THEMATICAL REVIEW

Nutritional adversity, sex and reproduction: 30 years of DOHaD and what have we learned?

Patrycja A Jazwiec1,2 and Deborah M Sloboda1,2,3

1Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada
2The Farncombe Family Digestive Diseases Research Institute, McMaster University, Hamilton, Canada
3Department of Pediatrics and Obstetrics and Gynecology, McMaster University, Hamilton, Canada

Correspondence should be addressed to D M Sloboda: sloboda@mcmaster.ca

This paper is part of a thematic section on 30 Years of Developmental Endocrinology of Health and Disease. The guest editors for this section were Sean Limesand, Kent Thornburg and Jane Harding

Abstract

It is well established that early life environmental signals, including nutrition, set the stage for long-term health and disease risk – effects that span multiple generations. This relationship begins early, in the periconceptional period and extends into embryonic, fetal and early infant phases of life. Now known as the Developmental Origins of Health and Disease (DOHaD), this concept describes the adaptations that a developing organism makes in response to early life cues, resulting in adjustments in homeostatic systems that may prove maladaptive in postnatal life, leading to an increased risk of chronic disease and/or the inheritance of risk factors across generations. Reproductive maturation and function is similarly influenced by early life events. This should not be surprising, since primordial germ cells are established early in life and thus vulnerable to early life adversity. A multitude of ‘modifying’ cues inducing developmental adaptations have been identified that result in changes in reproductive development and impairments in reproductive function. Many types of nutritional challenges including caloric restriction, macronutrient excess and micronutrient insufficiencies have been shown to induce early life adaptations that produce long-term reproductive dysfunction. Many pathways have been suggested to underpin these associations, including epigenetic reprogramming of germ cells. While the mechanisms still remain to be fully investigated, it is clear that a lifecourse approach to understanding lifetime reproductive function is necessary. Furthermore, investigations of the impacts of early life adversity must be extended to include the paternal environment, especially in epidemiological and clinical studies of offspring reproductive function.

Introduction

It is well established that our lifestyle and external environment, including nutrition and physical exercise, influences our overall health. However, seminal work completed nearly 35 years ago (Barker & Osmond 1986, Barker et al. 1989, 1993) as well as accumulating evidence from epidemiological and experimental studies have demonstrated that the early life environment, including the in utero environment experienced by the embryo/fetus, also influences health and disease risk later in life (reviewed in Chan et al. 2015b). The developing
organism is plastic, where regulation of gene expression and cell signaling pathways are sensitive to and thereby adapt to, environmental cues. This plasticity is most prevalent during key developmental windows, including the period during which gametes, germ/stem and somatic cells proliferate and differentiate forming organ systems (Gluckman et al. 2005). Signals received by a developing organism from its mother (particularly in mammals) are used to make changes in homeostatic pathways, most notably to reproductive, metabolic and stress-related homeostasis (Gluckman & Hanson 2004), in order to maximize fitness (reproductive and metabolic in this case) in the postnatal environment (Gluckman et al. 2005, Hanson & Gluckman 2014). It has been proposed, however, that when a mismatch exists between the developmental and predicted postnatal environments, these adaptations may negatively affect health and result in increased disease risk. The hypothesis that the early life environment influences later life disease susceptibility is now recognized as the Developmental Origins of Health and Disease (DOHaD).

Programming reproduction

It is now known that non-communicable chronic diseases that were previously associated with lifestyle and genetics have their origins early in life. Of note, these disease effects span multiple generations (Gluckman & Beedle 2007, Aiken & Ozanne 2014). Germ cells (oocytes/sperm) in the developing gonads appear to be similarly vulnerable to early life events and thus are likely to contribute to transgenerational disease risk. It is therefore plausible that since the gametes that will eventually give rise to grand-offspring form during development, that the long-term impacts of early life adversity and postnatal disease lies in the gonads – within the developing germ cells and their function.

If one looks at programming reproduction through an evolutionary biology lens, life history theory provides a framework to understand how early life adversity results in significant shifts in reproductive capacity and development (Barrett et al. 2012). An organism’s success is measured by the number of offspring that survive to pass on genetic information and thus relies on both reproductive capacity and survival (Barrett et al. 2012). At the basis of life history theory is the presence of trade-offs that exist between the benefits of reproducing (and ultimately fitness) and the price of reproduction, which is costly in terms of energy requirements and may result in resource distribution from one system to another (Stearns 1992, 2000, Speakman 2008). An example of this trade-off is when an immature organism allocates energy resources to growth, and it is not until the time of sexual maturity that energy resources become available for reproduction (Stearns 2000, Sloboda et al. 2011). Classically, this framework proposes that under postnatal conditions of low energy or scarce resources, organisms will delay reproductive maturation until a time when resources are adequate and reproductive activities will be successful (Tanner 1955, Ellison 1982, Garn 1987, Coall & Chisholm 2010). Human and experimental evidence, however, shows that prenatal, early life adversity is associated with early reproductive maturation (Sloboda et al. 2011), although until recently, the focus has largely been on female reproductive maturation and function. In both epidemiological and experimental studies, evidence exists that early life adversity results in significant changes in reproductive maturation and capacity. Poor nutrition, childhood adversity, psychosocial distress and uncertainty and poor familial relationships are associated with high mortality rates, and when life expectancy is low, life histories are fast (Chisholm et al. 1993, Kramer et al. 2009, Coall & Chisholm 2010, Nettle 2010). Therefore, one might argue that from this evolutionary perspective, early life adversity will lead to accelerated maturation, where the organism trades body size and longevity for earlier reproduction in a ‘threatening’ environment. Indeed, we and others have previously suggested that fetal growth restriction (FGR) might reflect a life history strategy where the anticipation of a shorter life and increased risk of disease and mortality results in a decreased allocation of energy resources into growth and increased energy investment into reproduction to ensure reproductive fitness (Ellison et al. 1993, Gluckman & Hanson 2006, Jasienska et al. 2006, Sloboda et al. 2009, 2011, Chan et al. 2015b, Sharpe 2018). In this article, we review the reproductive literature from a DOHaD perspective. We provide a brief overview of the development of gametes in male and female mammals, and how early life nutritional adversity impacts female and male reproductive development and function, and briefly suggest how epigenetics may be the basis for the mechanism of action.

Brief overview of gametogenesis

In mammals, the link between early life adversity and reproductive impacts is not difficult to imagine, as the female and male germ cells, the oocytes and spermatozoa, develop from a common precursor stem cell, the primordial...
germ cells (PGC), in the developing gonads during the early life period. After the primordial gonads differentiate into oocytes or testes, the PCGs develop into oocytes through oogenesis or spermatogenesis in spermatogenesis in males and females, respectively.

**Oogenesis**

The mammalian adult ovary is heterogeneous in composition, containing follicles at several developmental stages. Each follicle is composed of an oocyte surrounded by somatic granulosa cells (Hirshfield 1991). Follicle growth begins with a gonadotropin-independent phase. During this phase, ovarian follicles transition through the primordial, primary and secondary stages and then transition from the preantral to the early antral stage. Subsequently, follicles grow and undergo selection and ovulation during the gonadotropin-dependent phase (Sanchez & Smitz 2012). Follicle formation begins during early embryogenesis (Mamsen et al. 2012) where oogonia enter meiosis I and arrest at the diplotene stage of prophase I forming primary oocytes. The development of oocytes remains in meiotic arrest until pubertal onset (Pepling 2012). During folliculogenesis, the primary follicle develops from a primordial follicle. The presence of multiple layers of cuboidal granulosa cells is representative of the secondary follicle stage. Cells derived from surrounding stromal tissue, known as thecal cells, arise and form a cellular layer encompassing the granulosa cells and oocyte (Young & McNeilly 2010). The formation of the granulosa and theca cell layers are essential for establishing sensitivity to gonadotropins as well as for the production of androgens essential in follicle maturation and ovulation (Kumar et al. 1997). Once the secondary follicle grows to its maximum size, small cavities filled with follicular fluid merge into a larger fluid-filled cavity, known as the antrum, in the granulosa cell layer (Monniaux et al. 2014). The antral follicle may then undergo apoptosis or the final steps of maturation necessary for ovulation; this process is regulated by the hypothalamic–pituitary–gonadal (HPG) axis, through the hypothalamic secretion of gonadotropin-releasing hormone (GnRH), and resulting secretion of the pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and ovarian steroidal hormones estradiol (E$_2$) and progesterone (P$_4$) (McGee & Hsueh 2000), which occurs after the onset of puberty.

**Spermatogenesis**

Spermatogenesis is a continuous and dynamic process by which immature diploid spermatogonia produce mature haploid spermatozoa (sperm). Spermatogenesis occurs in the seminiferous tubules of the testis and initiates after pubertal onset following activation of the HPG axis. The production of sperm through spermatogenesis is dependent on the differentiation and maturation of spermatogonial stem cells (SSCs). After pubertal onset, FSH and LH produced by the pituitary gland stimulate the Leydig cells of the testes to produce testosterone and consequently initiate spermatogenesis. Spermatogenesis is composed of three main stages, which include spermatocytogenesis (mitotic phase), spermatidogenesis (meiotic phase), and spermiogenesis (maturation). During spermatocytogenesis, diploid Type A spermatogonia undergo a round of mitosis and produce two diploid spermatogonia, Type $A_d$ spermatogonia and Type $A_p$ spermatogonia. Type $A_d$ spermatogonia undergo division to produce identical daughter cells that replenish and maintain the SSC population. In contrast, Type $A_p$ spermatogonia form Type B spermatogonia, which serve to produce mature sperm. During spermatocytogenesis, Type B spermatogonia divide by mitosis to produce two diploid primary spermatocytes. Primary spermatocytes divide and form two secondary spermatocytes by meiosis I. During the next step of spermatogenesis, spermatidogenesis, the secondary spermatocytes undergo meiosis II, during which they divide and form two haploid spermatids, half of which contain an X chromosome and half of which contain an Y chromosome. The resulting spermatids undergo the final stage of spermatogenesis, known as spermiogenesis. During spermiogenesis, the spermatids develop into motile and mature spermatozoa. The key changes that occur during spermiogenesis include acrosomal cap and tail formation, removal of excess cytoplasm, as well as histone–protamine transition (Gilbert 2000). Following spermiogenesis, the mature sperm are released into the lumen of the seminiferous tubules of the testis (Clermont 1966, Culty 2009).

**Maternal nutritional impacts on offspring reproduction**

**Impacts in female offspring**

**FGR and maternal nutritional restriction**

**Epidemiological/clinical data on growth restriction and reproduction in human females** Numerous studies have shown that FGR or intrauterine growth restriction (IUGR) is associated with altered development and reproductive dysfunction in offspring (Cooper et al. 1996,
Sloboda et al. 2007, Ibanez et al. 2011). Indeed low birth weight (LBW) is associated with a number of morbidities often associated with aging in women, including early reproductive aging (Alexander et al. 2014). Since adequate nutrition during pregnancy is a key factor mediating fetal growth, it is not surprising that some of the first observations linking growth restriction and reproductive dysfunction come from studies of famine in large populations. One of the first sets of observations implicating the prenatal nutritional environment to impaired reproductive function was derived from the Dutch Hunger Winter Famine of 1944–1945 (Schulz 2010). Women exposed to famine throughout pregnancy gave birth to children that began bearing children at a younger age (Painter et al. 2008) and presented at an increased risk of experiencing menopause earlier in life (Elias et al. 2003, Yarde et al. 2013), although there is at least one report that some of these associations may not exist (Lumey & Stein 1997). Similarly, women that experienced acute malnutrition in utero as a result of the 1959–1961 famine in China experienced an increase in the risk of sterility, albeit a modest 1.1% increase, potentially resulting in permanent impaired fecundity (Song 2013), illustrating that similar phenotypes occur in different geographical locations across different populations.

Data from small selected populations have suggested underlying mechanisms. Ibanez and colleagues have published an extensive set of papers showing that in utero growth restriction in girls significantly impairs reproductive development. This groups has demonstrated that serum levels of FSH were increased twofold in girls born small-for-gestational age (SGA) compared to girls born appropriate-for-gestational age (AGA) (Ibanez et al. 2002), and FSH hypersecretion was also evident in adolescent females born SGA (Ibanez et al. 2000, Ibanez 2003). In addition to altered gonadotropin secretion, females born SGA show impaired ovarian development and reduced ovarian size (Ibanez et al. 2000, Ibanez 2003). Similarly, female fetuses that experienced growth restriction in utero show reduced ovarian size accompanied by a significant decrease in primordial follicle number (de Bruin et al. 1998). Other reports contradict these findings, however, showing similar primordial follicle counts in IUGR female fetuses compared to appropriately grown fetuses (de Bruin et al. 2001).

Girls born SGA achieved reproductive maturity earlier in comparison to girls born AGA (Cooper et al. 1996). Indeed, this relationship holds true across the normal birth weight spectrum (Sloboda et al. 2007). Precocious reproductive maturity in SGA girls manifested as exaggerated adrenarche (Ibanez et al. 1998, Veening et al. 2004), advanced menarche (Cooper et al. 1996, Veening et al. 2004, Ibanez & de Zegher 2006), reduced rates of ovulation (Ibanez & de Zegher 2006), premature onset of menopause, as well as increased secretion of adrenal androgens (Ibanez et al. 1998). However, it is currently unknown whether earlier reproductive maturity is associated with premature follicle loss. Studies suggest that girls growth-restricted in utero have an increased risk of infertility (Vikstrom et al. 2014); however, SGA is not always associated with increased risk of infertility in adulthood (Meas et al. 2010, Sadrzadeh-Broet et al. 2011), or with advanced menarche (Shim et al. 2013) or precocious menopause (Treloar et al. 2000). Overall, most reports suggest that IUGR and SGA are associated with reproductive dysfunction in females. The mechanisms underlying these associations are unclear, but since fetal nutrient supply significantly impacts fetal growth, prenatal nutritional manipulation has emerged as an important model, used to elucidate the relationship between fetal growth and postnatal reproductive function.

**Experimental animal models of nutritional restriction programming reproduction in females** Given the considerable amount of evidence from studies suggesting that reproductive function is influenced by early life events, particularly those that restrict fetal growth, animal models of nutrient restriction and FGR have been developed to understand underlying mechanisms. Using rodents models of maternal caloric restriction during pregnancy and/or lactation, we (Sloboda et al. 2009, Bernal et al. 2010, Chan et al. 2015a, 2018, Jazwiec et al. 2019) and others (Caron et al. 2012, Mossa et al. 2013, Khorram et al. 2015, Hoffman et al. 2018) have demonstrated that offspring born under conditions of nutrient restriction show early puberty, reproductive cycle irregularity and reductions in follicle subpopulations; clear indicators of early ovarian aging. Although some models of maternal caloric restriction have found that pubertal onset is delayed in female offspring (Engelbrecht et al. 2000, Sanchez-Garrido et al. 2013, Matsuzaki et al. 2018). It has been proposed that the susceptibility of oocytes to nutritional adversity during development results in impairments to folliculogenesis that translate to reproductive dysfunction in later life. We have recently shown that these impacts may occur very early in the development of the ovary, as early as day 4 of neonatal life in rats, effects that are associated with impaired reproductive cyclicity and ovulation by young adulthood (Chan et al. 2018). We showed that neonates born to nutrient restricted mothers
have accelerated primordial follicle recruitment, and that beyond this stage, impaired follicle growth factor and gonadotropin responsiveness resulted in increased follicular atresia by apoptosis, by 65 days of age (Chan et al. 2018). We propose that accelerated follicular recruitment is facilitated by an increase in the transcriptional driver FOXO3a, concomitant with a reduction in the brake on follicular recruitment via the AMH receptor, in neonatal ovaries born to undernourished mothers (Chan et al. 2018) (Fig. 1). We and others have shown that prenatal nutritional adversity results in ovarian oxidative stress (Aiken et al. 2013, Chan et al. 2015a), impaired mitochondrial function (Aiken et al. 2013) and together with a loss of ovarian follicles early in life (Sloboda et al. 2009, Aiken et al. 2013, Chan et al. 2015a) suggests that prenatal nutrient restriction results in early ovarian aging. Indeed in early sheep studies, oxidative stress was evident even in fetal oogonia and was associated with an increase in tumor suppressor/cell-cycle arrest modulator p53, suggesting that depending on the species studied, different windows of development may be more or less vulnerable to nutritional adversity (Murdoch et al. 2003).

Critical windows have been explored, suggesting that depending on the period of exposure to undernutrition, differential reproductive outcomes may occur. This has largely been explored in large animal models, since gestation length is long enough to explore different periods of exposure. Indeed, early studies in sheep have shown that an acute bout of undernutrition early in pregnancy impacts fetal follicular development where undernutrition during the first 30 days (of 150-day pregnancy) impaired growth of ovarian follicles in fetuses some 80 days later (Rae et al. 2001). Undernutrition late in pregnancy resulted in smaller ovarian follicles in juvenile lambs possibly due to lower insulin like growth factor (IGF) levels (Hoffman et al. 2018) and undernutrition throughout most of pregnancy resulted in a diminished response to GnRH in young lambs (Deligeorgis et al. 1996). Undernutrition for the first half of pregnancy had no effect on FSH profiles in females, basal LH profiles or gonadotrophin responses to GnRH, but ovulation rate was significantly reduced (Rae et al. 2002a).

It is likely that some of these ovarian changes are coupled with alterations in central processing and hypothalamic–pituitary drive. Coupled with changes in estrous cyclicity, maternal undernutrition in rats resulted in changes in hypothalamic gonadotrophin-releasing hormone (GnRH) transcript levels in 1-day-old neonatal rats, proposed to be mediated by hypothalamic leptin

Figure 1
Maternal undernutrition (UN) impacts on key signaling factors involved in primordial follicle recruitment in neonatal (P4) ovaries. Data from Chan et al. (2018). Reproduced, with permission, from Chan KA, Jazwiec PA, Gohir W, Petrik JJ & Sloboda DM; Maternal nutrient restriction impairs young adult offspring ovarian signaling resulting in reproductive dysfunction and follicle loss; Biology of Reproduction, 2018, volume 98, pages 664–682, by permission of Oxford University Press. Photographs represent the immunolocalization of phosphorylated (p)FOXO3, AMH receptor 2 (AMHR2) and AMH in P4 neonatal ovaries from either control fed (CON) or undernourished (UN) mothers. Brown color depicts protein localization. Inset circles represent the image at 20× magnification. Negative controls incubated in the absence of primary antibody showed no staining (data not shown). Scale bar represents 100 μm. Graphs represent semi-quantitative analysis of immunopositive staining for pFOXO3 (CON n = 5, UN n = 5); AMHR2 (CON; n = 5, UN; n = 5) and AMH (CON; n = 5, UN; n = 6). Data are presented as box and whiskers plots, min to max, where the center line represents the median. *P < 0.05.
levels (Khorram et al. 2015). Leptin is known to stimulate GnRH secretion, and it has been previously shown that maternal undernutrition alters the normal neonatal leptin surge in rodents (Yura et al. 2005, Delahaye et al. 2008) which likely impacts neuronal regulation of both metabolism and reproduction (Mela et al. 2015). It is thus possible that the link between energetics and reproductive function is dysregulated in neonatal life, and that later life outcomes, like ovarian follicle loss, are likely the result of this disrupted interaction. Central regulation of behavior is also vulnerable, since hypothalamic nuclei appear sensitive to early nutritional adversity. Sexual behavior is altered by early life nutritional adversity, where receptive sexual behavior in female offspring born to undernourished mothers was decreased and was associated with changes in transcript levels of hypothalamic progesterone receptors and oxytocin. These changes were associated with changes in pubertal onset and estrous cyclicity (Matsuzaki et al. 2018).

**Impacts of obesity, overweight and nutritional excess**


**Epidemiological/clinical data on the impacts of maternal obesity and nutrient excess on reproduction in human females**

Evidence from human cohorts suggests that maternal obesity predisposes offspring to reproductive dysfunction as adults. An overweight and an obese prepregnancy body mass index (BMI) was associated with early onset of menarche (first menstrual cycle) in daughters (OR 3.1; 95% CI 1.1–9.2) (Keim et al. 2009), where girls born to mothers with a BMI ≥22 kg/m² experienced menarche 4.1 months earlier than girls whose mothers had a BMI of 20.0–21.9 kg/m² (Mariansdatter et al. 2016), and also experienced earlier onset of secondary sex characteristics (Kubo et al. 2018). These findings suggest that exposure to maternal obesity, as well as maternal hyperglycemia, places girls at higher risk of earlier pubarche (Kubo et al. 2016). Excessive gestational weight gain (GWG) during pregnancy is also an independent risk factor for early menarche. Daughters whose mothers gained greater than 40 lbs of gestational weight had a 30% increased risk of experiencing menarche before 11 years of age (OR 1.27; CI 1.06–1.56) (Boynton-Jarrett et al. 2011). These findings are supported by other studies that report an inverse association between maternal prepregnancy BMI and GWG and age of menarche of their daughters (Shrestha et al. 2011, Deardorff et al. 2013, Jansen et al. 2015, Lawn et al. 2018). Both maternal prepregnancy BMI and GWG were inversely associated with daughter’s age at menarche and interestingly the associations remained unchanged after adjustment for many confounders, but were attenuated when adjusted for daughter’s prepubertal BMI (Lawn et al. 2018). These findings indicate that the association between prepregnancy BMI and GWG may be mediated by daughters’ prepubertal BMI (Lawn et al. 2018), an observation that we have also found when considering the relationship between birth weight and age at menarche (Sloboda et al. 2007).

**Experimental animal models of obesity and nutritional excess programming reproduction in females**

Animal models of diet-induced obesity (DIO) provide considerable insight into understanding the mechanisms governing maternal obesity-induced impacts on female offspring reproductive development and function. We and others have found that adult female rat offspring exposed to a high-fat diet (either n-6 polyunsaturated fatty acid or saturated fats) in utero reach puberty earlier compared to control counterparts (Hilakivi-Clarke et al. 1997, Sloboda et al. 2009, Connor et al. 2012), and these offspring have increased risk of reproductive (breast) cancer (Hilakivi-Clarke et al. 1998) possibly via elevated pregnancy estrogen levels in high-fat fed mothers. It has been proposed that these high levels of estrogen may alter mammary gland morphology, increasing terminal end bud number, and expression of fat- and/or estrogen-regulated genes in the offspring, thereby increasing breast cancer risk in offspring (Hilakivi-Clarke et al. 1999). Although estrogen levels were unaffected, we have demonstrated that female rat offspring of high-fat-fed mothers experienced irregular reproductive cycles (Connor et al. 2012).
There is no doubt that maternal obesity affects both the mother’s own fertility (Bermejo-Alvarez et al. 2012, Silvestris et al. 2018) as well as development of her offspring (Catalano & Shankar 2017). Obesity in women results in significant impairments in the oocyte, including mitochondrial dysfunction (Grindler & Moley 2013), lipotoxicity (Jungheim et al. 2011), elevated reactive oxygen species (ROS) (Wakefield et al. 2008, Igosheva et al. 2010) and ER stress (Wu et al. 2015b) damage. Weight loss, at least in mice, did not show reversal of these negative defects or reverse oocyte lipid accumulation, alterations in TCA cycle metabolite levels and mitochondrial membrane potential, or abnormalities in mitochondrial distribution, despite reversal of the metabolic phenotypes of obesity (Reynolds et al. 2015). But effects can be reversed with therapies specifically targeting the mitochondrial respiratory chain (Boots et al. 2016) or ER stress, and oocyte competence has been improved with voluntary exercise regimes (Boudoures et al. 2016). These impairments in oocytes are associated with poor blastocyst and embryo development, increased ROS production as well as mitochondrial dysfunction in embryos (Igosheva et al. 2010, Wu et al. 2010, Boudoures et al. 2017). Furthermore, defects in the mitochondria of maternal oocytes can be seen in offspring oocytes for at least two more generations, suggesting germline transmission (Saben et al. 2016) (Fig. 2). Similar defects in mitochondrial function have been seen in mouse embryos of pregnancies complicated by diabetes (Wang et al. 2009), although at least one study reports that normal blastocyst development was observed in fertile, obese females (Bermejo-Alvarez et al. 2012).

Longer-term impacts of maternal obesity and high-fat diet (HFD) exposure on female offspring reproduction is also evident. Exposure to HFD in utero is associated with a significant reduction in the number of primordial follicles in lambs (Da Silva et al. 2001). In our work, we show that fetuses of mothers fed a HFD during pregnancy have an 11% reduction in oocyte number (Tsoulis et al. 2016) – an effect that correlated significantly to maternal adiposity (Fig. 3). We proposed that maternal HFD intake during pregnancy resulted in an increase in primordial follicle assembly, amounting to more primordial and transitioning follicles early in life essentially changing the development of the primordial follicle pool in fetal life, but that these follicles are then lost to atresia as they transition to growing follicles (Tsoulis et al. 2016). Similar outcomes have been reported in rabbit offspring,
which had increased atretic follicle numbers without changes in the number of primordial, primary and second follicles (Leveille et al. 2014), although fertility was unaltered (Leveille et al. 2014). Depletion of the ovarian follicle reserve, also reported in other models of maternal obesity, has been attributed to mitochondrial dysfunction and increased lipid peroxidation (Aiken et al. 2016).

Despite numerous studies describing the impacts of maternal obesity on female reproductive function, currently little is known about molecular mechanisms. Changes in circulating levels of sex steroids in obese mothers (Maliqueo et al. 2017) has generated some novel hypotheses. Maternal obesity is associated with higher circulating androgen levels (Kallak et al. 2017), an effect that may be modulated by the sex of the fetus (Maliqueo et al. 2017). Since prenatal exposure to testosterone is associated with higher risk of polycystic ovarian syndrome in the offspring of many species (Abbott et al. 2005, Sun et al. 2012, Moore et al. 2013, Padmanabhan & Veiga-Lopez 2013), and potentially in humans (Sir-Petermann et al. 2002, Filippou & Homburg 2017), exposure to high levels of androgens at vulnerable developmental windows of ovarian development is a potential driver of offspring ovarian compromise. Changes in offspring sex steroid concentrations may also influence ovarian follicle development. Offspring from HFD-fed rats demonstrated elevated estradiol levels between day 1 and 60 of postnatal life, in association with a loss of ovarian antral follicles, increased follicular cysts as well as changes in hepatic metabolism of estradiol (Ambrosetti et al. 2016). Finally, recent work suggests that improving maternal insulin sensitivity during an obese pregnancy has positive impacts on offspring ovarian function (Álvarez et al. 2018).

Treating obese pregnant rats with metformin did not prevent advanced puberty in offspring, but returned the number of antral follicles, follicular cysts and multi-oocyte follicles to control values in the female offspring (Álvarez et al. 2018) suggesting that maternal obesity impacts on fetal ovarian development may be a combination of both the mother’s own metabolic dysfunction and changes in the her oocyte quality.

**Impacts in male offspring**

**FGR and maternal nutritional restriction**

**Epidemiological/clinical data on growth restriction and reproduction in human males** Poor maternal periconceptional nutrition impairs fetal growth, resulting in IUGR and LBW infants and, accordingly, birth weight has been used as a proxy measure for intrauterine nutritional adversity. One of the first studies published in 1953, establishing an association between fetal growth and male reproductive function was a case study of a male child born LBW that presented with, among many other complications, elevated urinary gonadotropins (Silver et al. 1953). Subsequent studies show that FGR is associated with alterations in hypothalamic–pituitary–gonadal (HPG) activity prior to pubertal onset. Male infants born SGA have a twofold elevation in serum FSH concentrations without changes in serum LH, inhibin B and free androgen index (Ibanez et al. 2002). Adolescent males born SGA have reduced testicular size, decrease testosterone levels and increased LH levels in adolescence, suggesting that changes in HPG activity observed in infants likely persist into post-pubertal life (Cicognani et al. 2002). Reports also exist however showing no differences in testosterone and inhibin B levels, LH/testosterone ratio, overnight secretory
patterns of gonadotropins or testicular size (Jensen et al. 2007, Ramlau-Hansen et al. 2007) or semen quality (Olsen et al. 2000, Ozturk et al. 2001, Ramlau-Hansen et al. 2007) in SGA males compared to control counterparts. Despite these apparent contradictory reports, IUGR has been reported to be associated with unexplained infertility in males in adulthood. Early studies show that 53% of men with abnormal sperm parameters are classified as having unexplained male infertility and compared to males with a normal semen and males with unexplained subfertility had a median birth weight SD score of −0.5 suggesting that LBW maybe associated with unexplained male infertility (Francois et al. 1997). In a cross-sectional, population-based sibling pair study, LBW was associated with decreased serum testosterone in adult men, (Vanbillemont et al. 2010) and low testosterone is often associated with poor reproductive outcomes (de Kretser 1979). Indeed, more recently, significant associations between birth weight, adult sperm DNA integrity and total spermat count have been reported (Faure et al. 2015).

In a relatively large cohort of men seeking infertility treatment, when compared with fertile men, infertile men showed a lower mean birth weight and a higher proportion of LBW. These infertile LBW men had reduced sperm motility and abnormal sperm morphology, lower total testosterone levels but higher FSH values, compared with average or high birth weight men. Interestingly, these LBW male patients reported a higher rate of significant morbidities and reduced left testicular volume and also presented with higher BMI (Boeri et al. 2016). It may be that part of association between reproductive deficits and LBW could be accounted for by an increased prevalence of obesity, which occurs concomitantly with metabolic dysfunction, since LBW increases obesity risk, and obesity in men is known to impair reproductive capacity (Palmer et al. 2012). Finally, maternal smoking during pregnancy (proposed to reduce fetal growth through placental hypoxia) significantly advances Tanner staging pubertal onset in boys (and girls) (Ernst et al. 2018).

Experimental animal models of nutrient restriction and growth restriction and reproduction in male offspring Maternal nutrient restriction-induced FGR has been shown to program reproductive development in male offspring, where in lambs the adolescent testosterone surge, a marker of pubertal onset, occurred 5 weeks later in growth-restricted male lambs (Da Silva et al. 2001), and although it is unclear whether prenatal undernutrition impairs testis development, it has been consistently reported that growth restriction is associated with reduced sperm quality in male offspring. In a model of placental insufficiency, prenatally growth-restricted male lambs had reduced testicular volume (Da Silva et al. 2001). Similarly, male bovine offspring born after prenatal nutrient restriction had an increased proportion of seminiferous tubules and reduced testicular blood vessel area (Copping et al. 2018). Maternal undernutrition was associated by reduced mean Sertoli cell numbers and reduced seminiferous tubule diameter (Kotsampasi et al. 2009). In a bovine model, male offspring born to heifers fed a low-protein diet during gestation presented reduced sperm quality, characterized by increased proportion of sperm with non-progressive motility and abnormal morphology (Copping et al. 2018). Similarly, the number of Sertoli cells were reduced by 20% in male offspring born to pregnant ewes fed a low-caloric diet (Bielli et al. 2001). These data have been replicated in other large animals models; in horses, maternal undernutrition impaired prepubertal testicular maturation (Robles et al. 2017). Despite these many reports, contrasting studies exist reporting that fetal testis weight and mean scrotal circumference were unaltered in adult lamb offspring undernourished in utero (Rae et al. 2002a,b, Kotsampasi et al. 2009). In rodents, in contrast, ano-genital distance, a developmental marker of intrauterine testosterone exposure, was increased by maternal protein restriction and postnally, offspring testicular descent was delayed, testes weight was reduced as well as LH and testosterone concentrations and fertility was reduced (Zambrano et al. 2005). In other work, prenatal undernutrition delayed sexual maturation, but did not suppress sexual behavior, in mature male rats (Matsuzaki et al. 2018). Intrauterine growth retardation (IUGR) induced by uterine artery ligation, delayed pubertal onset in rats (Engelbrecht et al. 2000).

Maternal obesity and nutritional excess

Epidemiological/clinical data on maternal obesity/nutritional excess reproduction in human males Very few studies have investigated the association between maternal obesity and reproductive development or function in male offspring. A small pilot study investigating the association between maternal BMI and offspring semen parameters found a weak association between semen quality and reproductive hormone levels in offspring and maternal obesity, but this study was small and underpowered (Ramlau-Hansen et al. 2007). In a much larger study, sons of obese mothers started on average to shave regularly 8.3 months earlier than
sons of normal weight mothers (Hounsgaard et al. 2014) suggesting that pubertal onset is earlier in sons of obese mothers. Maternal diabetes, in contrast, did not show a strong association between maternal GDM or type 2 diabetes with pubertal milestones (Lauridsen et al. 2018), although in a sub-analysis, after adjusting for potential confounders, daughters and sons of mothers with BMI >25 kg/m² and GDM entered puberty earlier than daughters and sons of mothers with BMI <25 kg/m² without GDM, suggesting that both GDM and BMI may together play a role in modulating pubertal onset in boys.

**Experimental animal models of maternal obesity and nutrient excess and reproduction in male offspring** Despite the fact that few human studies exist investigating the linking prenatal exposure to an obesogenic environment and reproductive compromise in males, many animal models suggest that maternal obesity affects reproductive function in male offspring. Adult male offspring born to pregnant rats consuming a high-calorie cafeteria diet throughout pregnancy and lactation had reduced plasma levels of LH, FSH and testosterone, as well as altered sexual behavior compared to control male offspring (Jacobs et al. 2014). Male rat offspring were deficient in testosterone, and this was accompanied by an overall reduced sperm count, with reductions in round and elongated spermatids, which may be mediated by testosterone deficiency, fueling the impaired formation and/or survival of spermatids and contributed to reduced sperm count (Navya & Yajurvedi 2017). The early neonatal window may also be a vulnerable time for sperm programming. Daily sperm production was reduced in adult male rats born to mothers exposed to HFD during pregnancy and lactation (Reame et al. 2014) but only when mothers were exposed to HFD during both pregnancy and lactation and not pregnancy alone (Reame et al. 2014, Rodriguez-Gonzalez et al. 2015). In addition to reducing sperm counts, sperm quality is also affected by prenatal adversity. In offspring born to obese mothers, sperm viability and motility was reduced and the number of sperm presenting with impaired oxidative stress management was increased; malondialdehyde (MDA) levels were increased, superoxide dismutase (SOD) was decreased and glutathione peroxidase (GPx) activity was increased in sperm and testes of males offspring, resulting in decreased fertility rates (Rodriguez-Gonzalez et al. 2015, Bautista et al. 2017). Collectively, suggesting that maternal obesity during pregnancy impairs the antioxidant defense system in testes, contributing to reduced reproductive capacity in adulthood.

**Paternal nutritional impacts on offspring reproduction**

Much of the work on programming of offspring reproductive function has focused on the mother and the maternal environment, while paternal impacts have been largely overlooked and understudied. To our knowledge, there have been no epidemiological studies that have investigated the impacts of paternal nutrition on their children’s reproductive outcomes. However, despite the lack of epidemiological evidence, experimental studies have recently begun exploring the contribution of paternal nutrition, specifically obesity, on reproductive outcomes in female and male offspring using rodent models of paternal DIO.

**Impacts in female offspring**

**Impacts of obesity, overweight and nutritional excess**

**Experimental animal models of obesity and nutritional excess programming reproduction in females** Rodent models of paternal DIO report detrimental effects on reproductive health in female offspring and grand-offspring. F1 females fathered by HFD-fed males presented with impaired oocyte quality, evident as reduced meiotic progression and altered mitochondrial function (Fullston et al. 2012). In addition to altered mitochondrial function, oocytes from F2 females had increased oxidative stress (Fullston et al. 2012). Whether impairments in these oocytes affected embryo quality is unknown. Females fathered by HF-fed males have delayed embryo development, reduced compaction rates and reduced proportion of embryos achieving blastocyst stage (Binder et al. 2012). Similarly, female offspring sired by HF-fed males generated embryos with delayed development and altered proportions of trophectoderm and inner cell mass cells in developing blastocyst (Fullston et al. 2015b). Collectively, the findings from these studies suggest that paternal HFD reduces oocyte quality in female offspring. In contrast, one report shows that paternal obesity did not significantly alter gonadotropin levels in the female offspring fed HFD, although these females displayed reduced LH responses to kisspeptin-10 (Fernandois et al. 2017). Given that changes in oocyte and embryo quality are observed in female offspring and grand-offspring in rodent models of paternal obesity, investigations using data from human cohorts are warranted.
Impacts in male offspring

Impacts of obesity, overweight and nutritional excess

Experimental animal models of obesity and nutritional excess programming reproduction in males Evidence from rodent models indicates that paternal obesity is also detrimental to the reproductive health of male offspring and grand-offspring. HF-fed males generated male offspring whose sperm had reduced progressive motility, increased ROS and DNA damage, as well as reduced binding and fertilization rates. Indeed, grand-offspring also presented a similar phenotype, where their sperm also had decreased progressive motility and increased ROS (Fullston et al. 2012). Male offspring born to F1 females, who displayed decreased oocyte quality, had decreased testis weights, decreased serum testosterone, as well reduced progressive motility and increased ROS in sperm (Fullston et al. 2012). These findings are supported by a follow-up study showing that paternal obesity results in offspring with reduced sperm motility, elevated intracellular ROS and decreased sperm–oocyte binding (Fullston et al. 2015a). Paternal obesity caused a decrease in LH levels and exacerbated the drop in circulating testosterone and gene expression of its key biosynthetic enzymes caused by HFD in the male offspring (Fernandois et al. 2017). Furthermore, central regulation of the HPG axis also appeared compromised where LH responses to central kisspeptin-10 administration were suppressed in HFD males from obese fathers (Fernandois et al. 2017). Efforts to mitigate these outcomes with dietary intervention in founders improved offspring sperm motility and mitochondrial markers of sperm health (decreased ROS and mitochondrial membrane potential) and improving sperm binding. Sperm binding and capacitation was also improved in F1 males born to a combined diet and exercise intervention in founders (McPherson et al. 2014).

Epigenetic mechanisms underlying reproductive programming

As reviewed earlier, it is well established that exposure to adverse parental nutritional environments programs impairments in reproductive health of offspring. There is now overwhelming evidence showing that the germ cells that will eventually give rise to grand-offspring are vulnerable to nutritional adversity, and while the exact cellular signaling pathways remain to be fully investigated, many have turned their research focus to processes that mediate gene expression very early life. Parental lifestyle factors, notably maternal and paternal diet/nutrition (as reviewed in Jiménez-Chillarón et al. 2012), have been shown to alter the epigenome and changes to epigenetic processes and signaling pathways, are associated with increased disease risk in offspring later in life and many studies now suggest that epigenetic mechanisms underpin programming of disease. The epigenome is a collection of heritable chemical modifications and proteins that regulate gene expression through transcription without changes to the DNA sequence (Skinner et al. 2010), modifications that may include DNA methylation, histone modifications, regulatory RNAs and transcription factors. Particularly relevant to the investigation of reproductive development and function is the fact that the epigenome is reprogrammed several times throughout the lifespan and thus likely plays a key role in modulating reproductive outcomes in circumstances of early life adversity. As there are many reports that review epigenetic reprogramming (Messerschmidt et al. 2014, Stuppia et al. 2015, Xu et al. 2015, Fraser & Lin 2016), this topic will not be extensively reviewed here and we will simply identify some of the processes that could underlie the link between nutritional adversity and offspring reproductive compromise.

The epigenome is first reprogrammed during gametogenesis. As PGCs proliferate and migrate to the genital ridges, methylation patterns are erased by active global demethylation. This loss of DNA methylation patterns enables establishment of sex-specific imprints in female and male germ cell precursor cells. Following sex determination, DNA remethylation occurs in germ cell precursors in female and male embryos. Specifically, in male embryos, de novo methylation initiates in mitotically arrested prospermatogonia and is completed prior to birth. In female embryos, however, DNA methylation initiates after birth when ovarian follicles are growing. Other forms of epigenetic reprogramming occur in PGCs concomitantly, such as histone deacetylation and histone variant H2A.Z replacement (Wu et al. 2015a). In males, epigenetic reprogramming continues into postnatal life. After birth, spermatogonia undergo rapid expansion and remain dormant until puberty. Spermatogenesis initiates after pubertal onset following activation of the HPG axis. Final DNA methylation patterns are established in spermatogonia during spermatocytogenesis. Subsequently, during the initial stages of spermiogenesis, histone–protamine exchange occurs and results in extensive chromatin remodeling (Wu et al. 2015a). At this point in development, both the paternal and maternal
genomes of the oocyte and sperm are hypermethylated and transcriptionally silent. Shortly after fertilization the paternal and maternal genomes are actively and passively demethylated, respectively, and generate a transcriptionally active totipotent zygote. However, methylation patterns are maintained at imprinted genes during this phase of reprogramming. Following implantation, a new methylation pattern is established in the embryo by de novo methylation (Wu et al. 2015a).

Given the above described epigenetic landscape, it is not surprising that adversity early in life is hypothesized to operate through epigenetic mechanisms that can have long-term impacts on offspring. Environments during pregnancy have been shown to perturb epigenetic remodeling in PGCs and embryos and result in lasting impacts on male and female offspring. In a similar manner, exposure to adverse paternal nutrition post-puberty, when epigenetic reprogramming is occurring in maturing sperm, can alter the sperm epigenome and program impairments in reproductive function in offspring (Lambrot et al. 2013, Desai et al. 2015, Gu et al. 2015, Ly et al. 2015, Siklenka et al. 2015, Whidden et al. 2016, Ly et al. 2017, Morgan et al. 2019). Although there is much work to be done in understanding the drivers of epigenetic modifications due to early life nutritional adversity, it seems that this will likely be a fruitful path of investigation linking early life adversity to reproductive impairments in offspring.

Conclusions and future directions

There is now overwhelming evidence that health and disease has its origins early in life and perturbations at vulnerable windows of development, particularly in germ cells, have impacts that span multiple generations (Gluckman & Beedle 2007, Aiken & Ozanne 2014, Pembrey et al. 2014). Evidence suggests that since germ cells that eventually give rise to grand-offspring form during development, and are vulnerable to nutritional adversity through epigenetics mechanisms, the link between early life adversity and postnatal disease likely lies within the developing gonads and their function. These PGCs give rise to alterations in numerous organ systems, and in the control of reproductive development and function – ultimately resulting in not only impairments in the first generations, but many generations thereafter (Fig. 4).

New approaches to understanding paternal and offspring health and disease have recently highlighted mechanisms other than epigenetics that need to be considered, including immune factors (Schjenken & Robertson 2015, Evans et al. 2019). While the mechanisms still remain to be fully investigated, it is clear that a lifecourse approach to understanding reproductive development and lifetime reproductive function is necessary. Furthermore, investigations of the impacts of early life adversity must be extended to include the paternal environment, especially in epidemiological studies and clinical studies of offspring reproductive function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
D M S is supported by the Canada Research Chairs Program and P A J is supported by the Natural Sciences and Engineering Research Council of Canada.

Author contribution statement
P A J wrote the manuscript, D M S edited and wrote the manuscript and compiled the figures.

Acknowledgements
The authors would like to thank Katharine Kennedy for her assistance in generating Figure 4.

References
Chan KA, Bernal AB, Vickers MH, Gohir W, Petrok J & Sloboda DM 2015a Early life exposure to undernutrition induces ER stress, apoptosis, and...
induced obese male mice display impaired blastocyst development with molecular alterations to their ovaries, oocytes and cumulus cells. *Journal of Assisted Reproduction and Genetics* **32** 725–735. (https://doi.org/10.1007/s10815-015-0470-x)


Gilbert SF 2000

Hanson MA & Gluckman PD 2014 Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiological Reviews* **94** 1027–1076. (https://doi.org/10.1152/physrev.00029.2013)


in adult female rat offspring. *Fertility and Sterility* **103** e291.e2–298.e2. (https://doi.org/10.1016/j.fertnstert.2014.09.026)


McPherson NO, Fullston T, Bakos HW, Setchell BP & Lane M 2014 Obese father’s metabolic state, adiposity, and reproductive capacity indicate son’s reproductive health. *Fertility and Sterility* **101** 865. e861–e861.


Received in final form 15 March 2019
Accepted 25 March 2019