021. The 4-day 2021 virtual meeting was highly successful in reuniting the SRB community and facilitating dissemination of new research across a wide range of areas. This summary of the 2021 annual meeting highlights novel advances being made in Australia, New Zealand, and globally to fundamental animal and human biology, assisted reproductive technologies, contraceptive technologies, and novel medical devices to aid in reproduction.

Novel technologies in reproduction

Speakers: Kylie Dunning, Robin Hobbs, Mariana Medina-Sánchez and Robert Gorkin III

Emerging knowledge on assisted conception, contraception and lifetime support of reproductive health form the basis for the development of novel technologies in reproduction. Indeed, 'reproduction' extends beyond egg and sperm to include endometrial health (Evans *et al.* 2016), immune and inflammatory responses (Schjenken and Robertson 2020), whole body systems and reproductive health including the prevention of STIs. This symposium discussed the use of light technologies to determine embryo health, regulation of spermatogonial stem cell fate, aiding sperm transport with medical microrobots, and a condom for both contraception and conception.

Embryo health underpins the success of *in vitro* fertilisation (IVF), however, there is a high incidence of euploid/aneuploid mosaicism in human blastocyst embryos (up to 17.3%). A high proportion of aneuploid cells is a predicted cause of early pregnancy loss (Macklon *et al.* 2002; Bolton *et al.* 2016; Li *et al.* 2020). Current diagnostics to measure embryo health are invasive and fail to diagnose the presence of aneuploid cells within the inner cell mass (ICM) of blastocysts. To address these limitations, Dr. Kylie Dunning (The University of Adelaide) and her team are developing a novel diagnostic strategy using non-invasive hyperspectral autofluorescence microscopy. Hyperspectral imaging uses multiple excitation and emission wavelengths of light to fluoresce natural compounds within cells. Aneuploid cells contain altered metabolic features, such as increased nicotinamide adenine dinucleotide (NAD(P)H). Such changes alter the autofluorescence signal of cells and can be quantified and correlated using mathematical approaches, thus enabling discrimination between different cell populations (Gosnell *et al.* 2016). Dr. Dunning's team has recently demonstrated that hyperspectral imaging can be used to successfully distinguish healthy euploid from aneuploid cells within the ICM of mouse preimplantation embryos (Tan *et al.* 2021). Future studies will determine the effectiveness of hyperspectral imaging at detecting aneuploidy in human embryos, which would ultimately provide an affordable and practical clinical tool for determining embryo health non-invasively. If successful, this tool has potential to greatly improve clinical IVF outcomes and may have merit for application in other areas of reproductive biology.

Approximately one in 15 males of reproductive age are infertile in Australia and New Zealand, thus understanding what governs male fertility is of critical importance (Duffy *et al.* 2021). Lifelong maintenance of male fertility is dependent on spermatogonial stem cells (SSCs), which self-renew and generate differentiating germ cells for spermatozoa production (Mäkelä and Hobbs 2019). Aging or damage following exposure to genotoxic therapies can compromise male fertility by perturbing SSC function (Ryu *et al.* 2006; La and Hobbs 2019). Despite their significance, molecular mechanisms regulating SSC function and maintenance are ill-defined. Associate Professor Robin Hobbs (Hudson Institute of Medical Research) aims to better define the genetic and cellular pathways that regulate mammalian SSC function. Using *in vitro* and *in vivo* models, his work has characterised key regulators of mammalian SSC activity and fate, including transcription factors PLZF and SALL4, and the mTORC1 signalling pathway (Hobbs *et al.* 2012; La and Hobbs 2019). More recently, single cell approaches to characterise cellular heterogeneity within the SSC and progenitor cell pool have revealed the dynamic nature of spermatogonial states. This has driven his team's current work in exploring potential clinical applications aimed at improving SSC regenerative capacity or enhancing spermatogenic output. For example, the potential for germline regeneration following chemotherapeutic drug exposure. Recent studies in the Hobbs group have suggested that the regenerative capacity of SSCs following germline damage is promoted by specific microenvironmental growth factors that induce their conversion to a regenerative state. This concept holds promise for application in humans assuming these mechanisms of regeneration are conserved. Understanding key mechanisms regulating SSC fate and function and germline maintenance and regeneration are essential for the development of interventions targeting male infertility.

Emerging technologies such as medical microrobots may aid in reproduction. Medical microrobots are devices a few micrometres in size, engineered from bioavailable polymers and powered by either (1) a catalytic reaction generating bubble-based propulsion, (2) external physical field-based propulsion, or (3) bio-hybrid propulsion wherein sperm, bacterium or other motile biological agents propel the synthetic structure (Medina-Sánchez and Schmidt 2017). These micromotors allow for *in vivo* propulsion, enabling *in situ* diagnosis and targeted cargo delivery of cells and drugs (Agrahari *et al.* 2020). Dr. Mariana Medina-Sánchez (IFW Dresden) highlighted three important applications of medical microrobots; (1) aiding sperm transport in IVF (Schwarz *et al.* 2019) with microrobots fabricated with sharp peaks for high penetration capacity, (2) embryo intrafallopian microrobotic transfer for recurrent implantation failure fabricated with soft terminations (Schwarz *et al.* 2020) and (3) drug delivery (Xu *et al.* 2018). Indeed, the treatment burden male infertility places on the female partner [IVF, intracytoplasmic sperm injection (ICSI)], could potentially be overcome by coupling existing sperm with fabricated micropropellers enabling sperm transport for asthenospermia (reduced sperm motility) (Medina-Sánchez *et al.* 2016) or through the use of guidance scaffolds to ensure rare motile sperm reach their target for oligospermia (low sperm count) (Striggow *et al.* 2020). Drug delivery applications include in gynaecological healthcare for drug delivery within the female reproductive tract and the *in situ* treatment of blood clots by loading microrobots with heparin liposomes to target treatment to regions of interest (Xu *et al.* 2018, 2020). This strategy may also have potential in the targeted treatment or contrast-enhanced imaging of endometriosis lesions (Filby *et al.* 2020). Potential barriers to translation include the immune response to residual microstructure debris, motion control, biocompatibility and regulation. Future work will utilise preclinical models to overcome some of these challenges, where first demonstrations visualizing and controlling microrobots using external magnetic fields in living mice have been already shown (Aziz *et al.* 2021).

In the developing world the rate of STIs and unplanned pregnancies remains very high (Mayaud and Mabey 2004; Bearak *et al.* 2020), whilst in higher income countries there is a need to enable pregnancies particularly in older couples with infertility, and to support lifelong intimate longevity where male and female interventions are democratically available (Woodsong and Koo 1999; Ross and Hardee 2017). Addressing this fertility dichotomy in developing (fertility gap) vs developed nations (fertility crises) is an important avenue for application of reproductive technology. Drawing on experience in both industry and academia, and with assistance of The NSW Medical Devices Fund and early support by The Gates Foundation, Professor Robert Gorkin III (University of Wollongong) launched the start-up Eudaemon, Greek for 'Human Flourishing', to investigate whether condom design could be reimagined to create a superior contraceptive; with the team identifying potential for the technology in conception and reproductive health applications. Using novel hydrogels, Eudaemon has created a condom prototype that, unlike latex, preserves sensation and thus if effective will overcome one of the main barriers to condom usage. Being a hydrogel, the prototype also has the potential of being embedded with compounds including lubricants and therapeutic agents. As a legacy device, condoms have existing specification guidelines to receive TGA or FDA approval. As such, to test the condom hydrogel prototype, advanced manufacturing and digital quality management systems were established by drawing on existing infrastructure in local condom testing facilities. However, most basic science creates new interventions for which there is often no blueprint. Therefore Prof. Gorkin advised scientists engage with both consumers and funders to ensure that there is good alignment of needs to justify investment for development. Contraception and conception both remain human rights and gender equality issues, and reproductive health and choices are essential to our economy. A platform materials technology offering solutions for both contraception and conception may create novel solutions enabling these dichotomous needs (Cook *et al.* 2021).

Collectively, these examples of innovative technologies in reproduction demonstrate our exciting capacity as researchers to use novel approaches to solve unmet needs in reproductive biology informed by robust knowledge in basic science. Application of new technologies are a vital component of reproduction research in Australia, New Zealand, and abroad and will be a key driving force of our research impact into the future.

Lessons from non-model species

Speakers: Jane Fenelon, Erica Todd, Taylor Pini, Paul Waters

The diversity and complexity of reproduction in non-model species offers unique insights into the fundamental mechanisms of sex determination, development, and fertility. This symposium discussed non-model species specific research on Y chromosome preservation in therian mammals, sex determination and development in monotremes and fish, and functions of seminal plasma in sheep.

The Y chromosome of therian mammals, such as humans, has long been considered a 'wimpy' X chromosome relic destined for inevitable demise. This theory is based on the idea that only a few key remaining functions have ensured its survival, and thus if mutations occur, the resultant genetic decay or removal of functional genes would lead to its loss. Despite this, the demise of the Y chromosome has only been recorded in a handful of therian mammals such as *Ellobius* (Mulugeta *et al.* 2016), and compared to other vertebrate lineages, the therian XY genetic sex determination system is uniquely persistent. Professor Paul Waters (The University of New South Wales) proposed a persistent Y hypothesis to explain the ongoing survival of the Y chromosome in therian mammals (Waters and Ruiz-Herrera 2020). Under this hypothesis, the Y chromosome contains meiotic executioner genes which play essential roles during meiosis but need to be silenced during the meiotic sex chromosome inactivation (MSCI) window because their expression is pachytene lethal (Royo *et al.* 2010). Thus, translocation of executioner genes from the Y chromosome to autosomal genomic regions leads to ectopic expression during the MSCI window and prevents progression through meiosis. Supporting this hypothesis, transgenic insertion of zinc finger protein Y-linked (*Zfy*) 1 and 2 onto an autosome, lead to ectopic expression of *Zfy1/2* and subsequent pachytene arrest in mice (Royo *et al.* 2010). Such a surveillance system inhibits the removal of executioner genes from the Y chromosome preventing the Y chromosome from being lost (Waters and Ruiz-Herrera 2020). The proposal of the persistent Y hypothesis improves our understanding of the mechanisms the Y chromosome employs to allow its persistence for millions of years.

In teleosts, sex determination is not as simple as becoming male or female. Sequential hermaphroditism, where an adult reproduces as one sex before undergoing a total sex change in response to social or environmental cues, highlights the natural plasticity of sexual fate (Devlin and Nagahama 2002). Through her research on wrasses, Dr. Erica Todd (Deakin University) aims to understand the drivers of this plasticity. Blue-head wrasse (*Thalassoma bifasciatum*) begin transitioning from female to male within minutes of the socially dominant male being removed (Todd *et al.* 2019). Early stages of this transition include a dramatic decrease in estrogen and an increase in 11-ketotestosterone (Todd *et al.* 2019). Aromatase, which is responsible for the conversion of androgen to estrogen (Piferrer and Blázquez 2005), is a common denominator in protogynous hermaphrodite species. Downregulation of the gonadal aromatase gene, *cyp19a1a*, occurs in rice field eels during sex change (Zhang *et al.* 2008; Liu *et al.* 2009), and implantation of aromatase inhibitors induces sex change in red spotted groupers (Li *et al.* 2006). Thus, the regulation of *cyp19a1a* expression may point to early triggers of sex change in protogynous species. Epigenetic remodelling is key to sexual transition, with whole methylome approaches showing the *cyp19a1a* transcription start site is hypermethylated and subsequently silenced during sex change (Todd *et al.* 2019). Social triggers likely induce widespread epigenetic reprogramming leading to an expression change of the aromatase gene, subsequent masculinization of the hormone profile and gonadal remodeling resulting in female to male transition within 20 days. By studying the mechanisms of sequential hermaphroditism, we can gain new understanding of how epigenetic and transcriptome-level changes can influence gonadal development and sexual fate.

Monotremes diverged from therians (marsupial and eutherian mammals) approximately 187 million years ago (Zhou *et al.* 2021) and provide key insights into understanding how mammalian reproductive traits evolved. Research by Dr. Jane Fenelon (The University of Melbourne) aims to elucidate how the sexual development of the short-beaked echidna (*Tachyglossus aculeatus*) differs from therian mammals. Similar to marsupials, echidnas are born very altricial with the majority of their development occurring after hatching (Griffiths 1968). For example at hatching, the echidna gonads are undifferentiated, similar to the tammar wallaby (*Macropus eugenii*) at birth (Griffiths 1968; Renfree *et al.* 1996). In the tammar, the first signs of morphological gonad differentiation are in the testis 2 days after birth with the ovary first recognisable 6 days later (Renfree *et al.* 1996). In contrast to the tammar, and all other mammals examined to date, the echidna ovary is the first to morphologically differentiate, 3 days after hatching (unpubl.). This is despite echidnas possessing an XY sex chromosome system (Rens *et al.* 2007) with many of the genetic sex determining pathway markers present in both gonads at this stage (unpubl.). However, monotremes lack *SRY*, the sex determining gene in almost all therian mammals, and the master sex determining gene in monotremes is unknown. This suggests SRY evolved after monotremes diverged from therian mammals (Wallis *et al.* 2008). Monotremes display a unique combination of mammalian and reptilian-like features (Grützner *et al.* 2003). In studying the development of reproduction in species like the echidna, we may better understand the evolution of reproduction across Animalia, gaining new insights into how and why different mechanisms evolve and what this could mean for future evolution.

Seminal plasma (SP) is an acellular mixed fluid produced by the accessory sex glands that sperm are first exposed to at ejaculation (Rolland *et al.* 2013). While not required for fertility, there is increasing evidence SP has important roles in improving reproductive success and Dr. Taylor Pini (The University of Queensland) is exploiting these functions to improve reproductive success in sheep assisted reproductive technology (ART). The sheep cervix has a unique physical structure, with a narrow and contorted lumen separated by several misaligned cervical rings, which presents a significant barrier during sperm transit. When ram epididymal spermatozoa are deposited in the cervix, exposure to SP is shown to significantly improve pregnancy rate compared to unexposed controls, by facilitating spermatozoa transit through the cervix (Rickard *et al.* 2014). In humans and other animal models, SP exposure promotes sperm function and assists their interactions with the female reproductive tract (Rodríguez-Martínez *et al.* 2011; Zhou and Dimitriadis 2020). However, many functions of SP are still unknown and species-specific functions likely exist. Through proteome comparisons between epididymal and ejaculated spermatozoa, Dr. Pini and her colleagues have revealed only three proteins in sheep are detected solely in the ejaculated spermatozoa (Pini *et al.* 2016). Of these detected proteins, binder of sperm protein (BSP)5 and its other family member BSP1 have suggested regulatory roles in sperm capacitation, acrosomal reaction and membrane integrity (Plante *et al.* 2016; Pini *et al.* 2018*a*). The physiological roles of SP have translational potential to improve ART outcomes in sheep, particularly when using cryopreserved spermatozoa. Cryopreservation causes freezing damage and cryocapacitation, which shortens the fertilizing life span of sperm. The addition of individual BSPs improved cryopreservation outcomes in ram spermatozoa (Pini *et al.* 2018*b*), demonstrating the supportive role these proteins play in the SP. Collectively, this work is an example of how proteomics can directly lead to translational outcomes, and reveals how research focused on improving ART within agricultural breeding systems can provide fundamental mechanistic insights into SP function.

Reproductive ageing and sustaining a pregnancy

Speakers: Alison Care, Stephen Tong, Dulama Richani, Craig Pennell

There are many challenges in reaching the end goal of a healthy live birth, with poor oocyte quality, pregnancy complications and pre-term birth among the most common issues. A mutual underlying risk factor for these conditions is maternal age (Cimadomo *et al.* 2018; Bouzaglou *et al.* 2020), which continues to increase in modern society (Australian Institute of Health and Welfare 2021). Due to their high frequency and significant consequences, poor oocyte quality, pregnancy complications and pre-term birth continue to be heavily investigated, and research is beginning to yield clinical solutions to these issues. This symposium discussed research which addresses the challenges faced by reproductively ageing women, and how therapies are being developed to improve outcomes for both the pregnant person and child.

Oocyte developmental competence (ODC) is defined as the ability of an oocyte to mature into a fertilisable egg which can sustain development to a foetus. ODC decreases with age (Cimadomo *et al.* 2018), and is a major cause of age related infertility and IVF cycle failure (Ubaldi *et al.* 2019). Interventions to improve ODC are highly sought after, with one promising approach discussed by Dr. Dulama Richani (The University of New South Wales) being support of the bi-directional communication between the oocyte and its surrounding cumulus cells (Richani *et al.* 2021). GDF9 and BMP15 are essential oocyte-secretory factors that are hypothesised to form a heterodimer called cumulin. Cumulin is a potent stimulator of granulosa cell proliferation *in vitro* and improves ODC of oocytes matured *in vitro* (Mottershead *et al.* 2015). Cumulin has significant effects on metabolic processes in both cumulus cells and oocytes, wherein it is believed to remodel oocyte mitochondria to maintain a quiescent state, thus improving ODC (Richani, unpubl. data, 2021). Importantly, the addition of cumulin to oocyte *in vitro* maturation (IVM) culture media increases blastocyst yield in murine (Stocker *et al.* 2020), porcine (Mottershead *et al.* 2015), equine (Metcalf *et al.* 2020), and human embryos (unpubl.), highlighting cumulin supplementation as a promising therapy to improve ODC in humans, and in livestock breeding. The development of therapies supporting the oocyte and the vital functions of its surrounding cumulus cells opens a promising avenue for improving ODC and thus improving clinical outcomes for IVM.

Beyond conception, pregnancy complications remain a significant medical and financial burden in human reproduction. Of these complications, preeclampsia is one of the most common and severe (Hogan *et al.* 2010; Wanderer *et al.* 2013). The pathogenesis of preeclampsia includes two stages; abnormal placentation early in the first trimester and subsequent excess circulating antiangiogenic factors (Rana *et al.* 2019). An aberrant maternal immune response is potentially a key determinant in the development of preeclampsia. Both adaptive and innate maternal immune cell subsets are key regulators of trophoblast invasion and spiral artery remodelling (Faas and De Vos 2018). Regulatory T cells (Tregs), a specialised $CD4^+$ T cell subset, are important mediators of maternal-foetal tolerance by regulating peri-implantation inflammation (Miller *et al.* 2021). Recent investigations, discussed by Dr. Alison Care (The University of Adelaide), demonstrated that induced Treg depletion during peri-implantation resulted in reduced pregnancy rates, with increased foetal resorption, elevated circulating inflammatory cytokines, and perturbed uterine arterial function demonstrated by enhanced conversion of inactive big endothelin-1 to the active vasoconstrictor endothelin 1 (Care *et al.* 2018). These data, along with evidence of perturbed Treg populations in preeclamptic patients (Robertson *et al.* 2019; Miller *et al.* 2021), suggest that Tregs play important roles in maintaining maternal vascular function during pregnancy. New evidence demonstrates a link between Treg depletion, uterine natural killer cell abundance and insufficient spiral artery remodelling (Care, unpubl. data, 2021), suggesting a potential mechanistic cascade from Treg disturbance to abnormal placentation and subsequent features of preeclampsia. The immune system is well known to play a pivotal role in pregnancy, thus understanding how imbalances in immune cell populations influence preeclampsia development and pregnancy success is key in developing screens to identify and support at-risk pregnancies.

While investigations into the fundamental pathogenesis of preeclampsia continue, there is also a concerted effort to produce meaningful diagnostic tests and effective clinical treatments. In a clinical setting, history and routine assessment are used to identify suspected preeclampsia, with diagnosis reliant on intensive, expensive monitoring (Rana *et al.* 2019). Given this context, early diagnostic biomarkers remain an important aspect of preeclampsia research. A decade of preclinical and clinical research has seen the anti-angiogenic factor, soluble fms-like tyrosine kinase-1 (sFlt-1) and pro-angiogenic, placental growth factor (PIGF) emerge as excellent candidates, with negative predictive values of up to 99.3% (Zeisler *et al.* 2016; Cerdeira *et al.* 2019; Cruickshank *et al.* 2021). There is now a push to include these biomarkers in the clinical definition of preeclampsia (Stepan *et al.* 2020). Beyond diagnosis, clinical treatments for preeclampsia remain limited to low dose aspirin and induced delivery (Fox *et al.* 2019). However, as discussed by Professor Stephen Tong (The University of Melbourne and Mercy Perinatal), both preclinical and clinical studies suggest that drugs such as esomeprazole, sulfasalazine (Binder *et al.* 2020), beta blockers (Binder *et al.* 2021) and in particular metformin, which is showing the most promise (Cluver *et al.* 2021), may provide an avenue to maximise gestation length in preeclamptic patients. In addition, as the importance of Tregs in preeclampsia continues to come to light, there is a drive for clinical treatments which centre around increasing abundance or restoring function of aberrant Tregs (Robertson *et al.* 2019). Being able to safely maximise the length of gestation in preeclamptic patients can have vast impacts on a baby's overall health, and current research is showing that with currently available therapies yielding promising results, there is optimism that implementation of these inventions is close.

Preterm birth is yet another pregnancy complication with a direct positive risk relationship to maternal age at conception (Astolfi and Zonta 1999; Fuchs *et al.* 2018). Preterm birth can result in a range of short-, intermediate-, and long-term complications for the child (Luu *et al.* 2016), therefore investigation of therapeutics to prevent preterm birth are critical. Research discussed by Professor Craig Pennell (The University of Newcastle) investigating the effect of omega-3 supplementation from 16 weeks gestation found that the number of preterm births (<34 weeks gestation) was reduced by 42% (Middleton *et al.* 2018), and preterm infants had longer gestation times and shorter hospital stays than those in the placebo group (Carlson *et al.* 2013). In addition, analysis of 41 randomised control trial studies found omega-3 supplementation resulted in an increased gestation length of 1.67 days (Middleton *et al.* 2018). However, intensive work has elucidated that the effectiveness of omega-3 supplementation to prevent pre-term birth is dependent on DHA serum levels of prior to 20 weeks gestation, with supplementation potentially increasing preterm birth risk in those who are already replete (Simmonds *et al.* 2020). Analysis of DHA level is costly and hence not feasible to be implemented as a standard test early in pregnancy. Development of cheaper testing methods and/or better predictive tools of a pregnant person's circulating DHA level is a key prerequisite to ensure targeted, beneficial use of omega-3 supplementation as a therapeutic for pregnancies at risk of preterm birth.

Maternal reproductive ageing presents a vast array of challenges from conception to placentation, to maintaining the pregnancy to term. Australian research is leading the way in identifying how these challenges arise and, importantly, how these pregnancies can be supported through safe therapeutic interventions to provide the best outcomes.

Advances in contraception

Speakers: Lee Smith, Darryl Russell, Timothy Jenkins, Damian Dowling

This session covered several different approaches that could be used to develop effective contraceptives to address the current urgent need for a broader range of safe and reversible fertility regulation methods.

Advancements in gene-therapy vectors are allowing for more rapid and cost-effective methods of studying and manipulating gene function in mice, compared to traditional transgenic and gene knockout mouse lines. Professor Lee Smith (University of Newcastle) and his team have been testing the utility of different gene therapies in selective genetic manipulation of somatic cell populations of the mouse testis. The ideal gene therapy is easy to deliver, long lasting, and cell specific, but has low immunogenicity and minimal side effects. To date, naked plasmid DNA, liposomes, lentiviruses, adenoviruses, adeno-associated viruses (AAV) and retroviruses have all been used as deliverable transgenic vectors. One of the main challenges in delivering gene-therapy vectors to the seminiferous tubule compartment of the testis, is that high pressure delivery systems disrupt testicular architecture, leading to inflammatory damage compromising the blood-testis barrier. Professor Smith's team bypassed this by injecting vectors into the rete testis under controlled pressure and found that lentiviral vectors delivered in this way specifically target Sertoli cells without transducing germ cells. Using this method, his team have been able to block spermatogenesis by inhibiting key Sertoli cell genes and conversely, rescue infertility in a Sertoli Cell Androgen Receptor Knockout mouse by restoring androgen receptor expression in Sertoli cells (Willems *et al.* 2015). His team is also optimising gene-therapy targeting of Leydig cells, having found that interstitial testis injections of lentiviruses, adenoviruses and AAVs result in transduction of only the Leydig population. However, lentiviral targeted Leydig cells underwent apoptosis 10 days after injection, and while adenoviral targeted Leydig cells did not undergo apoptosis, transgene expression was transient, and an immune response was elicited. More promisingly, co-injection of AAV-9 together with neuraminidase successfully targeted Leydig cells without causing apoptosis, and transgene delivery using this method was used to successfully reduce the expression of key steroidogenic genes (Darbey *et al.* 2021). This work provides proof of principle for future approaches to suppress spermatogenesis and androgen production, thus selectively manipulateing testis function.

The need for contraception of 218 million women of reproductive age worldwide remains unmet (Sully *et al.* 2020), while the current progestin based pharmaceutical contraceptives have increasingly recognised undesirable off-target effects. There is an urgent need for the development of new safer female contraceptives. Such a contraceptive must have minimal off-target effects and therefore associated health risks, as well as being reliable and reversible. By taking advantage of the fact that the cumulus-oocyte complex (COC) acutely acquires adhesive capacity prior to ovulation (Akison *et al.* 2012), Professor Darryl Russel (The University of Adelaide) and his team are developing scalable biosensor systems to screen large compound libraries for drugs and cell targets that can be further investigated as potential ovulation-blocking contraceptives. Additionally, the transcription factor progesterone receptor (PGR), which is highly expressed in the ovary during the peri-ovulatory window and displays tissue-specific expression in reproductive tissues (Dinh *et al.* 2019) is essential for ovulation. Through vertical integration of ATAC-seq, ChIP-seq and RNA-seq, the ovarian-specific transcriptional activity of PGR is being identified, and this specific mechanism can be targeted for the development of novel non-hormonal contraceptives.

Vasectomy is a leading method of permanent male contraception. Currently, there are no immediate post-surgical methods to establish that the connection between the testes and the rest of the male genital tract has been severed effectively. To address this, Assistant Professor Timothy Jenkins (Brigham Young University, USA) is developing an epigenetics based ‘cell of origin’ analysis. Epigenetics, in particular DNA methylation, is the fingerprint for a cell type as it discriminates between tissues. Distinct DNA methylation signatures set sperm apart from somatic cells and can be used to identify the cell of origin of a single molecule of DNA in a heterogenous cell mix, or in cell-free DNA (Pollard and Jenkins 2020). This cell of origin epigenetic analysis can thus be used to assess the success of vasectomy by demonstrating sperm derived cell free DNA is underrepresented in semen post-surgically. It can also be used as a non-invasive diagnostic method of non-obstructive azoospermia, where no cell free DNA is present in semen. Forensic medicine could also benefit from this analysis, since it enables rapid, quantitative confirmation of the presence of sperm.

Effective suppression of reproduction is also the goal of population control of pest species. Professor Damian Dowling (Monash University) has bridged evolutionary theory and reproductive manipulation with the concept of sex-specific selection of mitochondrial gene mutations (the Mother’s Curse hypothesis), and suggested implications in pest population control as well as a contraceptive measure. The Mother’s Curse is a hypothesis in which mutations in the mitochondrial DNA (mtDNA) that are specifically detrimental to male fertility are predicted to escape natural selection through the inheritance of solely maternal mitochondria during fertilisation (Dowling and Adrian 2019). A clear application to the Mother’s Curse phenomena is in pest control, in which ‘Trojan females’ carrying mtDNA mutations that are harmful to male fertility but are neutral or even beneficial to female viability are introduced into the wild population, allowing for effective and sustainable control of pest population (Wolff *et al.* 2017). To illustrate this, proof-of-concept experiments were performed on *Drosophila melanogaster* populations, where an introduced mtDNA haplotype known to be disadvantageous to male fertility led to a sustained, but not complete, reduction in population viability over generations. As Mother’s Curse mutations are predicted to be linked to a coevolved nuclear background that can neutralise the infertility effects of the Mother’s Curse (Yee *et al.* 2013), new male contraceptives that temporarily target such counter-adaptive genes may allow mitochondrial-driven male sterilisation to take effect.

In summary, this session highlighted the use of novel techniques, including gene delivery systems, genomic and epigenomic techniques and practical application of evolutionary concepts, that are currently being explored to develop safe, effective contraceptives for both males and females.

Bridging the gaps from discovery to impact

Speakers: Jeremy Thompson, John Newnham

Through the research impact stories of Professor Jeremy Thompson (The University of Adelaide) and Professor John Newnham (The University of Western Australia), this session explored novel insights when considering application of basic and translational research model discoveries to both business industry and government policy. Converting discovery to impactful translational outputs requires firm collaborative relationships between academic and industrial scientists (Kleiman and Ehlers 2019). Elucidating these relationships, Professor Thompson presented his personal journey in translating research discoveries into two start-up companies; ART Lab Solutions Pty Ltd (est. 2018; https://www.artlabsolutions.com/) and Fertilis (est. 2019; https://www.fertil.is/). His discovery-to-translation journey began with an opportunity to develop a formulated solution for cattle embryo recovery, cryopreservation and transfer for ICPbio™ (https://icpbiorepro.com/emcare-embryo-transfer-products.html); a product that remains in the marketplace 26 years later. Due to his active research career, Professor Thompson was also approached for consultancy and product development work by Cook Medical (https://www.cookmedical.com/). Reflecting on his time, the lessons he learnt were that there are always missed opportunities to further develop a product due to the restrictive nature of contract research; and, in industry the foundation for success is the fostering of relationships. Key pieces of advice Professor Thomson gives for success in research translation are a realistic understanding of the timeframe required to bring an idea to the marketplace; an understanding that regulatory bodies have a very different perspective to the scientist; and those relationships underpin the success of products.

Building from these experiences, Professor Thompson began independently creating medical devices by establishing an internal business unit (IVF Vet solutions) with the University of Adelaide. This support from the university created employment within the group, providing experience for the next generation of young researchers. Crucially, this experience guided the formation of his first start-up company, ART Lab Solutions, which develops reproductive technologies to accelerate livestock breeding. While small investments from the South Australian Government and The University of Adelaide helped initially, having a strong product was crucial for organic growth. Independence brought new challenges, chief amongst them the internal distribution of products for continued growth, and resilience in adapting to new market challenges. As put by Professor Thompson himself, it has taken his whole career to get to what his latest company, Fertilis, is today. The challenges faced along the way expose your true weakness and highlight the importance of the diversity of the team you assemble, both internally and externally, to ensure the success of your company. Professor Thompson's collective experiences, reveal the inherent trade-offs between engaging with established companies, versus taking the entrepreneurial route when commercialising research. Whilst the former involves less risk, it offers minimal intellectual control. Conversely, the risks are significantly increased in startups, but the implementation of ideas is unrestricted. If one has the passion and the entrepreneurship, one can start a business early in their career.

Recognising successful programs that have closed the gap between basic research discovery and implementation of policy in politics and practice allows researchers, academics, and clinicians to drive change within healthcare in Australia and New Zealand. This was the focus of Professor John Newnham's presentation. The Australian Preterm Birth Prevention Alliance (APBPA) is a collaborative group that demonstrate the potential impact of research discovery on preventative health. The alliance was the first Australian program designed to prevent preterm birth (PTB), and specifically addressed seven key areas of intervention to prevent PTB (Newnham *et al.* 2014). National outreach through 'The Whole Nine Months' campaign outlined these key clinical practice points. Consequently, and after implementing the PTB prevention program for 1 year, Western Australia saw a 7.6% reduction in the rate of singleton PTB (Newnham *et al.* 2017).

The APBPA group are now the first group in the world to implement PTB prevention strategies at a national level, with the alliance securing AUD13.7M in federal funding as part of the Australian Commission on Safety and Quality in Health Care. Unsurprisingly, the formation of the APBPA alliance presented challenges, the greatest of which was implementing policy change in determining the correct interventions in preventing PTB since decades of research show many interventions have no impact (Puthussery *et al.* 2018). Another challenge was the lack of contemporary relevance of research and acknowledging that the importance of some research may only be recognised in decades to come. Addressing these issues required reliance on, and production of, high-quality research that is irrefutable, thus avoiding introducing policy too early. Notably, a key initial step in building the APBPA alliance was development of a peer review framework, founded in high-quality research, outlining implementation strategies in prevention of PTB (Newnham *et al.* 2014). It was also important to acknowledge that failure to communicate research effectively also hinders impact of research (McKee 2019). Examples include the use of technical language incomprehensible to the greater audience, the use of language that renders findings obscure, and by failing to engage and disseminate to peers.

In summary, successful translation of scientific discovery into policy or industry practice, must be based on the development of a strong product or clear guidelines with sound scientific foundations. Establishment and maintenance of strong professional relationships is also of paramount importance as it opens the door for new opportunities.

Conclusion

Despite the restrictions imposed by a global pandemic, the 2021 annual SRB meeting continued to provide a platform for Australian and New Zealand reproductive biologists to disseminate advances in the field. The 2021 meeting highlighted the importance of research translation into both policy and industry practice and provided insights on how this can be achieved. Innovative approaches to contraception were presented, including the development of non-hormonal female contraceptives, gene therapy-based male contraception, and reproductive control of pest species. At the other end of the spectrum, research into conception was presented, with a focus on advancements in understanding what constitutes reproductive health and therapeutic innovations to improve outcomes for pregnant people and offspring. Research in non-model species provided significant new insights into the evolution of reproduction. Furthermore, the highly innovative research presented on the development of new diagnostic and ART technologies (such as diagnostic hyperspectral imaging, medical microrobots, and hydrogel condoms) highlighted the power of cross-disciplinary collaboration between scientists, clinicians, engineers, physicists, bioinformaticians, and mathematicians. The 2021 SRB virtual meeting was successful in uniting reproductive biologists from across Australia and New Zealand under challenging circumstances, thus providing fertile ground for the development of new ideas and for the progress of this close-knit community.

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