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Review

Reproductive health research in Australia and New Zealand: highlights from the Annual Meeting of the Society for Reproductive Biology, 2019

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Abstract. The 2019 meeting of the Society for Reproductive Biology (SRB) provided a platform for the dissemination of new knowledge and innovations to improve reproductive health in humans, enhance animal breeding efficiency and understand the effect of the environment on reproductive processes. The effects of environment and lifestyle on fertility and animal behaviour are emerging as the most important modern issues facing reproductive health. Here, we summarise key highlights from recent work on endocrine-disrupting chemicals and diet- and lifestyle-induced metabolic changes and how these factors affect reproduction. This is particularly important to discuss in the context of potential effects on the reproductive potential that may be imparted to future generations of humans and animals. In addition to key summaries of new work in the male and female reproductive tract and on the health of the placenta, for the first time the SRB meeting included a workshop on endometriosis. This was an important opportunity for researchers, healthcare professionals and patient advocates to unite and provide critical updates on efforts to reduce the effect of this chronic disease and to improve the welfare of the women it affects. These new findings and directions are captured in this review.

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Introduction

Our health during adulthood begins as early as fertilisation and time in the womb. Therefore, understanding the crucial factors governing reproductive health underlies our ability to improve the health of future generations. Leveraging this fundamental knowledge can open doors for the design of new diagnostic tools, therapeutics and intervention measures. However, after the 2019 Society for Reproductive Biology (SRB) Meeting, we understand more than ever that partnering this knowledge with diverse, cross-disciplinary teams is key to us ultimately improving reproductive outcomes, as a community. We saw numerous examples of collaborations between scientists, clinicians, engineers, bioinformaticians and mathematicians teaming up to advance reproductive health through revolutionary new approaches and technologies. This summary of the 2019 Annual Meeting highlights the novel advances being made to address topical reproductive health issues ranging from endometriosis and the improvement of assisted reproductive techniques (ARTs) to synthesising big data and understanding the effect of the environment on reproductive health. Importantly, the partnerships being forged to facilitate these advances are outlined in this review.

Inaugural SRB endometriosis workshop

Participating speakers: Linda Giudice, Susan Evans, Stuart Brierley, Caitlin Filby, Gita Mishra, Peter Rogers, Melissa Parker, Sally Mortlock, Sarah Holdsworth-Carson

The inaugural SRB endometriosis workshop, Australian Endometriosis Research: The State of the Art and What the Future Looks Like, was held as a satellite meeting in conjunction with the SRB Annual Scientific Meeting in Sydney at The Royal Hospital for Women. The meeting was co-organised by Louise Hull, Sarah Holdsworth-Carson, Jane Girling and Jason Abbott, bringing together scientists, clinicians and patient advocates from around Australia and New Zealand to showcase current research activities and updates.

Endometriosis is a chronic, inflammatory, oestrogen-dependent disease that affects an estimated 176 million women globally and is the leading cause of infertility. With a personal cost of nearly A$30 000 and an economic burden of A$9.6 billion each year in Australia (Armour *et al.* 2019), new clinical, basic science and government initiatives are needed to improve this situation. Peter Rogers (The University of Melbourne) and Cecilia Ng (Jean Hailes) were invited to provide an update about the National Action Plan for Endometriosis (NAPE, https://www1.health.gov.au/internet/main/publishing.nsf/Content/endometriosis, accessed 1 October 2019). After years of identifying the unmet needs and research priorities for endometriosis (Horne *et al.* 2017; Rogers *et al.* 2017; As-Sanie *et al.* 2019) and consumer-driven advocacy of federal politicians, the Parliamentary Friends of Endometriosis Awareness group was established to push for a policy response to address the disease. In early 2018, a National Round Table was held where patients, advocates, clinicians, scientists and government officials commenced development of the NAPE. Following a national consultation process, the final document was established that outlined three key areas requiring federal support: (1) awareness; (2) clinical management; and (3) research with measurable outcomes to include improved quality of life and a reduction in the effect of endometriosis on the individual and the community.

To date, the NAPE has delivered a total of A$15 million, with A$2.5 million towards the founding of a National Endometriosis Clinical and Scientific Trials (NECST) network that has engaged VCS Digital Health to build and integrate demographic, clinical outcomes, imaging, Patient Reported Outcome Measures (PROMS), and biobank information from endometriosis patients from across Australia. A further A$3.4 million has been allocated for the development of education and clinical guidelines, digital health and online patient advocacy platforms, and there is a Medical Research Future Fund targeted call for Endometriosis Research worth A$9 million. Within 5 years, the NAPE is expected to deliver significant improvements in patient diagnosis, treatment and quality of life. As part of developing the NAPE, a clearer picture of how many women experienced endometriosis in Australia was required. Gita Mishra (Director, Australian Longitudinal Study of Women's Health (ALSWH), University of Queensland), presented new data on the prevalence of endometriosis in Australia. Using data from ALSWH, Professor Mishra highlighted that endometriosis needs to be identified as a chronic disease because it exhibits prevalence rates higher than vascular disease (4.8%), type 2 diabetes (5%) and asthma (11.2%).

Endometriosis-related pain was a key topic of the day. Endometriosis research updates were opened by Linda Giudice from University of California San Francisco who was awarded the SRB Founder's Oration and Medal Lecture 2019. Professor Giudice presented an overview of endometriosis pathogenesis theories, including genetic, epigenetic and environmental factors, with a focus on the role of endocrine disruptors in the establishment of endometriosis through modulation of the epigenome. Of interest was the indication that endocrine disruptors such as dioxin induce transgenerational epigenome disruption (Manikkam *et al.* 2012; Nilsson *et al.* 2018). These findings suggest that maternal exposure to dioxin could alter the embryonic epigenome by altering methylation patterns of imprinted genes, resulting in adverse effects in subsequent generations. Although rodent and non-human primates have been used to demonstrate the role of dioxin in the establishment of endometriosis (Rier and Foster 2002), evidence linking dioxin exposure to endometriosis development in humans is limited (Simsa *et al.* 2010).

Research into the most debilitating consequence of endometriosis, chronic pelvic pain (CPP), was then discussed. Melissa Parker (Canberra Endometriosis Centre) described her latest research, which evaluated the effect of menstrual disturbance, including period pain, on adolescent health. This reflected data from a second cohort of 1000 15- to 19-year-old females who completed the Menstrual Disorder of Teenagers (MDOT) and Period ImPact and Pain Assessment (PIPPA) surveys. These data identified significant menstrual disturbance in 25% of girls surveyed, with the pain causing disruption in daily activities and increased absenteeism from both school and work. The PIPPA survey is currently being developed as an online resource to facilitate self-screening for menstrual disturbance using PIPPA and education about managing period pain, first-line measures and when to seek primary health care. The website aims to help

young women determine what is normal or not for menstrual periods and open constructive supportive dialogue with their carers about period pain.

Susan Evans (Pelvic Pain Foundation of Australia) then provided an overview of how we describe CPP in order to improve clinical understanding of pain syndromes and treatment. Dr Evans presented research about the comorbidities of dysmenorrhoea, stating that stabbing pain in association with endometriosis was highly associated with dysmenorrhoea severity, length of menstruation and pelvic and extrapelvic pain (Evans *et al.* 2018). The research also highlighted that symptoms associated with dysmenorrhoea can be independent of endometriosis and hormone use. This finding further supported the notion that CPP and endometriosis may be independent conditions that coexist, requiring treatment options suited to each condition. To round out the discussion on pain, Stuart Brierley (Flinders University and South Australian Health and Medical Research Institute) presented an overview of the neural pathways underlying abdominal and pelvic pain (Grundy *et al.* 2019), including the description of neuronal cross-organ sensitisation between the bowel, bladder and vagina. This overview also included details of a recent preclinical study describing how a gut-acting therapy like linaclotide (a synthetic guanylate cyclase 2C agonist, currently clinically approved for use in irritable bowel syndrome patients with constipation) reduced comorbid CPP in rodent models of endometriosis (Ge *et al.* 2019).

To finalise the day, several researchers provided updates on the phenotypes, risks and potential causes of endometriosis. Sally Mortlock (Queensland University) provided an update on the endometriosis genome-wide association studies (GWAS) research that, to date, has identified 19 independent single nucleotide polymorphisms (SNPs) at 14 distinct genomic loci associated with endometriosis risk (Fung and Montgomery 2018). Dr Mortlock also discussed recent research investigating the epigenome and methylation signatures of endometriosis patients (Mortlock *et al.* 2019). From her study, 4546 sentinel *cis*-methylation quantitative trait loci were identified, with one located in the endometriosis risk region near the growth regulating estrogen receptor binding 1 (*GREB1*) gene. Expansion of her research will involve the inclusion of patient phenotypic information (age, body mass index (BMI), pain, day of cycle) and cell-specific features (endometrial epithelium and stroma) to determine risk SNPs and genes related to endometriosis phenotypes. Sarah Holdsworth-Carson (The University of Melbourne) presented recent data on the heterogeneity of endometriotic lesions. Using pathology specimens and investigating microscopic cellular features, little correlation was observed between cell characteristics and patient clinical information (e.g. duration of symptoms or cycle stage). Dr Holdsworth-Carson also presented novel research investigating the spatial distribution of metabolites, proteins and N-glycans in endometriotic lesions, with early data also indicating significant heterogeneity between lesions and eutopic endometrium. From these findings, the future use of lesion descriptors and application of molecular markers as biomarkers of disease will need to consider overall lesion heterogeneity in order to be effective. Caitlin Filby (Hudson Institute of Medical Research) provided an update on the most recent research on the cellular origins of endometriosis (Valentijn *et al.* 2013; Gargett *et al.* 2014; Nguyen *et al.* 2017; Cousins *et al.* 2018), including investigation of the presence of endometrial stem and progenitor cells in peritoneal fluid and menstrual blood. This work was discussed in the context of recent publications on cancer-associated somatic mutations in the epithelial cells of deep infiltrating endometriotic lesions (Anglesio *et al.* 2015; Anglesio and Yong 2017; Suda *et al.* 2018) and advances in endometrial organoid technology (Boretto *et al.* 2017, 2019; Turco *et al.* 2017). Importantly, the ability to generate organoids from the lesions themselves is a major advance that is likely to aid our understanding of how endometriosis lesions form and how best to treat them using a personalised medicine approach.

The symposium was closed with a panel discussion including Grant Montgomery (University of Queensland), Caroline Gargett (Hudson Institute of Medical Research), Louise Hull, Cecilia Ng and Peter Rogers and moderated by Jane Girling highlighting the need for personalised treatment, novel screening and diagnostic tools, increasing the collection of patient phenotypic data, more multidisciplinary collaborations and increasing education and outreach opportunities to communities that are hindered by language, culture and location. By implementing the key findings of the panel discussion and aligning research to endometriosis patient needs, researchers are certain to make significant contributions to improving the lives of endometriosis patients in the coming years:

> Keep small successes going so larger successes can be achieved. [Linda Giudice, 2019]

Environmental effect on reproduction

Speakers: Bob Wong, Andrew Pask, Nicole McPherson

Environmental variables, both exogenous and endogenous, can modulate the behaviour and homeostasis of living organisms. Such variables are not only limited to physical, chemical and biological factors, but include lifestyle, diet and hormones (Bhargava *et al.* 2017). Across time, organisms and populations have continually adapted to changing environmental stresses and conditions. But unarguably the past few decades have brought an unprecedented rate and magnitude of detrimental changes in many ecosystems, exacerbated by human activities such as increasing chemical and pharmaceutical pollution, greenhouse gases and deforestation. These factors have drastically altered not only the natural habitats of many native species, but also their behaviour responses, development and reproduction. For example, 17β-trenbolone, a highly potent endocrine disruptor with androgen-mimicking activity, is commonly used as a hormonal growth promoter in the beef industry (Lagesson *et al.* 2019). This chemical enters aquatic ecosystems via livestock effluent run-off and alters the course of reproduction and sexual selection in freshwater fish. Thus, its capacity to alter behaviour in wildlife is a growing environmental concern. For example, in the guppy *Poecilia reticulata*, males exposed to 17β-trenbolone are frequently more aggressive towards rival males than their unexposed male counterparts (Tomkins *et al.* 2016). Exposed males also exhibit altered sexual behaviour, such as a decrease in courting behaviour and more sneak (i.e. coercive) mating attempts towards females. Similarly, female

guppies exposed to 17β-trenbolone show altered sexual responsiveness and are less discerning in their mate selection (Tomkins *et al.* 2016).

Another class of endocrine-disrupting chemicals (EDCs) mimics oestrogen and has strong potential to alter downstream endocrine functions, affecting male and female reproduction. The effect of EDCs is not limited to parental reproduction; their transgenerational effects on reproductive organs are also a major concern (Jeng 2014). Sustained or abnormal production of endogenous oestrogen can lead to cancers of reproductive organs, of which endometrial type I carcinoma (Yang *et al.* 2019) and cervical carcinomas (Chung *et al.* 2010) are common examples. Increasing exposure to EDCs has affected oestrogen signalling, which has not only been associated with reduced male fertility in terms of functional semen parameters (Rehman *et al.* 2018), but also resulted in developmental anomalies of the male reproductive tract (Bouty *et al.* 2015). Among such anomalies, hypospadias is one common condition reported to be higher in males born to mothers having been exposed to oestrogenic EDCs during pregnancy. This anomaly is represented by ectopic placement of the urethral opening and affects 1 in 125 live male births in developed countries (Paulozzi *et al.* 1997). However, the mechanism by which oestrogen-like EDCs cause hypospadias is not fully understood. Recently, altered expression in urethral patterning and keratin genes were reported in response to compromised oestrogen signalling in mice (Cripps *et al.* 2019). Notably, a loss of oestrogen signalling in mice deficient in the oestrogen receptor ERα also leads to mild hypospadias (Govers *et al.* 2019). A similar phenotype has been reported in aromatase (*Cyp19α1*)-knockout mice (Cripps *et al.* 2019), which show complete ablation of endogenous oestrogen. These findings indicate that physiological levels of oestrogen are necessary for normal closure of the penile urethra. Nonetheless, the rise in developmental anomalies of the male reproductive tract may be attributed to continuous and increased exposure to exogenous (contraceptive pill, oestrogen-replacement therapies, intrauterine contraceptive devices, oestrogen-rich products) or endogenous (oestrogen-secreting tumours or obesity) oestrogen.

Among endogenous factors, obesity in reproductive-age men is of great concern, because obesity rates have nearly tripled worldwide in the past 30 years and nearly one-quarter of all men are obese (Palmer *et al.* 2012). This coincides with an increase in male subfertility and infertility, evidenced by the increase in time to natural conception and the number of obese couples seeking ARTs. Numerous studies have demonstrated that male obesity and high BMI are associated with impaired sex steroid hormone production, reduced sperm quality and quantity, increased oxidative sperm DNA damage and changes in the epigenetic status of spermatozoa (McPherson and Lane 2015). However, a growing number of studies also demonstrate that higher adiposity and male obesity are associated with comorbidities, including metabolic syndrome, hypercholesterolaemia, hyperleptinaemia and a proinflammatory state, all of which are independently associated with male subfertility (for a review, see McPherson and Lane 2015). It remains unclear whether increased adiposity is the sole driver of impaired reproductive function in obese males or whether comorbidities also affect sperm quality, quantity and DNA integrity. In a rodent model of male obesity, both diet and exercise interventions improved sperm function (motility, DNA damage, reactive oxygen species (ROS) and mitochondrial function; Palmer *et al.* 2012). Interestingly, there was no change in adiposity levels in the exercise intervention relative to control group, but exercise did lead to improved glucose function, implicating metabolic dysfunction independent of increased adiposity as a causative factor in impaired sperm function (Palmer *et al.* 2012).

In humans, the effects of obesity or high BMI on semen parameters and male fertility are inconclusive (for a review, see Palmer *et al.* 2012). Some argue that increased fat mass is not a robust indicator of subfertility and report no change in sperm concentration (Pauli *et al.* 2008; Shayeb *et al.* 2011), whereas others have shown decreased sperm concentration in men with a high BMI (Stewart *et al.* 2009; Alshahrani *et al.* 2016). These findings further highlight that, in humans, adiposity alone is likely not the sole determinant of impaired sperm function ascribed to male obesity. Moreover, the correlation between the level of adiposity and BMI can vary between individuals, and does not always take into account differences in muscle mass, blood profile and other metabolic parameters; in time, these may prove to be more reliable indicators of subfertility and allow treatments to be tailored to each patient.

Together, these findings highlight that anthropogenic changes can have both blatant and subtle effects on natural populations. Lifestyle, extensive use of and thus continuous exposure to EDCs negatively affect reproductive capacity, and EDCs also carry potential consequences of transgenerational reproductive anomalies in animals and humans. Therefore, it is vital to understand and assess how organisms will cope in an increasingly human-dominated world, to forecast the likely fate of species in the longer term and, where possible, take the remedial actions necessary to counter the loss of biodiversity and minimise the unwanted effects.

Novel insights into implantation and embryo development

Speakers: Evdokia Dimitriadis, Alexandra Harvey

The intricacies of the implantation process have mystified scientists for years, such that our understanding of this critical phase of development remains virtually unchanged over the past decade. In addition to the requisite synchrony between a receptive endometrium and a good-quality blastocyst (Koot *et al.* 2012), our understanding of implantation reflects only the broader morphogenetic events of blastocyst apposition, attachment and invasion. This has hindered attempts to resolve the developmental origin and molecular aetiology of implantation failure, a leading contributor to infertility, affecting approximately 15% of all pregnancies worldwide (Zinaman *et al.* 1996; Koot *et al.* 2012). It therefore remains difficult to accurately identify and diagnose women at risk of experiencing this condition (Tan *et al.* 2005; Ford and Schust 2009; Simon and Laufer 2012; Coughlan *et al.* 2014). Indeed, current therapeutic strategies rely almost exclusively on embryo selection based on morphological criteria and the kinetics of cell division during early embryogenesis (Meseguer *et al.* 2011). However, neither parameter has proven sufficient to accurately predict embryo

implantation potential or pregnancy success. To address this, our speakers spearhead research into novel prognostic biomarkers to predict implantation potential and endometrial receptivity with the goal to improve ARTs.

One such strategy has been to explore the role and predictive capacity of embryo-borne non-coding RNAs (i.e. microRNAs (miRNAs)) in regulating endometrial receptivity (Cuman *et al.* 2015; Winship *et al.* 2018). This is perhaps not surprising because miRNAs mediate gene silencing by targeting protein-coding transcripts for degradation and, despite strict temporal and spatial control, dysgenesis of miRNAs is associated with numerous pathological processes, including in reproduction (AbdelHafez *et al.* 2010; Revel *et al.* 2011; Zhao *et al.* 2012; Dior *et al.* 2014; Ha and Kim 2014; Kropp *et al.* 2014; Rosenbluth *et al.* 2014; Cuman *et al.* 2015). In this context, the study of Cuman *et al.* (2015) reported that the miRNA profile derived from spent blastocyst media collected from an IVF clinic can be stratified based on implantation success. One miRNA in particular, miR-661, was detected exclusively in the media of embryos with reduced implantation potential; miR-661 has since been shown to be taken up by endometrial cells, where it significantly downregulates gene and protein production of poliovirus receptor-related 1 (PVRL1; also known as Nectin-1) leading to the inhibition of endometrial cell adhesiveness, as determined by a trophoblast spheroid attachment assay (Cuman *et al.* 2015). Fittingly, nectins have previously been shown to mediate crucial embryo implantation processes (Norwitz *et al.* 2001; Takai and Nakanishi 2003; Takai *et al.* 2003, 2008). In addition, a more recent study suggests that miR-661 may also downregulate mouse double minute homologue 2 (*MDM2*), similarly reducing endometrial epithelial cell adhesion (Winship *et al.* 2018). These data demonstrate that blastocyst-borne miRNAs actively participate in the implantation process; moreover, the disparity between miRNA profiles from implanting and non-implanting blastocysts may form an intriguing new avenue for their use as biomarkers of implantation potential or as targets to treat implantation failure and infertility.

Another novel strategy with significant potential to increase our understanding of this critical window of early development is the study of embryonic stem cells (ESCs). ESCs represent an important tool to study the molecular biology of the inner cell mass (ICM), established during the first lineage specification event before implantation. The ICM is defined by its pluripotent state and open chromatin structure, and will later give rise to all embryo tissues (Morris *et al.* 2012; Niakan *et al.* 2012). Consequently, all cells inherit the epigenetic information established during early lineage specification events, which has recently been shown to be regulated by the external nutrient environment (Moussaieff *et al.* 2015; Harvey *et al.* 2016*a*, 2016*b*), placing nutrient availability and cellular metabolism at the forefront of developmental regulation. The metabolic fingerprint of the blastocyst is characterised by glycolysis to support the formation and maintenance of the blastocoel cavity and provide biosynthetic precursors for proliferation (Gardner *et al.* 2011; Gardner and Wale 2013; Gardner and Harvey 2015). Similarly, ESCs rely on glycolytic metabolism to support ongoing proliferation (Zhang *et al.* 2011; Folmes *et al.* 2012; Lees *et al.* 2015; Gu *et al.* 2016; Harvey *et al.* 2016*a*, 2016*b*).

Despite significant evidence that nutrient availability affects embryo development, viability and differentiation spanning more than three decades (Gardner and Leese 1987; Lane and Gardner 1996; Harvey *et al.* 2004; Gardner *et al.* 2011), it remains underappreciated that *in vitro* embryo culture systems need to closely mimic the physiological environment of the female tract, especially regarding oxygen and nutritional conditions (Gardner 2016; Gardner and Schoolcraft 1999; Gardner *et al.* 2002). This requirement is also not taken into consideration during the culture of human pluripotent stem cells (PSCs), which can dramatically affect their physiology (for a review, see Lees *et al.* 2017) and subsequent differentiation capacity (Moussaieff *et al.* 2015; Shyh-Chang and Daley 2015). Changes in oxygen tension alone modulate human ESC metabolism, epigenetics and differentiation (Lees *et al.* 2015, 2018, 2019; Harvey *et al.* 2016*b*), with studies highlighting that *in vitro* culture conditions used for ESC derivation and maintenance, as well as handling practices, can negatively affect their stability and ability to respond to stimuli, a significant challenge given that they must appropriately respond to environmental cues to give rise to all cell types within the body. Importantly, cellular changes have only been detected using metabolic analysis, in the absence of overt changes in pluripotency, emphasising the need to assess cell physiology as a marker of cell health, and the sensitivity of early stages of development to their surrounding environment. This is further exemplified in induced pluripotent stem cells (iPSCs), adult cells that have been reprogrammed to ESC-like cells. iPSCs exhibit deficits in metabolic reprogramming, resulting in the retention of somatic metabolic memory (Harvey *et al.* 2018), the degree of which can be modulated, in part, by oxygen availability during the reprogramming process, with standard atmospheric oxygen conditions negatively affecting resultant metabolism, genomic stability and the epigenetic landscape (Spyrou *et al.* 2019).

The use of atmospheric oxygen remains the norm not only for stem cells, but also embryo culture. However, these data suggest that such an approach may contribute to long-term changes in cell physiology and health, particularly given the dynamic links between nutrient availability, metabolism and the epigenetic landscape (Moussaieff *et al.* 2015; Harvey *et al.* 2016*a*, 2016*b*). This novel approach has the potential to change everything we thought we knew about cell biology and shed new light on ICM regulation and differentiation. This is especially important for ensuring embryo quality of IVF embryos before transfer and the subsequent health of resultant offspring, in line with the developmental origins of adult health and disease hypothesis.

SRB–Australian and New Zealand Placental Research Association (ANZPRA) Symposium

Speakers: Amanda Sferruzzi-Perri, William Rawlinson, Jo James, Fiona Brownfoot

This session explored novel diagnostics for a range pregnancy complications. In particular, the function of the placenta as the interface between mother and fetus has been a central focus of this area of research. The placenta plays major vital functions during pregnancy. Two major functions are: (1) transporting nutrients and oxygen from the mother to the fetus to allow

healthy development of the growing fetus; and (2) producing hormones to maintain pregnancy and modulate the energy balance of the mother to aid fetal resource supply. In fact, altered resource allocation during pregnancy can lead to pregnancy complications affecting both mother and baby, including abnormal fetal growth and gestational diabetes. Insulin-like growth factor (IGF) 2 signalling via the phosphatidylinositol 3-kinase (PI3K) pathway is a key driver of fetal growth via the actions of IGF2 on the placenta under physiological and adverse pregnancy environments (for a review, see Sferruzzi-Perri *et al.* 2017). In response to environmental challenge, hypoxia and undernutrition interact via this pathway to control placental phenotype (Higgins *et al.* 2016). Genetic mouse models have enabled distinctions between maternal and fetal signals operating via IGF2/PI3K signalling that are responsible for adapting placental resource allocation (Sferruzzi-Perri *et al.* 2016). Recent evidence indicates that there is a threshold at which the mother and placenta may not be able to adapt to optimise fetal resource allocation and growth, although this depends on the specific gestational environment (Sferruzzi-Perri *et al.* 2019). Finally, via the production of hormones that are downstream of IGF2, the placenta may signal to the mother to alter her allocation of resources and control fetal growth.

The placenta also facilitates the transport of bacteria and viruses to the developing fetus. Congenital cytomegalovirus (CMV) is the most common, yet under-recognised, infectious cause of neonatal malformations in developed countries, in severe cases causing pre-eclampsia and fetal and neonatal death (Kenneson and Cannon 2007). A lack of uniform guidelines has impaired efforts to decrease the effects of CMV globally. Consequently, a recommendations group was convened in 2015 to establish guidelines for prevention and diagnosis (for a review, see Rawlinson *et al.* 2017). Ethical and practical barriers studying fetal infection have led to a lack of vaccine or pharmaceutical treatments, highlighting the ongoing need to understand the viral pathogenesis. Congenital CMV results from direct fetal infection, as well as indirect effects through placental infection (Hamilton *et al.* 2012). *Ex vivo* primary term and first trimester placental explant studies implicate altered dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) and Wnt signalling as central to CMV infection-mediated placental dysfunction. Specifically, CMV infection decreased trophoblast migration via elevated expression of Wnt-binding Receptor Tyrosine Kinase Like Orphan Receptor 2 (ROR2) (Huynh *et al.* 2019). Meanwhile, CMV replication within the placenta is dependent on DYRK signalling (Hamilton *et al.* 2018). Now, inhibitor studies are required to determine whether either or both targets could pave the way for the generation of the first therapeutic intervention. Early studies *in vitro* suggest such inhibitors reduce CMV replication and movement, paving the way for a new way of preventing placental and fetal damage (Hutterer *et al.* 2016; Hahn *et al.* 2018).

During pregnancy the placenta modifies the uterine vasculature to facilitate a 15-fold increase in maternal blood delivery to the placenta. Poor adaptation of the uterine circulation leads to decreased fetal nutrient and oxygen delivery, and consequently fetal growth restriction (FGR). However, the dynamic structural changes that occur in the uterine circulation during pregnancy and their functional consequences are poorly understood. Pathological pregnancies are detected by the presence of a uterine artery Doppler notch, which is generally attributed to inadequate spiral artery remodelling implicated in the pathogenesis of FGR. However, the contributions of the larger vessels in the uterine circulation that also undergo significant trophoblast-independent remodelling during pregnancy have largely been overlooked. A new collaboration encompassing physiological and bioengineering research has led to the development of virtual models of uteroplacental blood flow during pregnancy (Clark *et al.* 2018). These *in silico* models incorporate the arteriovenous anastomoses (AVA) in the maternal–fetal circulation for the first time and, in so doing, highlight a novel and potentially important role for the radial arteries in regulating volumetric blood delivery to the placenta (Clark *et al.* 2018; James *et al.* 2018). Indeed, models of the uterine artery Doppler waveform demonstrate that the radial arteries, rather than the spiral arteries, are the dominant contributors to the uterine artery Doppler notch frequently used to detect FGR pregnancies (Clark *et al.* 2018). These data also highlight an underappreciated role of the trophoblast plugs in the remodelling of the uterine vasculature (James *et al.* 2018; Saghian *et al.* 2019). Spiral and radial artery remodelling could be haemodynamically linked and should be considered as a complete and dynamic system in the diagnosis of FGR and to reduce the risk of stillbirths.

Maternal reporting of fetal movement is currently used a determinant of possible stillbirth, but with little efficacy. The partnering of clinical expertise with electrical engineers has led to the design of an electric sensing device that continuously monitors fetal heartbeat (electrocardiogram (ECG)). So far, a small pilot study ($n = 10$ pregnant women) (F. Brownfoot, unpubl. data) has developed predictive algorithm to remove noises coming from the maternal heartbeat and other interference from the fetal ECG. Now, a larger cohort of pregnant women will test a wearable patch allowing continuous monitoring of the fetal ECG from home (F. Brownfoot, unpubl. data). The day of birth is currently the most dangerous day in a lifetime associated with a high risk of mortality (Walker *et al.* 2014). Surprisingly, current available techniques to monitor fetal distress during labour are untimely and wrong 50% of the time. Therefore, there is a great need to develop new technologies to detect fetal distress in labour. Fiona Brownfoot, together with physicists and chemical engineers, has developed a device that measures a direct marker of fetal distress (F. Brownfoot, unpubl. data). This sensor will soon be tested in a preclinical fetal sheep model of hypoxia. Understanding the links between placental dysfunction and pregnancy complications continues to help unravel the pathophysiology of these disorders, which is a key prerequisite to developing improved diagnostics and therapeutics.

Big data in reproduction

Speakers: Claire Roberts, Katie Ayers, Grant Montgomery

Many researchers across the world of reproductive biology are now dealing with enormous datasets to further understand the complex regulation of various aspects of male and female

fertility. The utility of such data, analysed together with new datasets, will likely pave the way for elucidating the causes of infertility states in both sexes, as well as pregnancy complications. There is also a need for the stringent analysis and mining of these data, with promising results seen as discussed below.

It is now well established that early life events shape lifelong health and that the placenta plays an integral role by regulating fetal health (for reviews, see Tarrade *et al.* 2015; Burton *et al.* 2016). One in four women experiences a pregnancy complication during their first pregnancy, which emphasises the need for further research into this area to understand the underlying causes. As part of a National Institutes of Health (NIH) Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Human Placenta Project study (unpubl. data), Claire Roberts and her team have collected a multitude of placental tissue and blood samples across all three trimesters of pregnancy from both normal and complicated (e.g. gestational diabetes, pre-eclampsia, preterm birth and intrauterine growth restriction) pregnancies. They have implemented a multiomics approach to survey the DNA, RNA and protein extracted from these samples, which is allowing them to investigate gene expression during normal development and any abnormalities in gene expression onset with pathologies of pregnancy. The mining of such data for biomarkers of healthy or aberrant pregnancies will be a major step forward in understanding the causes of these pathologies during pregnancy, as well as identifying the major changes occurring during the three trimesters in normal pregnancies. Interestingly, they report that the placenta is a comparatively highly hypothmethylated organ (Chatterjee *et al.* 2016), with increases in methylation gradually to term. However, in conditions such as pre-eclampsia, precocious methylation occurs, and this may contribute to the causative mechanisms of this disease (C. Roberts, unpubl. data). Using RNA sequencing, Roberts and her colleagues have also identified a temporal expression of genes regulating the immune response, as well as sex-specific differences in miRNA expression (C. Roberts, unpubl. data). The data generated from this RNA sequencing study and associated projects will be of major clinical benefit for states of pregnancy complication.

Disorders of sex development (DSD) represent a large spectrum of anatomical, gonadal or chromosomal sex abnormalities that affect both men and women (Ohnesorg *et al.* 2014; Croft *et al.* 2016). These disorders encompass common conditions such as hypospadias, where men have ectopic urethral openings (0.4–0.8% of men; Bouty *et al.* 2015) and are often diagnosed during childhood, meaning children suffer serious psychological and reproductive consequences, as well as rare conditions like 46,XX testicular DSD (sex reversal). Although DSD are largely genetic in nature, their cause remains unexplained in at least 50% of cases (Délot *et al.* 2017), with less than 13% of patients actually receiving a genetic diagnosis as part of their clinical care. To address this issue, Ayers and her colleagues have used a massive parallel sequencing approach in a cohort of 327 patients to target more than 1000 candidate genes believed to be required for normal sex development. This approach led to a highly successful genetic diagnostic rate in 43% of DSD patients, with 93 novel genetic variants reported across 28 genes (K. Ayers *et al.*, unpubl. data). This raised the diagnostic rates threefold and, due to this success, this novel gene panel has been integrated into the standard screening pipeline within the Victorian Clinical Genetics Laboratories in Melbourne (Australia) and is now available to all patients nationally and internationally. Furthermore, to identify a genetic cause for the remaining idiopathic cases, Ayers and colleagues are now implementing whole-exome sequencing to probe for additional genetic variants across all protein-coding genes. Novel genetic variants identified with this more inclusive approach are currently being tested for their ability to cause DSD and affect sex development using various functional genomics models, including the fruit fly *Drosophila* (K. Ayers *et al.*, unpubl. data).

Although increased use of next-generation sequencing techniques has improved our understanding of the complex genetic causes of certain disease states of male and female infertility (Maxwell *et al.* 2016; Oud *et al.* 2017), there is a subtle nature to many genetic risk factors that may be overlooked. Furthermore, there is likely a complex relationship between multiple genetic variants and/or epigenetic regulation that is causative for many infertility phenotypes. To further investigate, Grant Montgomery and his team have been undertaking GWAS in women with endometriosis (Fung *et al.* 2015). Many 'hits' identified within the GWAS have been localised to the non-coding regions of the genome, identifying a complex regulation of gene expression that is associated with the onset of this condition. The development of GWAS catalogues for disease types will likely create excellent sources for associations of certain genetic variants with particular diseases. However, the need for stringent testing of these associations will be required, including an assessment of multiple testing to minimise false-positive results. Another key area to address for the success of these approaches will be the specialised training of staff to understand bioinformatics and advanced statistics.

Conclusion

The SRB annual conference has long provided a platform for the dissemination of important contributions by SRB members in the field of reproductive science. The 2019 meeting has highlighted how these contributions are being translated to improve human health, agriculture and the conservation of species, with a focus on environmental effects on reproduction, the use and potential effects of big data in this field and key insights from researchers focusing on gamete biology, placental biology and embryogenesis. Importantly, several presentations showcased the transition from basic research to innovation and new technology, and how these technologies are being used to improve animal breeding efficiency, particularly in the horse, as well as for assisted reproduction in humans and for the protection of fertility during cancer treatment. The inclusion of the Endometriosis Workshop held immediately before the SRB meeting provided a critical update on how this key issue is being tackled at both a political and scientific level. This satellite meeting also served to unite scientists, clinicians and patient advocates from across Australia and New Zealand. Ultimately, tackling issues of reproduction from both a legislative and

scientific angle will enable us to more rapidly turn discoveries into novel healthcare procedures and alleviate the financial and health burden of reproductive diseases such as endometriosis.

Conflict of interest

The authors declare no conflicts of interest.

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References

AbdelHafez, F. F., Desai, N., Abou-Setta, A. M., Falcone, T., and Goldfarb, J. (2010). Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. *Reprod. Biomed. Online* **20**, 209–222. doi:10.1016/J.RBMO.2009.11.013

Alshahrani, S., Ahmed, A. F., Gabr, A. H., Abalhassan, M., and Ahmad, G. (2016). The impact of body mass index on semen parameters in infertile men. *Andrologia* **48**, 1125–1129. doi:10.1111/AND.12549

Anglesio, M. S., and Yong, P. J. (2017). Endometriosis-associated ovarian cancers. *Clin. Obstet. Gynecol.* **60**, 711–727. doi:10.1097/GRF.0000000000000320

Anglesio, M. S., Bashashati, A., Wang, Y. K., Senz, J., Ha, G., Yang, W., Aniba, M. R., Prentice, L. M., Farahani, H., Li Chang, H., Karnezis, A. N., Marra, M. A., Yong, P. J., Hirst, M., Gilks, B., Shah, S. P., and Huntsman, D. G. (2015). Multifocal endometriotic lesions associated with cancer are clonal and carry a high mutation burden. *J. Pathol.* **236**, 201–209. doi:10.1002/PATH.4516

Armour, M., Lawson, K., Wood, A., Smith, C. A., and Abbott, J. (2019). The cost of illness and economic burden of endometriosis and chronic pelvic pain in Australia: a national online survey. *PLoS One* **14**, e0223316. doi:10.1371/JOURNAL.PONE.0223316

As-Sanie, S., Black, R., Giudice, L. C., Gray Valbrun, T., Gupta, J., Jones, B., Laufer, M. R., Milspaw, A. T., Missmer, S. A., Norman, A., Taylor, R. N., Wallace, K., Williams, Z., Yong, P. J., and Nebel, R. A. (2019). Assessing research gaps and unmet needs in endometriosis. *Am. J. Obstet. Gynecol.* **221**, 86–94. doi:10.1016/J.AJOG.2019.02.033

Bhargava, A., Pathak, N., Sharma, R. S., Lohiya, N. K., and Mishra, P. K. (2017). Environmental impact on reproductive health: can biomarkers offer any help? *J. Reprod. Infertil.* **18**, 336–340.

Boretto, M., Cox, B., Noben, M., Hendriks, N., Fassbender, A., Roose, H., Amant, F., Timmerman, D., Tomassetti, C., Vanhie, A., Meuleman, C., Ferrante, M., and Vankelecom, H. (2017). Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability. *Development* **144**, 1775–1786. doi:10.1242/DEV.148478

Boretto, M., Maenhoudt, N., Luo, X., Hennes, A., Boeckx, B., Bui, B., Heremans, R., Perneel, L., Kobayashi, H., Van Zundert, I., Brems, H., Cox, B., Ferrante, M., Uji, I. H., Koh, K. P., D'Hooghe, T., Vanhie, A., Vergote, I., Meuleman, C., Tomassetti, C., Lambrechts, D., Vriens, J., Timmerman, D., and Vankelecom, H. (2019). Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. *Nat. Cell Biol.* **21**, 1041–1051. doi:10.1038/S41556-019-0360-Z

Bouty, A., Ayers, K. L., Pask, A., Heloury, Y., and Sinclair, A. H. (2015). The genetic and environmental factors underlying hypospadias. *Sex Dev.* **9**, 239–259. doi:10.1159/000441988

Burton, G. J., Fowden, A. L., and Thornburg, K. L. (2016). Placental origins of chronic disease. *Physiol. Rev.* **96**, 1509–1565. doi:10.1152/PHYSREV.00029.2015

Chatterjee, A., Macaulay, E. C., Rodger, E. J., Stockwell, P. A., Parry, M. F., Roberts, H. E., Slatter, T. L., Hung, N. A., Devenish, C. J., and Morison, I. M. (2016). Placental hypomethylation is more pronounced in genomic loci devoid of retroelements. *G3 (Bethesda)* **6**, 1911–1921. doi:10.1534/G3.116.030379

Chung, S. H., Franceschi, S., and Lambert, P. F. (2010). Estrogen and ERalpha: culprits in cervical cancer? *Trends Endocrinol. Metab.* **21**, 504–511. doi:10.1016/J.TEM.2010.03.005

Clark, A. R., James, J. L., Stevenson, G. N., and Collins, S. L. (2018). Understanding abnormal uterine artery Doppler waveforms: a novel computational model to explore potential causes within the utero-placental vasculature. *Placenta* **66**, 74–81. doi:10.1016/J.PLACENTA.2018.05.001

Coughlan, C., Ledger, W., Wang, Q., Liu, F., Demirol, A., Gurgan, T., Cutting, R., Ong, K., Sallam, H., and Li, T. C. (2014). Recurrent implantation failure: definition and management. *Reprod. Biomed. Online* **28**, 14–38. doi:10.1016/J.RBMO.2013.08.011

Cousins, F. L., Msc, D. F. O, and Gargett, C. E. (2018). Endometrial stem/progenitor cells and their role in the pathogenesis of endometriosis. *Best Pract. Res. Clin. Obstet. Gynaecol.* **50**, 27–38. doi:10.1016/J.BPOBGYN.2018.01.011

Cripps, S. M., Mattiske, D. M., Black, J. R., Risbridger, G. P., Govers, L. C., Phillips, T. R., and Pask, A. J. (2019). A loss of estrogen signaling in the aromatase deficient mouse penis results in mild hypospadias. *Differentiation* **109**, 42–52. doi:10.1016/J.DIFF.2019.09.001

Croft, B., Ayers, K., Sinclair, A., and Ohnesorg, T. (2016). Review disorders of sex development: the evolving role of genomics in diagnosis and gene discovery. *Birth Defects Res. C Embryo Today* **108**, 337–350.

Cuman, C., Van Sinderen, M., Gantier, M. P., Rainczuk, K., Sorby, K., Rombauts, L., Osianlis, T., and Dimitriadis, E. (2015). Human blastocyst secreted microRNA regulate endometrial epithelial cell adhesion. *EBioMedicine* **2**, 1528–1535. doi:10.1016/J.EBIOM.2015.09.003

Délot, E. C., Papp, J. C., The DSD-TRN Genetics Workgroup, Sandberg, D. E., and Vilain, E. (2017). Genetics of disorders of sex development: the DSD-TRN experience. *Endocrinol. Metab. Clin. North Am.* **46**, 519–537. doi:10.1016/J.ECL.2017.01.015

Dior, U. P., Kogan, L., Chill, H. H., Eizenberg, N., Simon, A., and Revel, A. (2014). Emerging roles of microRNA in the embryo–endometrium cross talk. *Semin. Reprod. Med.* **32**, 402–409. doi:10.1055/S-0034-1376359

Evans, S. F., Brooks, T. A., Esterman, A. J., Hull, M. L., and Rolan, P. E. (2018). The comorbidities of dysmenorrhea: a clinical survey comparing symptom profile in women with and without endometriosis. *J. Pain Res.* **11**, 3181–3194. doi:10.2147/JPR.S179409

Folmes, C. D., Nelson, T. J., Dzeja, P. P., and Terzic, A. (2012). Energy metabolism plasticity enables stemness programs. *Ann. N. Y. Acad. Sci.* **1254**, 82–89. doi:10.1111/J.1749-6632.2012.06487.X

Ford, H. B., and Schust, D. J. (2009). Recurrent pregnancy loss: etiology, diagnosis, and therapy. *Rev. Obstet. Gynecol.* **2**, 76–83.

Fung, J. N., and Montgomery, G. W. (2018). Genetics of endometriosis: state of the art on genetic risk factors for endometriosis. *Best Pract. Res. Clin. Obstet. Gynaecol.* **50**, 61–71. doi:10.1016/J.BPOBGYN.2018.01.012

Fung, J. N., Rogers, P. A., and Montgomery, G. W. (2015). Identifying the biological basis of GWAS hits for endometriosis. *Biol. Reprod.* **92**, 87. doi:10.1095/BIOLREPROD.114.126458

Gardner, D. K., and Schoolcraft, W. B. (1999). *In vitro* culture of human blastocysts. In 'Towards Reproductive Certainty: Fertility and Genetics Beyond 1999', (Eds R. Jansen and D. Mortimer.) pp. 378–388. (CRC Press: Boca Raton)

Gardner, D. K. (2016). The impact of physiological oxygen during culture, and vitrification for cryopreservation, on the outcome of extended culture in human IVF. *Reprod. Biomed. Online* **32**, 137–141. doi:10.1016/J.RBMO.2015.11.008

Gardner, D. K., and Harvey, A. J. (2015). Blastocyst metabolism. *Reprod. Fertil. Dev.* **27**, 638–654. doi:10.1071/RD14421

Gardner, D. K., and Leese, H. J. (1987). Assessment of embryo viability prior to transfer by the noninvasive measurement of glucose uptake. *J. Exp. Zool.* **242**, 103–105. doi:10.1002/JEZ.1402420115

Gardner, D. K., and Wale, P. L. (2013). Analysis of metabolism to select viable human embryos for transfer. *Fertil. Steril.* **99**, 1062–1072. doi:10.1016/J.FERTNSTERT.2012.12.004

Gardner, D. K., Lane, M., and Schoolcraft, W. B. (2002). Physiology and culture of the human blastocyst. *J. Reprod. Immunol.* **55**, 85–100. doi:10.1016/S0165-0378(01)00136-X

Gardner, D. K., Wale, P. L., Collins, R., and Lane, M. (2011). Glucose consumption of single post-compaction human embryos is predictive of embryo sex and live birth outcome. *Hum. Reprod.* **26**, 1981–1986. doi:10.1093/HUMREP/DER143

Gargett, C. E., Schwab, K. E., Brosens, J. J., Puttemans, P., Benagiano, G., and Brosens, I. (2014). Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis. *Mol. Hum. Reprod.* **20**, 591–598. doi:10.1093/MOLEHR/GAU025

Ge, P., Ren, J., Harrington, A. M., Grundy, L., Castro, J., Brierley, S. M., and Hannig, G. (2019). Linaclotide treatment reduces endometriosis-associated vaginal hyperalgesia and mechanical allodynia through viscerovisceral cross-talk. *Pain* **160**, 2566–2579. doi:10.1097/J.PAIN.0000000000001657

Govers, L. C., Phillips, T. R., Mattiske, D. M., Rashoo, N., Black, J. R., Sinclair, A., Baskin, L. S., Risbridger, G. P., and Pask, A. J. (2019). A critical role for estrogen signaling in penis development. *FASEB J.* **33**, 10383–10392. doi:10.1096/FJ.201802586RR

Grundy, L., Erickson, A., and Brierley, S. M. (2019). Visceral pain. *Annu. Rev. Physiol.* **81**, 261–284. doi:10.1146/ANNUREV-PHYSIOL-020518-114525

Gu, W., Gaeta, X., Sahakyan, A., Chan, A. B., Hong, C. S., Kim, R., Braas, D., Plath, K., Lowry, W. E., and Christofk, H. R. (2016). Glycolytic metabolism plays a functional role in regulating human pluripotent stem cell state. *Cell Stem Cell* **19**, 476–490. doi:10.1016/J.STEM.2016.08.008

Ha, M., and Kim, V. N. (2014). Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **15**, 509–524. doi:10.1038/NRM3838

Hahn, F., Hutterer, C., Henry, C., Hamilton, S. T., Strojan, H., Kraut, A., Schulte, U., Schutz, M., Kohrt, S., Wangen, C., Pfizer, J., Coute, Y., Rawlinson, W. D., Strobl, S., and Marschall, M. (2018). Novel cytomegalovirus-inhibitory compounds of the class pyrrolopyridines show a complex pattern of target binding that suggests an unusual mechanism of antiviral activity. *Antiviral Res.* **159**, 84–94. doi:10.1016/J.ANTIVIRAL.2018.09.012

Hamilton, S. T., Scott, G., Naing, Z., Iwasenko, J., Hall, B., Graf, N., Arbuckle, S., Craig, M. E., and Rawlinson, W. D. (2012). Human cytomegalovirus-induces cytokine changes in the placenta with implications for adverse pregnancy outcomes. *PLoS One* **7**, e52899. doi:10.1371/JOURNAL.PONE.0052899

Hamilton, S. T., Hutterer, C., Egilmezer, E., Steingruber, M., Milbradt, J., Marschall, M., and Rawlinson, W. D. (2018). Human cytomegalovirus utilises cellular dual-specificity tyrosine phosphorylation-regulated kinases during placental replication. *Placenta* **72–73**, 10–19. doi:10.1016/J.PLACENTA.2018.10.002

Harvey, A. J., Kind, K. L., Pantaleon, M., Armstrong, D. T., and Thompson, J. G. (2004). Oxygen-regulated gene expression in bovine blastocysts. *Biol. Reprod.* **71**, 1108–1119. doi:10.1095/BIOLREPROD.104.028639

Harvey, A. J., Rathjen, J., and Gardner, D. K. (2016*a*). Metaboloepigenetic regulation of pluripotent stem cells. *Stem Cells Int.* **2016**, 1816525. doi:10.1155/2016/1816525

Harvey, A. J., Rathjen, J., Yu, L. J., and Gardner, D. K. (2016*b*). Oxygen modulates human embryonic stem cell metabolism in the absence of changes in self-renewal. *Reprod. Fertil. Dev.* **28**, 446–458. doi:10.1071/RD14013

Harvey, A. J., O'Brien, C., Lambshead, J., Sheedy, J. R., Rathjen, J., Laslett, A. L., and Gardner, D. K. (2018). Physiological oxygen culture reveals retention of metabolic memory in human induced pluripotent stem cells. *PLoS One* **13**, e0193949. doi:10.1371/JOURNAL.PONE.0193949

Higgins, J. S., Vaughan, O. R., Fernandez de Liger, E., Fowden, A. L., and Sferruzzi-Perri, A. N. (2016). Placental phenotype and resource allocation to fetal growth are modified by the timing and degree of hypoxia during mouse pregnancy. *J. Physiol.* **594**, 1341–1356. doi:10.1113/JP271057

Horne, A. W., Saunders, P. T. K., Abokhrais, I. M., Hogg, L., and Group Endometriosis Priority Setting Partnership Steering (2017). Top ten endometriosis research priorities in the UK and Ireland. *Lancet* **389**, 2191–2192. doi:10.1016/S0140-6736(17)31344-2

Hutterer, C., Hamilton, S., Steingruber, M., Zeittrager, I., Bahsi, H., Thuma, N., Naing, Z., Orfi, Z., Orfi, L., Socher, E., Sticht, H., Rawlinson, W., Chou, S., Haupt, V. J., and Marschall, M. (2016). The chemical class of quinazoline compounds provides a core structure for the design of anticytomegaloviral kinase inhibitors. *Antiviral Res.* **134**, 130–143. doi:10.1016/J.ANTIVIRAL.2016.08.005

Huynh, K. T., van Zuylen, W. J., Ford, C. E., and Rawlinson, W. D. (2019). Selective modulation of Wnt-binding receptor tyrosine kinase ROR2 expression by human cytomegalovirus regulates trophoblast migration. *J. Gen. Virol.* **100**, 99–104. doi:10.1099/JGV.0.001179

James, J. L., Saghian, R., Perwick, R., and Clark, A. R. (2018). Trophoblast plugs: impact on utero-placental haemodynamics and spiral artery remodelling. *Hum. Reprod.* **33**, 1430–1441. doi:10.1093/HUMREP/DEY225

Jeng, H. A. (2014). Exposure to endocrine disrupting chemicals and male reproductive health. *Front. Public Health* **2**, 55. doi:10.3389/FPUBH.2014.00055

Kenneson, A., and Cannon, M. J. (2007). Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev. Med. Virol.* **17**, 253–276. doi:10.1002/RMV.535

Koot, Y. E. M., Teklenburg, G., Salker, M. S., Brosens, J. J., and Macklon, N. S. (2012). Molecular aspects of implantation failure. *Biochim Biophys Acta.* **1822**, 1943–1950. doi:10.1016/J.BBADIS.2012.05.017

Kropp, J., Salih, S. M., and Khatib, H. (2014). Expression of microRNAs in bovine and human pre-implantation embryo culture media. *Front. Genet.* **5**, 91. doi:10.3389/FGENE.2014.00091

Lagesson, A., Saaristo, M., Brodin, T., Fick, J., Klaminder, J., Martin, J. M., and Wong, B. B. M. (2019). Fish on steroids: temperature-dependent effects of 17beta-trenbolone on predator escape, boldness, and exploratory behaviors. *Environ. Pollut.* **245**, 243–252. doi:10.1016/J.ENVPOL.2018.10.116

Lane, M., and Gardner, D. K. (1996). Selection of viable mouse blastocysts prior to transfer using a metabolic criterion. *Hum. Reprod.* **11**, 1975–1978. doi:10.1093/OXFORDJOURNALS.HUMREP.A019527

Lees, J. G., Rathjen, J., Sheedy, J. R., Gardner, D. K., and Harvey, A. J. (2015). Distinct profiles of human embryonic stem cell metabolism and mitochondria identified by oxygen. *Reproduction* **150**, 367–382. doi:10.1530/REP-14-0633

Lees, J. G., Gardner, D. K., and Harvey, A. J. (2017). Pluripotent stem cell metabolism and mitochondria: beyond ATP. *Stem Cells Int.* **2017**, 2874283. doi:10.1155/2017/2874283

Lees, J. G., Gardner, D. K., and Harvey, A. J. (2018). Mitochondrial and glycolytic remodeling during nascent neural differentiation of human pluripotent stem cells. *Development* **145**, dev168997. doi:10.1242/DEV.168997

Lees, J. G., Cliff, T. S., Gammilonghi, A., Ryall, J. G., Dalton, S., Gardner, D. K., and Harvey, A. J. (2019). Oxygen regulates human pluripotent stem cell metabolic flux. *Stem Cells Int.* **2019**, 8195614. doi:10.1155/2019/8195614

Manikkam, M., Tracey, R., Guerrero-Bosagna, C., and Skinner, M. K. (2012). Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. *PLoS One* **7**, e46249. doi:10.1371/JOURNAL.PONE.0046249

Maxwell, S. M., Colls, P., Hodes-Wertz, B., McCulloh, D. H., McCaffrey, C., Wells, D., Munne, S., and Grifo, J. A. (2016). Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. *Fertil. Steril.* **106**, 1414–1419e5. doi:10.1016/J.FERTNSTERT.2016.08.017

McPherson, N. O., and Lane, M. (2015). Male obesity and subfertility, is it really about increased adiposity? *Asian J. Androl.* **17**, 450–458.

Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K. M., Ramsing, N. B., and Remohi, J. (2011). The use of morphokinetics as a predictor of embryo implantation. *Hum. Reprod.* **26**, 2658–2671. doi:10.1093/HUMREP/DER256

Morris, S. A., Grewal, S., Barrios, F., Patankar, S. N., Strauss, B., Buttery, L., Alexander, M., Shakesheff, K. M., and Zernicka-Goetz, M. (2012). Dynamics of anterior–posterior axis formation in the developing mouse embryo. *Nat. Commun.* **3**, 673. doi:10.1038/NCOMMS1671

Mortlock, S., Restuadi, R., Levien, R., Girling, J. E., Holdsworth-Carson, S. J., Healey, M., Zhu, Z., Qi, T., Wu, Y., Lukowski, S. W., Rogers, P. A. W., Yang, J., McRae, A. F., Fung, J. N., and Montgomery, G. W. (2019). Genetic regulation of methylation in human endometrium and blood and gene targets for reproductive diseases. *Clin. Epigenetics* **11**, 49. doi:10.1186/S13148-019-0648-7

Moussaieff, A., Kogan, N. M., and Aberdam, D. (2015). Concise review: energy metabolites: key mediators of the epigenetic state of pluripotency. *Stem Cells* **33**, 2374–2380. doi:10.1002/STEM.2041

Nguyen, H. P. T., Xiao, L., Deane, J. A., Tan, K. S., Cousins, F. L., Masuda, H., Sprung, C. N., Rosamilia, A., and Gargett, C. E. (2017). N-Cadherin identifies human endometrial epithelial progenitor cells by *in vitro* stem cell assays. *Hum. Reprod.* **32**, 2254–2268. doi:10.1093/HUMREP/DEX289

Niakan, K. K., Han, J., Pedersen, R. A., Simon, C., and Pera, R. A. (2012). Human pre-implantation embryo development. *Development* **139**, 829–841. doi:10.1242/DEV.060426

Nilsson, E. E., Sadler-Riggleman, I., and Skinner, M. K. (2018). Environmentally induced epigenetic transgenerational inheritance of disease. *Environ. Epigenet.* **4**, dvy016. doi:10.1093/EEP/DVY016

Norwitz, E. R., Schust, D. J., and Fisher, S. J. (2001). Implantation and the survival of early pregnancy. *N. Engl. J. Med.* **345**, 1400–1408. doi:10.1056/NEJMRA000763

Ohnesorg, T., Vilain, E., and Sinclair, A. H. (2014). The genetics of disorders of sex development in humans. *Sex Dev.* **8**, 262–272. doi:10.1159/000357956

Oud, M. S., Ramos, L., O'Bryan, M. K., McLachlan, R. I., Okutman, Ö., Viville, S., de Vries, P. F., Smeets, D. F. C. M., Lugtenberg, D., Hehir-Kwa, J. Y., Gilissen, C., van de Vorst, M., Vissers, L. E. L. M., Hoischen, A., Meijerink, A. M., Fleischer, K., Veltman, J. A., and Noordam, M. J. (2017). Validation and application of a novel integrated genetic screening method to a cohort of 1,112 men with idiopathic azoospermia or severe oligozoospermia. *Hum. Mutat.* **38**, 1592–1605. doi:10.1002/HUMU.23312

Palmer, N. O., Bakos, H. W., Owens, J. A., Setchell, B. P., and Lane, M. (2012). Diet and exercise in an obese mouse fed a high-fat diet improve metabolic health and reverse perturbed sperm function. *Am. J. Physiol. Endocrinol. Metab.* **302**, E768–E780. doi:10.1152/AJPENDO.00401.2011

Pauli, E. M., Legro, R. S., Demers, L. M., Kunselman, A. R., Dodson, W. C., and Lee, P. A. (2008). Diminished paternity and gonadal function with increasing obesity in men. *Fertil. Steril.* **90**, 346–351. doi:10.1016/J.FERTNSTERT.2007.06.046

Paulozzi, L. J., Erickson, J. D., and Jackson, R. J. (1997). Hypospadias trends in two US surveillance systems. *Pediatrics* **100**, 831–834. doi:10.1542/PEDS.100.5.831

Rawlinson, W. D., Boppana, S. B., Fowler, K. B., Kimberlin, D. W., Lazzarotto, T., Alain, S., Daly, K., Doutre, S., Gibson, L., Giles, M. L., Greenlee, J., Hamilton, S. T., Harrison, G. J., Hui, L., Jones, C. A., Palasanthiran, P., Schleiss, M. R., Shand, A. W., and van Zuylen, W. J. (2017). Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. *Lancet Infect. Dis.* **17**, e177–e188. doi:10.1016/S1473-3099(17)30143-3

Rehman, S., Usman, Z., Rehman, S., AlDraihem, M., Rehman, N., Rehman, I., and Ahmad, G. (2018). Endocrine disrupting chemicals and impact on male reproductive health. *Transl. Androl. Urol.* **7**, 490–503. doi:10.21037/TAU.2018.05.17

Revel, A., Achache, H., Stevens, J., Smith, Y., and Reich, R. (2011). MicroRNAs are associated with human embryo implantation defects. *Hum. Reprod.* **26**, 2830–2840. doi:10.1093/HUMREP/DER255

Rier, S., and Foster, W. G. (2002). Environmental dioxins and endometriosis. *Toxicol. Sci.* **70**, 161–170. doi:10.1093/TOXSCI/70.2.161

Rogers, P. A., Adamson, G. D., Al-Jefout, M., Becker, C. M., D'Hooghe, T. M., Dunselman, G. A., Fazleabas, A., Giudice, L. C., Horne, A. W., Hull, M. L., Hummelshoj, L., Missmer, S. A., Montgomery, G. W., Stratton, P., Taylor, R. N., Rombauts, L., Saunders, P. T., Vincent, K., Zondervan, K. T., and Wes Werf Consortium for Research Priorities in Endometriosis (2017). Research priorities for endometriosis. *Reprod. Sci.* **24**, 202–226. doi:10.1177/1933719116654991

Rosenbluth, E. M., Shelton, D. N., Wells, L. M., Sparks, A. E., and Van Voorhis, B. J. (2014). Human embryos secrete microRNAs into culture media – a potential biomarker for implantation. *Fertil. Steril.* **101**, 1493–1500. doi:10.1016/J.FERTNSTERT.2014.01.058

Saghian, R., Bogle, G., James, J. L., and Clark, A. R. (2019). Establishment of maternal blood supply to the placenta: insights into plugging, unplugging and trophoblast behaviour from an agent-based model. *Interface Focus* **9**, 20190019. doi:10.1098/RSFS.2019.0019

Sferruzzi-Perri, A. N., Lopez-Tello, J., Fowden, A. L., and Constancia, M. (2016). Maternal and fetal genomes interplay through phosphoinositol 3-kinase(PI3K)–p110alpha signaling to modify placental resource allocation. *Proc. Natl Acad. Sci. USA* **113**, 11255–11260. doi:10.1073/PNAS.1602012113

Sferruzzi-Perri, A. N., Sandovici, I., Constancia, M., and Fowden, A. L. (2017). Placental phenotype and the insulin-like growth factors: resource allocation to fetal growth. *J. Physiol.* **595**, 5057–5093. doi:10.1113/JP273330

Sferruzzi-Perri, A. N., Higgins, J. S., Vaughan, O. R., Murray, A. J., and Fowden, A. L. (2019). Placental mitochondria adapt developmentally

and in response to hypoxia to support fetal growth. *Proc. Natl Acad. Sci. USA* **116**, 1621–1626. doi:10.1073/PNAS.1816056116

Shayeb, A. G., Harrild, K., Mathers, E., and Bhattacharya, S. (2011). An exploration of the association between male body mass index and semen quality. *Reprod. Biomed. Online* **23**, 717–723. doi:10.1016/J.RBMO.2011.07.018

Shyh-Chang, N., and Daley, G. Q. (2015). Metabolic switches linked to pluripotency and embryonic stem cell differentiation. *Cell Metab.* **21**, 349–350. doi:10.1016/J.CMET.2015.02.011

Simon, A., and Laufer, N. (2012). Assessment and treatment of repeated implantation failure (RIF). *J. Assist. Reprod. Genet.* **29**, 1227–1239. doi:10.1007/S10815-012-9861-4

Simsa, P., Mihalyi, A., Schoeters, G., Koppen, G., Kyama, C. M., Den Hond, E. M., Fulop, V., and D'Hooghe, T. M. (2010). Increased exposure to dioxin-like compounds is associated with endometriosis in a case-control study in women. *Reprod. Biomed. Online* **20**, 681–688. doi:10.1016/J.RBMO.2010.01.018

Spyrou, J., Gardner, D. K., and Harvey, A. J. (2019). Metabolomic and transcriptional analyses reveal atmospheric oxygen during human induced pluripotent stem cell generation impairs metabolic reprogramming. *Stem Cells* **37**, 1042–1056. doi:10.1002/STEM.3029

Stewart, T. M., Liu, D. Y., Garrett, C., Jorgensen, N., Brown, E. H., and Baker, H. W. (2009). Associations between andrological measures, hormones and semen quality in fertile Australian men: inverse relationship between obesity and sperm output. *Hum. Reprod.* **24**, 1561–1568. doi:10.1093/HUMREP/DEP075

Suda, K., Nakaoka, H., Yoshihara, K., Ishiguro, T., Tamura, R., Mori, Y., Yamawaki, K., Adachi, S., Takahashi, T., Kase, H., Tanaka, K., Yamamoto, T., Motoyama, T., Inoue, I., and Enomoto, T. (2018). Clonal expansion and diversification of cancer-associated mutations in endometriosis and normal endometrium. *Cell Rep.* **24**, 1777–1789. doi:10.1016/J.CELREP.2018.07.037

Takai, Y., and Nakanishi, H. (2003). Nectin and afadin: novel organizers of intercellular junctions. *J. Cell Sci.* **116**, 17–27. doi:10.1242/JCS.00167

Takai, Y., Irie, K., Shimizu, K., Sakisaka, T., and Ikeda, W. (2003). Nectins and nectin-like molecules: roles in cell adhesion, migration, and polarization. *Cancer Sci.* **94**, 655–667. doi:10.1111/J.1349-7006.2003.TB01499.X

Takai, Y., Miyoshi, J., Ikeda, W., and Ogita, H. (2008). Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. *Nat. Rev. Mol. Cell Biol.* **9**, 603. doi:10.1038/NRM2457

Tan, B. K., Vandekerckhove, P., Kennedy, R., and Keay, S. D. (2005). Investigation and current management of recurrent IVF treatment failure in the UK. *BJOG* **112**, 773–780. doi:10.1111/J.1471-0528.2005.00523.X

Tarrade, A., Panchenko, P., Junien, C., and Gabory, A. (2015). Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. *J. Exp. Biol.* **218**, 50–58. doi:10.1242/JEB.110320

Tomkins, P., Saaristo, M., Allinson, M., and Wong, B. B. M. (2016). Exposure to an agricultural contaminant, 17beta-trenbolone, impairs female mate choice in a freshwater fish. *Aquat. Toxicol.* **170**, 365–370. doi:10.1016/J.AQUATOX.2015.09.019

Turco, M. Y., Gardner, L., Hughes, J., Cindrova-Davies, T., Gomez, M. J., Farrell, L., Hollinshead, M., Marsh, S. G. E., Brosens, J. J., Critchley, H. O., Simons, B. D., Hemberger, M., Koo, B. K., Moffett, A., and Burton, G. J. (2017). Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat. Cell Biol.* **19**, 568–577. doi:10.1038/NCB3516

Valentijn, A. J., Palial, K., Al-Lamee, H., Tempest, N., Drury, J., Von Zglinicki, T., Saretzki, G., Murray, P., Gargett, C. E., and Hapangama, D. K. (2013). SSEA-1 isolates human endometrial basal glandular epithelial cells: phenotypic and functional characterization and implications in the pathogenesis of endometriosis. *Hum. Reprod.* **28**, 2695–2708. doi:10.1093/HUMREP/DET285

Walker, K. F., Cohen, A. L., Walker, S. H., Allen, K. M., Baines, D. L., and Thornton, J. G. (2014). The dangers of the day of birth. *BJOG* **121**, 714–718. doi:10.1111/1471-0528.12544

Winship, A., Ton, A., Van Sinderen, M., Menkhorst, E., Rainczuk, K., Griffiths, M., Cuman, C., and Dimitriadis, E. (2018). Mouse double minute homologue 2 (MDM2) downregulation by miR-661 impairs human endometrial epithelial cell adhesive capacity. *Reprod. Fertil. Dev.* **30**, 477–486. doi:10.1071/RD17095

Yang, B., Chen, R., Liang, X., Shi, J., Wu, X., Zhang, Z., and Chen, X. (2019). Estrogen enhances endometrial cancer cells proliferation by upregulation of prohibitin. *J. Cancer* **10**, 1616–1621. doi:10.7150/JCA.28218

Zhang, J., Khvorostov, I., Hong, J. S., Oktay, Y., Vergnes, L., Nuebel, E., Wahjudi, P. N., Setoguchi, K., Wang, G., Do, A., Jung, H. J., McCaffery, J. M., Kurland, I. J., Reue, K., Lee, W. N., Koehler, C. M., and Teitell, M. A. (2011). UCP2 regulates energy metabolism and differentiation potential of human pluripotent stem cells. *EMBO J.* **30**, 4860–4873. doi:10.1038/EMBOJ.2011.401

Zhao, Y., Zacur, H., Cheadle, C., Ning, N., Fan, J., and Vlahos, N. F. (2012). Effect of luteal-phase support on endometrial microRNA expression following controlled ovarian stimulation. *Reprod. Biol. Endocrinol.* **10**, 72. doi:10.1186/1477-7827-10-72

Zinaman, M. J., Clegg, E. D., Brown, C. C., O'Connor, J., and Selevan, S. G. (1996). Estimates of human fertility and pregnancy loss. *Fertil. Steril.* **65**, 503–509. doi:10.1016/S0015-0282(16)58144-8