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Reproduction, Fertility and Development, 2016; OnlinePubl:A-G

Journal compilation © CSIRO 2016

Originally Published at: <http://dx.doi.org/10.1071/RD16382>

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7 April 2017

<http://hdl.handle.net/2440/104351>

**The Current State of Reproductive Biology Research in Australia and New Zealand:
Core Themes from the Society for Reproductive Biology Annual Meeting, 2016**

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Abstract

~~Reproduction is central to the understanding of all biology. Dysregulation of reproductive processes, particularly gametogenesis, implantation and pregnancy, can contribute to a variety of disease states in offspring through to adulthood. The Society for Reproductive Biology (SRB) 2016 conference held on the Gold Coast (QLD, Australia) displayed the breadth of reproductive research currently underway in Australia and New Zealand with additional insights from world leaders in the field. This conference review provides a broad summary of the key questions, emerging ideas, and novel technologies that were presented by symposia. We highlight the importance of reproductive biology research in furthering basic knowledge fundamental to reproduction, as well as improving the long term health of the mother, the fetus, and the child into adulthood. In addition we highlight the success of conservation biology research in this country. More disciplines should consider how reproductive biology intersects with, and potentially influences, other biological networks given its fundamental importance to all life.~~

As reproduction is essential for all life, it is central to our understanding of all aspects of biology. The Society for Reproductive Biology (SRB) 2016 conference held on the Gold Coast (QLD, Australia) displayed the current breadth of reproductive research in Australia and New Zealand, with additional insights from world leaders in the field. This conference review provides a focused summary of the key questions, emerging ideas, and novel technologies that were presented in the symposia. Presented research demonstrated key advances in how stem cell biology may allow us to better understand pluripotency, as well as how environmental and lifestyle factors such as circadian disruption, smoking, alcohol and diet impact on gametogenesis, embryo implantation, placental function, and reproductive capacity. Sessions also highlighted the role of reproductive biology in providing insight into

the mechanisms and processes governing a wide range of biological science disciplines, including cancer research and therapies, oncofertility, conservation of native species, and chronic non-communicable diseases. Recurring themes included the importance of male and female gamete quality for reproductive potential and the critical and varied roles of the placenta in the maintenance of a healthy pregnancy. Dysregulation of reproductive processes can contribute to a variety of pathological states that impact on future health, fertility and fecundity. Research being conducted by the SRB has the potential to impact not only the fertility of the current generation, but also the health and reproductive viability of future generations.

For Review Only

Introduction

The Society for Reproductive Biology (SRB) aims to foster and promote basic and applied research in reproduction, fertility, and development directed towards improving reproductive outcomes, lifelong health, and species conservation. This conference highlighted current research from these respective fields with a range of national and international invited speakers.

This review presents the overarching themes of the conference symposia and highlights the latest and most exciting research in reproductive biology, predominantly in Australia but also throughout the world. Our goal is to inform a wider audience and place this research into a broader scientific and societal context.

Big Ideas in Pluripotency and Reprogramming (Public Forum)

Speakers: Palpant N, Nottle M, Wells C, Gargett CE and Western P.

Stem cell science is rapidly advancing with the goal to answer fundamental questions about health and development and to ultimately cure disease. This session provided the background to the current state of stem cell research in Australia.

The key to advancing our knowledge in the factors underpinning cell fate is utilisation of emerging 'NextGen' technologies and the production, analysis, and integration of 'big data' sets. Current methods in sequencing provide insights into nuclear architecture, chromatin dynamics, gene expression, and much more. The standard approach to elucidating the molecular basis of phenotypes has relied on gene expression profiling. However, determining the differential expression of genes (often defined by researcher imposed parameters) is restrictive, relying on informatics approaches looking at gene ontology or pathway analysis. Technological advances such as single-cell sequencing increase our power to determine individual changes at the cellular level but still retain limitations related to expression

profiling. Transcriptomics data needs to be integrated with other ‘omic’ approaches to capture the full extent of molecular complexity.

The contribution of the epigenome must also be considered as modifications in chromatin involving the methylation and acetylation of histones provide us with a more fundamental view of the regulation behind gene expression. Dysregulation of chromatin regulators can result in pathologies such as cancer (Luna-Zurita and Bruneau 2013; Palpant *et al.* 2013). Chromatin dynamics helps reveal how genomic regions interact with one another and has allowed us to distinguish cell structure/function genes from those controlling cell identity (Paige *et al.* 2012). Moving forward, the integration of multiple omics approaches will be an important method to determine the basis of cell identity, fate, and function.

Many challenges in stem cell biology remain. One key question being investigated is “what is the best process to isolate pluripotent stem cells?” This question is confounded by the fact that the loss of pluripotency and the onset of cell differentiation occur at different stages for different species (at least for mice, humans, and pigs); suggesting caution is required when drawing conclusions from experimental models. For example, mouse embryonic stem cells (ESC) are more pluripotent than human embryonic stem cells at equivalent stages of embryo development (Vassiliev *et al.* 2010; Davidson *et al.* 2015). Furthermore, there are limited naïve human ESC lines used for research, restricting the full potential of future ESC therapies (Ware 2014). The recent discovery of mesenchymal stem cells (MSC) in human endometrium has proven exciting, as these cells can now be easily isolated with a minimally invasive procedure and, importantly, maintain multipotency in culture (Darzi *et al.* 2016). These cells can be sourced from various tissues and are being clinically trialled as a cell-based therapy for many pathologies, particularly chronic inflammatory and immunological disorders (*e.g.* Graft vs Host Disease).

In order to continue to advance stem cell biology, collaborative frameworks need to be developed, particularly between systems biologists, bioinformaticians, and biostatisticians, to navigate the complex web of factors involved in stem cell pluripotency and differentiation. This knowledge, coupled with significant research investment, offers exciting promise for future therapies.

Pathologies of Pregnancy (Joint SRB/ANZPRA Symposium)

Speakers: Redman C, James J, Kaitu'u-Lino T and Mark P.

The placenta is the fundamental conduit between mother and fetus which, when not functioning appropriately, leads to pregnancy disorders. Furthermore, a dysfunctional placenta can lead to poor fetal growth which may increase disease risk in pregnancies that persist to term and impair offspring health. This session provided a context of the past, present and future of research into pregnancy pathologies in relation to the placenta. Preeclampsia is a common pregnancy complication affecting 5–10% of pregnancies in Australia. Current evidence demonstrates that the syncytiotrophoblast plays a critical role in disease pathogenesis. When the syncytiotrophoblast layer undergoes cellular stress, a number of factors are secreted into the maternal circulation, resulting in endothelial dysfunction and symptoms of pre-eclampsia (Redman and Staff 2015). Emerging evidence indicates that all post-term pregnancies may show symptoms of preeclampsia, suggesting that as pregnancies progress, syncytial stress increases as the placenta reaches full capacity and late-onset preeclampsia may develop (Redman and Staff 2015).

Many pregnancy disorders, including preeclampsia and intrauterine growth restriction (IUGR), originate in early gestation as a result of poor placental development and angiogenesis. Innovative collaborations between mathematicians and biologists are currently underway to create a multiscale model of the normal feto-placental vasculature (Clark *et al.*

2015) and then adapting this to represent an IUGR phenotype. This is providing insight into how the anatomical changes seen in the IUGR placenta relate to functional differences in placental perfusion and fetal growth. Additionally, novel therapeutics have been proposed that would simultaneously target the production of factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sENG) that are implicated in the pathogenesis of preeclampsia (Brownfoot *et al.* 2016). Novel research into placental mesenchymal stromal cells is also defining their role in placental angiogenesis and potential contribution to IUGR (Mando *et al.* 2016).

Clearly fetal growth can also be influenced by the maternal environment. Factors such as maternal obesity can lead to impaired fetal development and programmed disease in offspring. Circadian disruption can also contribute, as maternal light exposure and temperature during pregnancy regulates maternal, placental, and fetal circadian rhythms. This in turn can affect the mother and her offspring through dysregulation of hormone rhythmicity and placental regulation of maternal physiology. For example, expectant mothers who undertake shift-work during their pregnancy are at risk of having a small for gestational age baby (Abeyseena *et al.* 2009). Potential disruption of circadian rhythmicity and its associated implications for health is also an important consideration during the post-natal period in pre-term infants exposed to inappropriately prolonged periods of light, as occurs in neonatal intensive care units (NICU) around the world (Morag and Ohlsson 2013).

With so many pregnancy disorders originating from placental dysfunction, studies such as those presented in this symposium highlight the need for continued research into this underappreciated organ that is central to a healthy life.

Insights into the Regulation of Implantation, Pregnancy and Parturition

Speakers: Dimitriadis E, Pringle K, Hart R and Sharkey D.

Successful *in utero* development and optimal health early in life underpins ongoing offspring fitness. Interactions at the maternal-fetal interface and how these promote pregnancy success was a strong recurring theme in this session. Research was presented on seminal events at copulation, factors mediating implantation success, novel risk-factors triggering spontaneous preterm birth, and the use of data linkage of these early events to predict determinants of adult health. Preparation for pregnancy is essential within the female immune system, including prior to fertilization, and requires establishment of an immunological response that promotes tolerance towards the semi-allogenic fetus (Robertson and Sharkey 2016). However, an appropriately 'primed' reproductive tract is not enough, with the blastocyst itself contributing to successful implantation. Exciting Australian research using human blastocysts is revealing unique biomarkers, such as miRNAs secreted by the blastocyst, to predict implantation success, and suggests that human embryos actively participate in their destiny to implant (Cuman *et al.* 2015). In terms of pregnancy maintenance once implantation has occurred, fascinating evidence implicates the renin-angiotensin system, better known for facilitating blood pressure homeostasis, in the maintenance of fetal membrane integrity and may be associated with risk for preterm labour and premature rupture of membranes in women carrying a female fetus (Pringle *et al.* 2015). However, sex-biased responses during pregnancy are not unique to the amnion, with an abundance of data supporting altered responses to stress in early and late pregnancy. Typically male embryos are more robust prior to implantation (Green *et al.* 2016), while female embryos perform better when challenged post-implantation (Bruckner *et al.* 2015).

Research in this session also highlighted the synergies that result from integrating cutting-edge basic research with robust clinical studies. For example, large clinical cohorts and superb data linkage are helping to address important long-term health outcomes for patients diagnosed with Polycystic Ovary Syndrome (PCOS). PCOS is associated with an increased

risk of vascular, metabolic, and psychological disease suggesting that health care resources should be directed accordingly to reduce these risks (Hart and Doherty 2015).

This session provided many examples of the interdependency of health events throughout an entire life-course. Understanding the critical links between conception, parturition, and long-term health is a field in which researchers from the SRB are excelling.

Determinants of Gamete Quality

Speakers: Bowles J, Russell D, Loveland K and Homer H.

The development and maturation of gametes is exquisitely sensitive to endogenous signals in the gonads and exogenous or environmentally-derived stimuli. Although the key mediators of these processes aren't fully elucidated, a complex balance of signals appears to be important for determining prenatal germ cell fate – a complexity that has many knowledge gaps that the latest research presented in this session aims to address. For example, what induces or suppresses germ cell entry into meiosis during development? Only female germ cells are induced to enter meiosis during development while this is suppressed in male germ cells, with elegant knock-out studies revealing some of the major factors in this process include retinoic acid, CYP26B1 and ALDH1A1 (Bowles *et al.* 2016).

Also highlighted in this session was the crucial molecular signals and intrafollicular changes required for successful ovulation. Emerging evidence indicates that oocytes themselves promote expression of key genes in the surrounding cumulus cells which mediate ovulation, thus through this interaction oocyte quality is a key determinant of ovulation (Russell and Robker 2007). While oocyte signalling is permissive, the critical trigger of the ovulatory cascade is the progesterone receptor in granulosa cells, which controls the final stages of follicle/oocyte maturation and subsequent ovulation (Robker *et al.* 2009). Current studies using global transcriptional approaches aim to identify other major players. These factors

may have clinical implications as indicators of oocyte quality and developmental potential (Gebhardt *et al.* 2011).

But what about male gametes: Are there specific markers which identify the developmental potential of sperm? One possibility may be specific members of the Importin family of nuclear transporters which play critical roles in sperm maturation and development. Each has a distinct cellular localisation and expression profile in the testis, correlating with specific developmental switches in spermatogenesis (Loveland *et al.* 2015). This has been similarly demonstrated within the ovary (Mihalas *et al.* 2015). The levels of one of these, Importin- α 4, correlates directly with the capacity for spermatids to survive under conditions of oxidative stress (Young *et al.* 2013), highlighting one example of how Importins can control gamete fate.

As discussed throughout the session, germ cell development during fetal life can have intergenerational impacts on adult health and fertility. Gamete age, both in terms of a mother or father's age or the length of time since collection, is a great predictor of pregnancy success. But why does oocyte quality and female fertility decline at such a relatively young age (~mid-thirties), at least a decade before male fertility shows any appreciable change? BubR1 is critical for proper oocyte maturation (Homer *et al.* 2009) and levels decline with ageing in human oocytes (Riris *et al.* 2014). Interestingly, BubR1 is stabilised by one of the Sirtuin family members to combat ageing (North *et al.* 2014). Sirtuins have additional anti-ageing properties and are therefore exciting targets to investigate for modulating oocyte quality.

Endocrine Implications of Cancer Treatment on Fertility (Joint ESA/SRB Symposium)

Speakers: Ledger W, Tanwar P and Grossmann, M.

Post-cancer survival rates have never been higher. But besides the direct health effects of the cancer itself, there are additional health risks post-treatment (Cheung *et al.* 2013; Ramchand *et al.* 2016). Patients who may not yet have considered their reproductive future may be faced with infertility as a result of cancer therapies (Koch and Ledger 2013). To preserve female fertility, ovarian tissue can be removed and cryopreserved before treatment, and subsequently re-implanted following recovery of the patient. Despite successful pregnancies, this technique can possibly reintroduce cancer cells back into the patient. *In vitro* culture of ovarian tissue circumvents this risk and offers an exciting alternative but exhaustive efforts have highlighted the difficulty in growing developmentally competent human oocytes completely *in vitro* (Telfer and McLaughlin 2011). The consensus now is that *in vitro* culture of ovarian tissue needs to be performed in discrete steps, beginning with primordial follicle activation and preantral follicle development, growth of isolated antral follicles, and culminating in the maturation and fertilisation of oocytes. Presently one group has been able to produce metaphase II oocytes using this technique (Xiao *et al.* 2015).

Alternatively, a better understanding of the basic mechanisms of cancer development in those predicted to be at most risk may allow for earlier detection and targeted treatments. Ovarian cancer, for example, which is commonly diagnosed late, has much better survival rates with early diagnosis. However, early diagnosis is challenging as the site of origin of ovarian cancer is usually unclear (Dubeau and Drapkin 2013) and mouse models of ovarian cancer have proven to be difficult to develop. This creates a niche for investigating potential early markers of cancer development in women with known genetic predisposition to cancer, for example, *BRCA* gene mutations and Wnt signalling (Nagendra *et al.* 2016).

Many questions remain: Is there a way to protect oocytes from damage by cancer therapies *in vivo*? Are there stem cells in the ovary that could be harnessed as a fertility preservation option? Recent initiatives such as Future Fertility Alliance (Anazodo *et al.* 2016), which

offers a partnership between oncologists, paediatricians, and fertility specialists, offer rare opportunities for increased translational research, potentially resulting in additional treatment options and better patient outcomes.

Environmental Impacts on Reproduction

Speakers: McLaughlin E, Akison L, Roman S and McPherson N/Lane M.

An ongoing legacy of lifestyle choices made by expectant parents is carried through to future generations. Given that germ cell development begins in fetal life, there is great potential for the maternal environment to impact on the health and fertility of subsequent generations. Although this has been suspected for some time, it is difficult to examine the impact of specific individual insults during pregnancy while controlling for other lifestyle or socioeconomic factors that confound health outcomes for offspring. Compelling evidence for parental influence on offspring health has been demonstrated using sophisticated pre-clinical models that closely mimic human scenarios of exposure. Two examples are cigarette smoking and alcohol consumption which, despite known health risks, still occur in a large number of pregnancies (Thrift *et al.* 2011; Roberts *et al.* 2014). A unique mouse model, in which cigarette smoke is applied using a 'nose-only' apparatus (Camlin *et al.* 2014), mimics human smoking. Use of this model demonstrates that smoking during pregnancy and lactation impairs ovarian development, oocyte quality, and reproductive capacity in female offspring (Camlin *et al.* 2016) and impacts spermatogenesis in male offspring (Sobinoff *et al.* 2014). A rat model of alcohol exposure confined to the periconceptional period mimics the common scenario of alcohol exposure before pregnancy detection (Gardebjer *et al.* 2014) and results in similar follicle number and ovarian growth reductions in offspring.

Our modern diets may also impact our reproductive function, with both paternal and maternal contributions. In mice, human-equivalent doses of acrylamide, a toxicant found in high levels

in some foods such as fried carbohydrates and roasted coffee beans, has been found to induce sperm DNA damage in males without affecting fertility, with sperm damage persisting in offspring (Katen *et al.* 2016a). Most of the reproductive toxicity associated with acrylamide is due to the actions of the CYP2E1 enzyme, which converts acrylamide to glycidamide. Current studies are testing CYP2E1 inhibitors, such as resveratrol, for their capacity to inhibit sperm DNA damage (Katen *et al.* 2016b). However despite some success, resveratrol's non-specific effects are resulting in the consideration of alternative targets. A number of studies have investigated the impact of maternal obesity, but few focus on paternal obesity, which has been shown to impair fertility in F1 and F2 offspring in a mouse model (Fullston *et al.* 2013). Importantly, diet and exercise interventions can ameliorate these phenotypes (McPherson *et al.* 2015), which provides evidence that intervention aimed at reducing obesity in men can also repair detrimental health outcomes in offspring.

A convergent mechanistic pathway for all of the above studies appears to be via oxidative stress, which can be improved through antioxidants, dietary supplementation, and exercise. Thus, this could provide a potential common therapeutic target to counter a wide range of reproductive and transgenerationally damaging environmental factors. Only by better understanding how mothers and fathers can modulate their prenatal environment can we mitigate the lifelong disease burden in their children and improve the health of future generations.

Reproduction Down Under

Speakers: Johnston SD, Whitworth D and Baird A.

With more than 7,000 species worldwide (>500 in Oceania) currently listed as endangered on the IUCN Red List, there is a pressing need to further our knowledge of reproductive processes in non-traditional research species as this is critical to species preservation

strategies. Within Australia, there are groups of researchers dedicated to understanding reproductive processes of native species, with their findings having tremendous implications for conservation. In this session, a recurrent theme was the importance of continually building knowledge about reproduction in wildlife. Perhaps surprisingly, we still lack knowledge on key reproductive processes in Australian fauna. For example, we are only beginning to understand the highly-conserved factors (Pou5f1/Oct4, Nanog, Sox2) that promote cellular stemness and pluripotency across eutherian and non-eutherian species (Whitworth 2016). Understanding these key early developmental processes is crucial in conservation and development of technologies to safeguard the future of our unique native species. Exciting research projects investigating koalas, echidnas, and wombats are providing insight into methods to appropriately store and maintain gametes in a state capable of fertilisation; revealing the fascinating longevity of koala sperm for use in *in vitro* technologies (Johnston 2016). This knowledge has implications for endangered species breeding programs, with potential to expand populations using advancing technologies like *in vitro* maturation (IVM), or even develop gametes from induced pluripotent stem cells.

This session also included compelling research on a group of marine invertebrates – corals. Until recently, the key mediators of coral spawning remained elusive. Recent research indicates that water temperature, particularly sudden increases in temperature, plays a crucial role in the spawning of many species of coral worldwide (Keith *et al.* 2016). Of concern, is that the potential water temperature changes predicted due to climate change might impact coral spawning. If there are dramatic changes in water temperature over a relatively short period, and reefs survive these climatic events, what will be the long-term impact on coral populations? In order for research reported in this session to be effective, accurate monitoring of the potential impacts of climate change on survival of not only Australian native species, but also on species globally, should be a priority.

Conclusions

The future is bright for reproductive biology research in Australia, with the 2016 SRB annual conference highlighting the importance of basic reproductive knowledge in understanding how to preserve native species, improve human health, and mitigate disease. The conference highlighted the innovative nature of Australian reproductive biology, with groups pioneering novel technologies spanning the breadth of research from gamete development through to the end of our reproductive lifespan. Importantly, reproductive biology is paving the way to understanding the biological mechanisms that underpin and contribute to intergenerational transmission of health and disease, particularly for conditions such as obesity and diabetes, which are becoming increasingly prevalent, and will ultimately alleviate the tremendous financial burden on the Australian health care system of preventable, chronic disease.

Acknowledgements

The authors acknowledge the following speakers who presented their research in the symposia and thank them for supplying their talks: Lisa Akison, Josephine Bowles, Hayden Homer, Stephen Johnston, Nathan Palpant and Deanne Whitworth (University of Queensland); Michelle Lane, Nicole McPherson, Mark Nottle, Darryl Russell and David Sharkey (University of Adelaide); Mathis Grossmann, Tu'uhevaha Kaitu'u-Lino and Christine Wells (University of Melbourne); Caroline Gargett and Kate Loveland (Hudson Institute of Medical Research and Monash University); Evdokia Dimitriadis and Patrick Western (Hudson Institute of Medical Research); Chris Redman (University of Oxford); Joanna James and Eileen McLaughlin (University of Auckland); Roger Hart and Peter Mark (University of Western Australia); Kirsty Pringle, Shaun Roman and Pradeep Tanwar (University of Newcastle); William Ledger (University of NSW); and Andrew Baird (James Cook

University). We also acknowledge the SRB President, Chris O'Neill, and the Reproduction, Fertility and Development Editor-in-Chief for their support of this initiative.

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