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Periconceptional Environment and the Developmental Origins of Disease

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Abstract

The concept emerging from Professor David Barker's seminal research on the developmental origins of later life disease has progressed in many directions since first published. One critical question is *when* during gestation might environment alter the developmental programme with such enduring consequences. Here, we review the growing consensus from clinical and animal research that the period around conception, embracing gamete maturation and early embryogenesis, might be the most vulnerable period. We focus on four types of environmental exposure shown to modify periconceptional reproduction and offspring development and health: maternal overnutrition and obesity; maternal undernutrition; paternal diet and health; and assisted reproductive technology. These conditions may act through diverse epigenetic, cellular and physiological mechanisms to alter gene expression and cellular signalling and function in the conceptus affecting offspring growth and metabolism and leading to increased risk for cardiometabolic and neurological disease in later life.

Key words

Embryo; sperm; parental nutrition; assisted reproductive technology (ART); epigenetics; cardiometabolic disease.

Introduction

The concept of the early origins of disease associated with *in utero* environmental factors has been advanced in both clinical and biological directions since the pioneering and ground-breaking epidemiological discoveries by Professor David Barker and his colleagues. Developmental programming of disease has been tested experimentally across global populations providing confirmation of its veracity. In addition, numerous animal models have been generated for insight on mechanisms across physiological, cellular, molecular and epigenetic levels. Much progress on the understanding of the hypothesis, now known as the Developmental Origins of Health and Adult Disease (DOHaD) concept, has been achieved as evidenced by the varied reviews in this special issue of *J Endocrinology* dedicated to Professor Barker's seminal work. One critical issue and the subject of our review is the question of **when** environment may interact with reproduction to initiate a change in the developmental programme leading to DOHaD-related responses and later disease risk.

A growing consensus has emerged that the period around conception is critical in DOHaD. This consensus has come from both animal and human studies, ranging across different environmental exposures from the quality of maternal and paternal nutrition to assisted reproductive technology (ART) (**Figure 1**). The stages of gamete maturation, fertilisation and early embryo development are collectively known as the **periconceptional** period. These are characterised by the parental genomes being superseded by the new embryonic genome and the establishment and differentiation of early cell lineages from a pluripotent cellular stock, required for development of the new organism (Graham and Zernicka-Goetz 2016; Li, et al. 2013). Such processes involve significant epigenetic, cellular and metabolic activity (Gardner and Harvey 2015; Lim, et al. 2016; White, et al. 2016) and, from fertilisation, occur within the confines of the maternal oviduct and uterine lumens, long recognised to facilitate the stepwise progression in gamete and embryo maturation culminating in implantation (Coy, et al. 2012; Ghersevich, et al. 2015; Matsumoto 2017).

It has become apparent that these periconceptional stages in reproduction are vulnerable to environmental factors that may cause changes, either through perturbation or via adaptive compensatory responses, which may persist beyond the periconceptional period affecting phenotype across the lifespan. We have recently reviewed the vulnerability of periconception in the context of adverse developmental programming with a focus on the consequences of maternal and paternal over- and under-nutrition and of ART in human and animal models (Fleming, et al. 2018). Maternal or paternal lifestyle factors such as nutritional quality will influence parental physiology in many ways and there is evidence that diet can modify oviduct and uterine transport activities and thereby alter the nutrient composition of luminal compartments and the direct environment experienced by early embryos (Eckert, et al. 2012; Jordaens, et al. 2017). A similar disturbance to the seminal tubule and sperm microenvironment by paternal diet has also been reported (Fan, et al. 2015). Given the clinical implications raised for next generation health from a time when many women may not know they are pregnant, these discoveries of environmental susceptibility of periconceptional stages have contributed to the call for preconception health of both partners to be considered before pregnancy (Barker, et al. 2018; Stephenson, et al. 2018).

Here, we summarise the key processes, mechanisms and DOHaD-induced outcomes during the periconceptional window with respect to maternal and paternal nutrition and ART. We focus in particular on new understanding of themes previously presented in our earlier review (Fleming et al. 2018), reflecting the dynamic nature of this subject.

Maternal Overnutrition and Obesity

High maternal body mass index (BMI) and obesity has long been associated with reduced fertility and the occurrence of obesity in children, mediated by raised maternal metabolites such as glucose and insulin promoting increased placental transport of macronutrients and subsequent increased

fetal growth in late gestation (Godfrey, et al. 2017; Musial, et al. 2017; Nam, et al. 2017; Nicholas, et al. 2016). The risk of metabolic syndrome in offspring from obese mothers has been substantiated mechanistically in animal models (Nicholas et al. 2016; Samuelsson, et al. 2008).

The periconceptional period is critical in the transmission of disease risk from maternal obesity to offspring. Women with high BMI transfer excess metabolites and hormones such as insulin, triglycerides, leptin and lactate from the circulation into ovarian tissue and especially the follicular fluid of maturing follicles (Robker, et al. 2009). These metabolites subsequently accumulate within oocytes, affecting their metabolic function and leading to diminished embryo developmental potential after fertilisation (Yang, et al. 2012). Interestingly, increased lipid accumulation within human follicular fluid coincides with increased inflammatory mediators that may contribute to the reduced potential of embryos from obese mothers (Gonzalez, et al. 2018). Notably, the size of human oocytes is reduced by high maternal BMI and lead to poorer quality embryos with excess triglycerides and diminished glucose consumption (Leary, et al. 2015).

Animal models have been used to identify the metabolic defects in oocytes and early embryos caused by maternal overnutrition. Mitochondria become severely affected in their structure and organisation of cristae, in their cellular distribution and rate of biogenesis, and critically in their capacity for generating energy in response to maternal overnutrition (Igosheva, et al. 2010; Luzzo, et al. 2012). These defective mitochondria are more likely to be preserved in embryos since obesity further reduced mitophagy (Boudoures, et al. 2017). Moreover, accumulating lipids in oocytes induces endoplasmic reticulum and oxidative stress, impairing developmental potential and increasing aneuploidy (Hou, et al. 2016; Igosheva et al. 2010; Luzzo et al. 2012). Maternal diabetes may similarly modulate embryo metabolism, recently investigated in a rabbit model of developmental programming. Here, significant remodelling of several metabolic pathways occurred with a critical role identified for adiponectin in generating lipid accumulation leading to oxidative

metabolic stress (Fischer, et al. 2017). Further evidence of periconceptional metabolic induction of programming from maternal overnutrition has come from supplementing the diet of obese mice with coenzyme Q10 injection which restored mitochondrial functioning (Boots, et al. 2016). Animal *in vitro* studies have also confirmed that increased levels of fatty acids impairs follicular maturation and oocyte potential leading to blastocysts with altered transcription and epigenome profiles (Desmet, et al. 2016; Van Hoeck, et al. 2013). Such studies also demonstrate fatty acid modulation of oviductal barrier function to influence embryo exposure to nutrient levels (Jordaens et al. 2017). Epigenetic effects have also been demonstrated in the oocytes from obese mouse dams with altered levels of DNA and histone methylation regulators (Hou et al. 2016). Epigenetic change associated with genes regulating metabolic health in offspring has also been shown in an ovine model of maternal overnutrition (Nicholas, et al. 2013).

Recent mouse studies have identified a role for PGC7/Stella protein in mediating maternal obesity effects on adverse programming of embryos (Han, et al. 2018). Stella is known to regulate the asymmetry in global DNA demethylation between paternal and maternal genomes and protect imprinted genes from demethylation (Nakamura, et al. 2007) and becomes depleted in oocytes from obese mothers coinciding with global hypomethylation of the embryonic genome (Han et al. 2018). Notably, restoring Stella expression reverses both the epigenetic status of embryos from obese dams and their developmental defects (Han et al. 2018). A further study has identified reduced expression of TIGAR (TP53-induced glycolysis and apoptosis regulator) in oocytes from obese mothers which may contribute to the increased oxidative stress and meiotic spindle defects in such oocytes (Wang, et al. 2018).

These metabolic perturbations induced in oocytes and embryos by maternal overnutrition persist during later development. Mouse fetuses from obese mothers exhibit an altered growth trajectory and give rise to offspring with increased adiposity and metabolic dysfunction such as glucose

intolerance (Jungheim, et al. 2010). Such physiological responses also coincides with underlying transcriptional and epigenetic changes both in the fetus and placenta (Mahany, et al. 2018). Moreover, metabolic dysfunction in offspring from maternal obesity has been shown to persist over three mouse generations, likely reflecting the inheritance of defective maternally-derived mitochondria (Saben, et al. 2016).

The importance of the periconceptional origin of adverse programming from maternal obesity has been demonstrated using embryo transfer to healthy recipients in mouse and sheep models with the persistence of fetal and postnatal metabolic dysfunction despite a normal uterine environment (Luzzo et al. 2012; Nicholas et al. 2013). A similar periconceptional origin of adverse programming in response to maternal diabetes has been shown by mouse transfer of zygotes to healthy recipients (Wyman, et al. 2008). Lastly, consistent with the above, in assisted conception practice, there is some evidence that the maternal BMI of oocyte donors negatively influences reproductive outcomes despite not carrying the pregnancy (Cardozo, et al. 2016).

Maternal Undernutrition

The original datasets revealing adverse adult health outcomes derived from *in utero* experience by David Barker and colleagues implicated maternal undernutrition during pregnancy followed by accelerated 'catch-up' growth postnatally as causative (Barker and Thornburg 2013). Supporting human evidence linking maternal undernutrition and subsequent adult health risks linked to cardiometabolic and neurological dysfunction have come from well-researched historical famines, particularly the Dutch Hunger Winter of 1944-45 and the Chinese Great Famine over 1959-61 (Liu, et al. 2018; Roseboom, et al. 2011; van den Broek and Fleischmann 2017). Whilst such human epidemiological studies are complex and wide-ranging, it has been possible to identify early gestation and the periconceptional period as a vulnerable window for adverse programming. Thus, those individuals conceived during the 5-month Dutch famine exhibit poorer cardiometabolic and

neurological outcomes in adulthood, including accelerated aging that those where the famine experience occurred later in their gestation (Franke, et al. 2018; Roseboom et al. 2011; Tobi, et al. 2014). A similar increased risk of first trimester exposure has also been shown in the Chinese famine (Wang, et al. 2012; Zimmet, et al. 2018). In addition, the Dutch famine research has shown that periconceptional exposure leads to epigenetic dysregulation of genes involved in growth and metabolism such as conserved hypomethylation of the imprinted *IGF2* gene into adulthood (Tobi et al. 2014).

A further critical human dataset linking maternal periconceptional undernutrition with later adult disease has come from studies on populations in The Gambia. Here, nutritional quality is seasonal and associated with later life mortality and health risk. The quality of maternal nutrition at conception has been shown to alter the pre-gastrulation epigenome at metastable epialleles, domains characterised by inter-individual variation in DNA methylation, in a manner that persists into childhood and adolescence (Waterland, et al. 2010). Such alterations in epigenetic signatures further associate with genomic regions predictive of immune status, obesity risk and tumorigenesis (Kuhnen, et al. 2016; Silver, et al. 2015). Indeed, metastable epialleles are present in human early embryos and may provide a suitable epigenetic basis for environment to induce persistent phenotypic change during developmental programming (Kessler, et al. 2018).

Animal DOHaD studies involving rodents, sheep and cattle have further demonstrated the close association between maternal undernutrition and later-life risk of poor health and again underscore the criticality of the periconceptional period (Fleming et al. 2018; Hansen, et al. 2016; Sinclair and Watkins 2013). From our own work, a maternal low protein diet, effectively 50% of normal protein recommendation, targeted exclusively to the mouse and rat preimplantation period of embryo development (Emb-LPD) has been shown sufficient to cause adult offspring cardiovascular, metabolic and behavioural dysfunction, especially in female progeny (Gould, et al. 2018; Kwong, et

al. 2000; Watkins, et al. 2008). The stepwise mechanistic pathway responsible for Emb-LPD adverse programming has been closely examined. The diet results in reduced concentrations of circulating insulin and amino acids (especially the branched-chain amino acids (BCAAs), leucine, isoleucine and valine) within dams that, through analysis of uterine luminal fluids, also changed the metabolite milieu of the immediate environment of embryos (Eckert et al. 2012). Insulin and BCAAs are potent activators of the mTOR signalling pathway regulating cellular growth (Wang and Proud 2009) and, as a consequence of dietary-induced reduction in these metabolites, blastocyst mTOR activity is reduced by Emb-LPD (Eckert et al. 2012). This early maternal-embryo interaction is critical since it activates later adverse programming as shown both by an *in vitro* culture model in medium reduced in insulin and BCAAs (Velazquez, et al. 2018) and by embryo transfer of Emb-LPD blastocysts into control, normal-fed, recipients (Watkins et al. 2008).

The subsequent development of the Emb-LPD blastocyst after maternal dietary induction is altered and in distinct ways for extra-embryonic (trophectoderm, TE; primitive endoderm, PrE) and embryonic (epiblast) cell lineages. These phenotypic modulations impact on the growth trajectory of the fetus which in turn positively correlates with later adult disease risk (Watkins et al. 2008). Both TE and PrE cell lineages, in response to maternal Emb-LPD, undergo cellular changes that collectively are compensatory, likely to augment nutrient delivery to the developing embryo and fetus. These include increased proliferation of the lineages and their capacity for endocytosis of extracellular fluids, thought to increase nutrient supply (Eckert et al. 2012; Sun, et al. 2014). The TE also adopts a more invasive migratory phenotype likely to enhance endometrial implantation (Eckert et al. 2012; Watkins, et al. 2015). Extra-embryonic adaptations induced by maternal protein restriction persist through pregnancy with evidence of improved nutrient delivery via the chorioallantoic placenta (Coan, et al. 2011) and visceral yolk sac (Watkins et al. 2008), the latter coinciding with altered epigenetic regulation of the *Gata6* transcription factor that has a central role in PrE differentiation (Sun, et al. 2015).

In contrast to extra-embryonic lineages, the somatic tissues of the fetus derived from the epiblast, such as liver and kidney, alter their growth trajectory to match prevailing maternal nutrient availability. This is achieved via the rate of ribosome biogenesis, the fundamental unit of biosynthesis, and specifically ribosomal RNA (rRNA) transcription, which is reduced if the maternal dietary restriction is maintained, but increased beyond control levels, if the dietary challenge is lifted as in Emb-LPD. The manipulation of ribosome biogenesis is regulated epigenetically through the level of DNA methylation at the rDNA gene promotor and coincides with altered expression of the ribosome factor Rrn3, known to link ribosome biogenesis with mTOR nutrient signalling (Denisenko, et al. 2016). Thus, the combination of extra-embryonic and embryonic lineage adaptations to maternal Emb-LPD from implantation, comprising increased extra-embryonic nutrient delivery and increased capacity for fetal biosynthesis, in addition to improved maternal protein diet, all act to promote late fetal overgrowth as a basis for postnatal disease derived from periconceptional environment (Fleming et al. 2018; Watkins et al. 2008).

Recent work has shown that Emb-LPD and sustained LPD treatment throughout pregnancy have a negative influence on neurogenesis. Both treatments lead to a decline in neural stem cells (NSCs) during fetal development through reduced proliferation and increased apoptosis. The loss of NSCs coincides with an altered rate of neural differentiation and a postnatal phenotype of altered cortex thickness and short-term memory loss in both males and females (Gould et al. 2018). These findings extend earlier behavioural outcomes from the mouse Emb-LPD model (Watkins et al. 2008) and confirm periconceptional maternal undernutrition as critical in DOHaD for postnatal health across diverse systems.

Assisted reproductive technologies

Assisted reproductive technology (ART) refers to any technique that interferes with the normal biological pathways of reproductive-related events and/or structures in order to contribute to the establishment of pregnancy with the final goal of producing healthy offspring. In general, ART manipulates events and/or structures related to ovulation, fertilization and embryo development (Velazquez 2008). Current estimates from the International Committee Monitoring for Assisted Reproductive Technologies (ICMART) indicate that since the first ART-derived baby in 1978 over 8 million babies have been born through ART worldwide (De Geyter 2018). It should be emphasised that most ART-derived babies appear healthy. But giving the adverse effects associated to ART reported in some human and animal studies (see below), there is an active effort to ensure an efficient and safe application of human ART, including monitoring of the health status of the resultant offspring.

Data from Finland indicated that children up to 4 years of age whose mothers were subjected to ovulation induction with or without intrauterine insemination (IUI) showed an increased risk of cerebral palsy, allergy and asthma, along with longer periods of hospitalization (Klemetti, et al. 2010). A Danish study found that the risk of developing type 1 diabetes during childhood was increased in children conceived through the use of FSH in ovulation induction protocols or in combination with IUI (Kettner, et al. 2016). Analysis of UK data revealed that babies derived from ARTs such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), IUI, gamete intra-fallopian transfer (GIFT) and ovulation induction had an increased risk of developing respiratory distress and infection during the first week of life when compared to naturally conceived counterparts (Waynforth 2018). Similarly, a meta-analysis of 45 studies suggested that the risk of developing birth defects can be increased by IVF and ICSI (Hansen, et al. 2013), something that has been confirmed in a more recent meta-analysis (Zhao, et al. 2018).

Another recent meta-analysis indicated that children conceived by IVF and ICSI showed a lower weight during the first 4 years of age, with the difference disappearing afterwards (Bay, et al. 2018), indicating an enhanced growth velocity during early development. Rapid growth during early childhood can increase the risk of developing obesity and hypertension later in life (Lei, et al. 2015; Mihrshahi, et al. 2011). Indeed, IVF children with rapid growth during early childhood (1-3 years of age) showed higher blood pressure levels compared to spontaneously conceived counterparts at 8-18 years of age (Ceelen, et al. 2009). Increases in blood pressure in IVF/ICSI-derived children has been detected in several studies (Meister, et al. 2018; Sakka, et al. 2010; Scherrer, et al. 2012; Valenzuela-Alcaraz, et al. 2013; Valenzuela-Alcaraz, et al. 2018). Reproductive potential seems to be affected as well, especially in males. Young adults conceived through ICSI showed low sperm concentration and motile sperm count compared to men born after spontaneous conception (Belva, et al. 2016). Interestingly, the impaired sperm production was not associated with significant changes in reproductive hormones (Belva, et al. 2017).

Current evidence seems to indicate that the incidence of certain diseases and some developmental features might not be strongly affected by ART. For instance, the available data indicate that the overall cancer risk does not seem to be increased in ART-derived children, although some studies found a small increased risk for specific types of cancer (Chen and Heilbronn 2017; Wainstock, et al. 2017; Williams, et al. 2018). Studies in the Netherlands reported that behavioural and cognitive performance was not affected in ICSI-derived children at 5 years of age when compared to the general Dutch population (Meijerink, et al. 2016) and that subfertility rather than ART *per se* seems to be the underlying cause of impaired cognitive and behavioural development during childhood observed in some ART-derived children (Schendelaar, et al. 2016). A recent study from the UK also found that IVF and ICSI do not seem to impair children's early cognitive outcomes up to age 11 years (Barbuscia and Mills 2017). Similarly, a recent systematic review revealed that ART treatments such as preimplantation genetic diagnosis/screening do not seem to affect cognitive and

behavioural development, but they can mildly affect psychomotor development (e.g. dysregulation in posture, muscle tone) of children in their first two years of life. However this subtle psychomotor dysfunction was not detected in follow up studies in children up to 9 years of age (Natsuaki and Dimler 2018).

Although these results have been taken as reassuring for ART outcomes affecting offspring mental health (Meijerink et al. 2016), these studies were carried out during early childhood and the truly long-term consequences (i.e. in adulthood) for mental health remain to be determined. Furthermore, there is more uncertainty with some neurodevelopmental disorders. For instance, the occurrence of autism and cerebral palsy in IVF/ICSI-derived children was found to be increased in some (Goldsmith, et al. 2018; Kamowski-Shakibai, et al. 2015; Lehti, et al. 2013; Sandin, et al. 2013; Schieve, et al. 2017; Stromberg, et al. 2002) but not all studies (Fountain, et al. 2015; Kallen, et al. 2010; Kissin, et al. 2015; Reid, et al. 2010). Both autism (Fountain et al. 2015) and cerebral palsy (Goldsmith et al. 2018) has been strongly associated with multiple births in ART pregnancies highlighting the need to reduce multiple pregnancies in women undergoing ART (Pinborg 2018).

Most of the above-discussed studies used as comparison group children naturally conceived by fertile couples, which has been suggested not to be the best control group. Instead, naturally conceived children from sub-fertile parents that managed to achieve pregnancy while waiting for ART treatment will be a more appropriate comparison group (Zhao et al. 2018). Although studies using this control group are available, a substantial proportion of human ART studies still have methodological limitations that hamper the ability to provide reliable conclusions (Guo, et al. 2017; Liu, et al. 2017; Rumbold, et al. 2017), to the point that some authors believe their findings (e.g. increased risk of type diabetes due to ovulation induction protocols) are a statistical artefact (Kettner et al. 2016).

Nevertheless, animal models have provided experimental evidence supporting the notion that cardiovascular (Rexhaj, et al. 2013; Watkins, et al. 2007), metabolic (Cerny, et al. 2017; Chen, et al. 2014; Feuer, et al. 2014), immunological (Karimi, et al. 2017), reproductive (Calle, et al. 2012), and behavioural (Lopez-Cardona, et al. 2015) activity during postnatal development can be affected by ART. These postnatal alterations can be induced by the microenvironment to which embryos are exposed to during *in vitro* procedures. For example, mice and bovine models have demonstrated that *in vitro* exposure during the preimplantation period to specific constituents of culture media such as metabolic hormones (e.g. insulin), amino acids, pyruvate, lactate, and growth factors can induced alterations in birth weight, body growth rate, and cardiovascular function (Banrezes, et al. 2011; Kannampuzha-Francis, et al. 2015; Velazquez et al. 2018). A similar situation has been found in humans, where the culture medium composition induced changes in birth weight (Kleijkers, et al. 2016) and body weight and body mass index examined at 9 years of age (Zandstra, et al. 2018). Importantly, animal models have revealed that culture media modification (e.g. melatonin supplementation) can reverse some of these altered phenotypes (e.g. cardiovascular dysfunction) (Rexhaj et al, 2015).

The current consensus is that the effects of ART on offspring health may have an epigenetic origin (Huntriss, et al. 2018). Indeed, a meta-analysis revealed that the incidence of rare imprinting disorders in IVF/ICSI-derived children is higher than in spontaneously conceived children, although the exact underlying epigenetic mechanism is unknown (Lazaraviciute, et al. 2014). Nevertheless, compared to methylation levels in somatic and embryonic stem cells, a perturbed methylation of imprinted genes such as SNRPN, KCNQ1OT1 and H19 was found in ART-derived human preimplantation embryos (White, et al. 2015). Similarly, changes in DNA methylation were observed in the placenta (Choufani, et al. 2018; Katari, et al. 2009; Melamed, et al. 2015) and cord blood (Katari et al. 2009; Melamed et al. 2015) from ART-derived babies when compared to naturally conceived counterparts. A study comparing natural conception with oocyte donation (i.e.

young fertile oocyte donors/no male infertility) also found differences in placental DNA methylation levels between the groups, suggesting a strong effect of ART and not infertility (Song, et al. 2015). Several regulatory regions, metastable epialleles and imprinted genes, including IGF2, were hypomethylated in blood spots from ART-conceived newborns relative to those conceived naturally (Estill, et al. 2016). The methylation levels of SNRPN, a paternal imprinted gene, were increased in the buccal cells of 2 year old children conceived by ICSI, but not by IVF. This hypermethylation is believed to be associated with the greater degree of *in vitro* manipulation taking place during ICSI (Whitelaw, et al. 2014).

These epigenetic changes are partially attributed to the microenvironment in which embryos are cultured in, as animal models have revealed that media culture composition can alter DNA methylation profiles in preimplantation embryos (Canovas, et al. 2017; Market-Velker, et al. 2010). Furthermore, oxygen tension (5% vs 20%) during culture and type of embryo transferred (fresh vs frozen) have the capacity to alter placental methylation levels from ART-conceived babies when compare to natural conception. Importantly, data from pigs indicate than modification of culture media to resemble *in vivo* composition can induced methylation levels in preimplantation embryos more similar to those of produced *in vivo* (Canovas et al. 2017).

In contrast, DNA methylation was not affected in blood from prepubertal children conceived through IVF (Oliver, et al. 2012). This suggest that ART-induced changes in DNA methylation could be gene- and/or tissue-specific or that postnatal environment masked any subtle changes in DNA methylation induced by ART. The latter emphasises the complexity of epigenetic studies in humans and the need to consider several methodological issues to produce useful epigenetic data (Lazaraviciute et al. 2014). Also, a critical step in elucidating the long-term effects of ART in human populations is the development of databases for ART surveillance (i.e. health monitoring of ART-derived offspring), something that has been implemented just in a few countries (Pinborg

2018). The first ART-derived baby turned 40 years just recently, hence the long-term repercussions (or lack of) of ART for healthy aging are far from being elucidated. This highlights the current need for more research throughout the lifespan of ART-derived offspring.

Paternal origin of periconceptional programming

In contrast to the substantial epidemiological and animal model research linking maternal well-being with offspring programming, our understanding of how a father influences the development and cardiometabolic health of his offspring has been largely overlooked. However, there is now a significant body of data indicating paternal physiological status, lifestyle and environmental exposure to a range of factors not only impact on sperm quality, but also affect the long-term health of his offspring (Fleming et al. 2018). In line with maternal programming studies, animal models have become critical tools for not only defining the underlying paternal mechanisms involved but also identifying central biomarkers of paternal programming ahead of studies using human samples. Studies from humans and animal models have revealed the complexity of both sperm and the seminal plasma, identifying novel processes by which perturbed paternal health at the time of conception affect a dynamic range of reproductive and developmental processes and ultimately, long-term offspring health.

Paternal reproductive health and sperm quality are impaired in response to paternal physiological and lifestyle factors. Mirroring changes in oocyte quality in response to maternal obesity, elevated paternal BMI has been associated with reduced semen volume, sperm number and sperm motility (Chavarro, et al. 2010; Ma, et al. 2018). Furthermore, sperm from overweight or obese men show higher levels of DNA damage when compared to sperm from normal weight males (Campbell, et al. 2015; Kort, et al. 2006). As obesity is associated with multiple disturbances in metabolic profile including elevated levels of inflammatory markers and metabolic intermediates, the detrimental effects of increasing male BMI on sperm quality is believed to be mediated through increased

oxidative damage. Indeed, in both men and rodents, obesity has been shown to result in increased reactive oxygen species generation (Palmer, et al. 2011; Tunc, et al. 2011) and sperm DNA damage (Duale, et al. 2014; Zhao, et al. 2014). Furthermore, consumption of high energy diets have also been associated with reduced sperm morphology, motility and DNA integrity (Agbaje, et al. 2007), perturbed testicular metabolism (Rato, et al. 2013) and reduced fertility (Bener, et al. 2009) in both mice and men. Similar to the effects of paternal overnutrition, deficiency of specific nutrients, or even nutritional imbalance also affect sperm quality. Many macronutrients such as zinc, vitamins and glutathione act as antioxidants to prevent excessive damage from reactive oxygen species. Sperm from infertile men show higher rates of DNA damage which can be reduced following treatment with supplement of selenium and vitamin E (Moslemi and Tavanbakhsh 2011). In mice, the negative effects of paternal undernutrition on sperm DNA damage can be prevented through dietary supplementation with vitamins and minerals (McPherson, et al. 2016).

Poor paternal health not only impacts on sperm quality, but can also affect post-fertilisation development and offspring well-being. In men, some studies have identified associations between obesity and reduced rates of blastocyst development and live birth following IVF (Bakos, et al. 2011). Such observations are supported by a recent, large meta-analysis in which the link between paternal obesity and live birth rates after ART cycles was examined in 115,158 patients (Campbell et al. 2015). Here, the authors reported a significant negative impact of increased male BMI on non-viable pregnancy outcomes. In mice, paternal obesity has been reported to increase rates of one-cell block, decrease blastocyst cell number and perturb embryo carbohydrate metabolism (Binder, et al. 2012; Mitchell, et al. 2011). Our own studies have revealed that a paternal low protein diet (LPD) decreased blastocyst expression of multiple genes involved in the 5' AMP-activated protein kinase (AMPK) pathway including genes for metabolism, regulation of transcription and protein synthesis (Watkins, et al. 2017). Interestingly, similar decreases in several of these AMPK pathway genes were still evident in late gestation fetal liver tissues and associated with increased rates of fetal

growth (Watkins et al. 2017). As in studies of poor maternal diet during pregnancy, we observed that the enhanced fetal growth programmed by paternal LPD was associated subsequently with increased adiposity, impaired glucose metabolism, hypotension and vascular dysfunction in adult offspring (Watkins and Sinclair 2014). Separately, other studies have shown significant changes in fetal (Carone, et al. 2010; Lambrot, et al. 2013) and postnatal offspring development and metabolic health (Anderson, et al. 2006; McPherson et al. 2016; Ryan, et al. 2018) in response to paternal diet or food intake in mice. Interestingly, recent studies have demonstrated robust transgenerational effects of chronic paternal stress on offspring well-being and hypothalamic pituitary adrenal axis function (Gapp, et al. 2014; Rodgers, et al. 2015).

The fact that many paternal programming studies identify consistent transgenerational programming effects (Fullston, et al. 2013; Gapp et al. 2014) indicates changes in sperm epigenetic status as one potential mechanism linking paternal well-being with offspring development. Over recent years the epigenetic complexity of mammalian sperm has been revealed. In contrast to the oocyte, sperm contain almost no cytoplasm and the DNA is packaged using protamines rather than histones. Inappropriate protamine packaging of the sperm DNA, or perturbed histone to protamine transition can be indicative of impairments in the fundamental process of spermatogenesis (Sakkas, et al. 2002) or damage due to excessive exposure to reactive oxygen species (Sakka et al. 2010). Furthermore, atypical chromosome packaging and localisation within the sperm or perturbed telomere-centromere interactions has been associated with infertility in some men (Zalensky and Zalenskaya 2007), while sperm chromatin maturation level has been link with pregnancy establishment rates (de Lamirande, et al. 2012). While the majority of the sperm DNA is re-packaged with protamines, specific genomic sequences retain their histone marks. What is interesting is that the location of these retained histones is not random, but specific to important developmental genes (Hammoud, et al. 2009) and retrotransposable long and short interspersed nuclear elements in both men and mice (Samans, et al. 2014). Furthermore, some of these sperm-

specific histones have been shown to be retained within the oocyte and contribute to the zygotic genome (van der Heijden, et al. 2008).

In addition to sperm chromatin structure, differential profiles of DNA methylation have also been linked to sperm quality in infertile men (Hammoud, et al. 2010). In studies looking at success rates of women undergoing IVF, the genome-wide methylation profile of their partner's sperm correlated with embryo quality (Aston, et al. 2015) and was indicative of pregnancy failure (Benchaib, et al. 2005). In mice, significant changes in sperm DNA methylation profiles have also been identified in response to paternal obesity (Fullston et al. 2013), low protein (Carone et al. 2010) or low folate (Lambrot et al. 2013) diets. Our own studies have showed that feeding male mice a LPD results in global sperm hypomethylation associated with reduced testicular expression of central regulators of DNA methylation and 1-carbon metabolism (Watkins, et al. 2018). Interestingly, analysis of the sperm DNA hypomethylation revealed significant reductions at multiple genes involved in calcium signalling which correlated with our earlier reported impairments in cardiovascular function and cardiac calcium signalling gene expression in adult offspring of LPD fed males (Watkins and Sinclair 2014). In addition to histone and DNA modifications, sperm have been shown to contain a range of RNA species including mRNA, micro-RNA, short and long non-coding RNA and small interfering RNAs (Colaco and Sakkas 2018). The significance of sperm-derived RNAs for post-fertilisation development has been demonstrated in animal models where the depletion of specific sperm micro RNAs results in developmental delay of the zygote (Liu, et al. 2012). In addition, injection of tRNA-derived small RNAs from sperm of high fat diet fed male mice into control zygotes resulted in impaired glucose metabolism and insulin secretion in the resultant offspring (Chen, et al. 2016).

Separate to the epigenetic status of the sperm, fathers may also influence the development of their offspring via seminal plasma-specific modulations of the maternal reproductive tract environment

(Robertson and Sharkey 2016). In both mice and women, deposition of seminal plasma within the reproductive tract initiates a significant inflammatory and immunological response culminating in uterine vascular remodelling, the recruitment of leukocytes and the priming of regulatory T cells (T-regs) and the production of a myriad of cell signalling molecules such as colony stimulating factor-2 (CSF2), leukemia inhibitory factor (LIF) and interleukin 6 (IL-6) (Schjenken and Robertson 2014). Interestingly, studies have demonstrated positive associations between a woman's unprotected exposure to her partner's seminal plasma and a reduced risk for her developing preeclampsia during pregnancy (Robillard, et al. 1994). In mice, lack of seminal plasma at the time of conception has been shown to impair embryo development, fetal growth and adult offspring cardiometabolic health (Bromfield, et al. 2014). Our own studies have shown that offspring growth and metabolic health appear equally compromised in response to either sperm or seminal plasma from male mice fed a LPD (Watkins et al. 2018).

Conclusions

It is clear from the above four types of exposure during periconceptional reproduction that altered developmental programming may emerge from diverse environments (summarised in **Table 1**). Whilst here we focus on parental nutrition *in vivo* and embryo manipulations *in vitro*, the spectrum of exposures with enduring consequences is undoubtedly broader. For example, periconceptional maternal alcohol consumption prior to embryo implantation in a rat model resulted in abnormal trophoblast placental function, altered expression of epigenetic regulators for DNA methylation in the fetal liver, culminating in postnatal glucose and insulin intolerance and increased risk of offspring obesity (Gardebjer, et al. 2015; Gardebjer, et al. 2018; Kalisch-Smith, et al. 2016). In another example, maternal sickness and systemic inflammation at the time of conception has been shown in a mouse model to alter blastocyst morphogenesis with long-term consequences for adult offspring immune function (Williams, et al. 2011). Here, reproductive function and embryo implantation are in part regulated by the activity of maternal immune cells and the balance of pro-

and anti-inflammatory cytokines can have significant influence not only on embryo survival but long-term health of offspring (Robertson, et al. 2015).

The extent to which periconceptional exposure can associate with adult DOHaD consequences is also influenced by intrinsic processes such as maternal ageing. Whilst it is well established that fertility declines with age, the developmental potential of oocytes with advancing age is also affected. In a recent mouse study, preimplantation embryos from aged versus young mothers, both sired by young males and transferred to young recipients to carry the pregnancy, gave rise to offspring with altered growth and increased cardiometabolic dysfunction (Velazquez, et al. 2016). Oocytes from older mothers exhibit mitochondrial dysfunction and perturbed energy homeostasis (Dumesic, et al. 2015) which may indicate adverse programming derives from similar processes as occurs following maternal overnutrition, although mechanisms are underexplored.

A consistent feature across the research field of periconceptional programming has been the involvement of epigenetic dysregulation as a means by which effects on gene expression and cellular phenotype may persist through gestation and later life (Steegers-Theunissen, et al. 2013). Manipulation of periconception maternal diet composition to reduce the availability of methyl donors for DNA and histone methylation via one-carbon metabolism has been shown to alter the offspring epigenome with accompanying cardiometabolic disease outcomes (Sinclair, et al. 2007). Provision of methyl donors can also reverse adverse programming mediated through the rat maternal LPD model (Lillycrop, et al. 2005). Animal oocytes and early embryos are known to express key enzymes in the methionine/folate cycles (Kwong, et al. 2010) and a role for mTOR signalling has been identified for sensing the levels of folate available for placental development and fetal growth (Gupta and Jansson 2018; Rosario, et al. 2017). Variability across individuals and ethnic groups in regulatory genes involved in one-carbon metabolism may contribute to the relative susceptibility to adverse programming (Clare, et al. 2018). What is clear is that health of both

parents in terms of diet and physiological condition is an important factor to establish before conception rather than later in pregnancy to protect the health of the next generation.

Declaration of interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure legends

Figure 1.

Summary diagram of the periconceptional period covering gamete maturation and early embryogenesis with key developmental stages and events identified, shown both in vivo and during ART, and with long-term risks for offspring health from adverse exposures listed.

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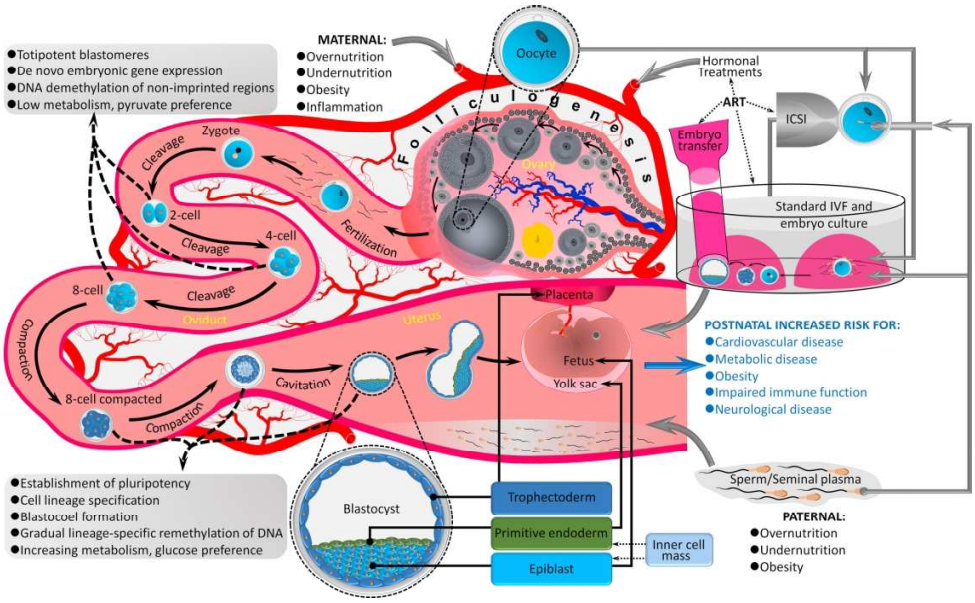

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Insult

	Maternal overnutrition	Maternal undernutrition	Paternal nutrition	Assisted Reproductive Technologies (ART)
Impact on gamete quality and parental environment	• Excess follicular metabolite concentration • Reduction in oocyte size and embryo quality • Increased oocyte lipid accumulation, ER stress and mitochondrial dysfunction • Perturbed expression of epigenetic regulators	• Altered uterine metabolite concentrations	• Elevated sperm DNA damage • Altered sperm epigenome • Altered sperm RNA content • Altered seminal plasma composition	
Impact on embryo development	• Increased oxidative metabolic stress • Altered profiles of transcription	• Reduced blastocyst mTOR signalling • Extra-embryonic cellular adaptations to enhance nutrient retrieval	• Reduced APMK gene expression • Altered maternal uterine immunological environment	• Altered epigenetic status
Impact on offspring phenotype and health	• Increased fetal growth • Altered placental epigenetic status • Increased offspring adiposity • Cardio-metabolic dysfunction	• Altered epigenetic status • Altered ribosome biogenesis • Increased fetal growth • Increased adiposity • Cardio-metabolic dysfunction • Neurodevelopmental dysfunction • Perturbed imprinted gene epigenetic status	• Perturbed fetal growth • Increased offspring adiposity • Cardio-metabolic dysfunction	• Altered birthweight • Increased early life growth • Poorer cardio-metabolic health • Reduced sperm counts • Increased rates of imprinting disorders

Table 1. Summary of main environmental exposures discussed in the review and their impact during development and health outcomes in later life.