Early-life nutritional effects on the female reproductive system

K A Chan1,*, M W Tsoulis1,* and D M Sloboda1,2,3

Departments of 1Biochemistry and Biomedical Sciences, 2Pediatrics and 3Obstetrics and Gynecology, McMaster University, 1280 Main Street West HSC 4H30A, Hamilton, Ontario, Canada L8S 4K1
*(K A Chan and M W Tsoulis contributed equally to this work (co-first authors))

Abstract

There is now considerable epidemiological and experimental evidence indicating that early-life environmental conditions, including nutrition, affect subsequent development in later life. These conditions induce highly integrated responses in endocrine-related homeostasis, resulting in persistent changes in the developmental trajectory producing an altered adult phenotype. Early-life events trigger processes that prepare the individual for particular circumstances that are anticipated in the postnatal environment. However, where the intrauterine and postnatal environments differ markedly, such modifications to the developmental trajectory may prove maladaptive in later life. Reproductive maturation and function are similarly influenced by early-life events. This should not be surprising, because the primordial follicle pool is established early in life and is thus vulnerable to early-life events. Results of clinical and experimental studies have indicated that early-life adversity is associated with a decline in ovarian follicular reserve, changes in ovulation rates, and altered age at onset of puberty. However, the underlying mechanisms regulating the relationship between the early-life developmental environment and postnatal reproductive development and function are unclear. This review examines the evidence linking early-life nutrition and effects on the female reproductive system, bringing together clinical observations in humans and experimental data from targeted animal models.

Key Words

- developmental programming
- reproduction
- puberty
- ovary
- maternal nutrition
- IUGR

Introduction

Disease risk is established well before birth. Life-style associated diseases, including obesity and type 2 diabetes, are known to be influenced by fetal adaptations to in utero conditions and critically, these disease effects span multiple generations (Gluckman et al. 2007, Aiken & Ozanne 2014). Although science has made significant advances in understanding how this occurs, the exact signaling pathways still remain unclear. As germ cells (oocytes) in the growing fetal ovary are vulnerable to prenatal events, it is likely that modifications in fetal gonadal development contribute to transgenerational disease risk.

The developing organism is capable of adapting to various environments. This phenomenon has led to the hypothesis that disease risk is the result of complex gene–environment interactions (Bouchard 2008, Andreasen & Andersen 2009). This ‘environment’ includes the period encompassing the developmental milieu within which gametes, germ/stem, and somatic cells will not only differentiate into established organ systems but also give rise to the next generation. It is this inherent developmental plasticity of an organism that allows it to respond to cues that will ultimately determine the adult
phenotype. However, under some circumstances, developmental adaptations to early-life insults may lead to negative effects on long-term health (Gluckman & Hanson 2007). In this regard, epidemiological and experimental data have indicated that there is a relationship between the in utero environment and the risk of developing chronic disease later in life (Gluckman & Hanson 2004, 2007). A number of differential insults that induce developmental adaptations have been shown to modify disease risk (Champagne 2011, Rosenfeld 2012, Desai et al. 2013, Reynolds 2013, Reynolds et al. 2013, Sinclair & Watkins 2013, Tarantal & Berglund 2014) and to modulate reproductive function (Savabieasfahani et al. 2006, Sloboda et al. 2009, Connor et al. 2012, Schöpper et al. 2012, Lie et al. 2013, Barra et al. 2014, Zhou et al. 2014, Zhuo et al. 2014). As the gametes that will eventually give rise to grand-offspring form during fetal life, it is possible that the link between early-life adversity and postnatal disease lies in the developing ovary – involving the developing germ cells and their function. In this review, we consider the female reproductive system, how perinatal adversity modifies fetal reproductive development and the long-term effects of early-life adversity on female reproductive function. As the effects of environmental toxins and chemicals (Walker & Gore 2011, Gopinath 2013, Marques-Pinto & Carvalho 2013) on the male reproductive system have been reviewed elsewhere (Mori 2001, Hampi et al. 2013), this review focuses on the effects of early-life nutritional insults on the female reproductive system.

Establishment of female reproductive function: a brief overview

Central to female fertility is the ovary, which consists of oocytes surrounded by somatic cells (follicles). Two pools of follicles exist within the ovary: the resting follicle pool and the growing follicle pool. The resting follicle pool is made up of primordial follicles, which are oocytes surrounded by a single layer of flattened (squamous) granulosa cells (Hirshfield 1991). The majority of primordial follicles remain quiescent; however, a small subset is recruited to supply the growing follicle pool throughout reproductive life (McGee & Hsueh 2000). Oocytes within primordial follicles originate from primordial germ cells (PGCs) that have migrated from the hindgut to the gonadal ridge during embryonic life (Mamsen et al. 2012, Sánchez & Smitz 2012). Upon arrival at the gonadal anlage, PGCs proliferate by mitosis to form oogonia; however, incomplete cytokinesis results in the formation of multi-nucleated syncitia, consisting of multiple oogonia connected by intercellular cytoplasmic bridges surrounded by somatic cells, also known as germ cell cysts (Pepling & Spradling 1998, Tingen et al. 2009, Haglund et al. 2011, Pepling 2012, Sánchez & Smitz 2012). Subsequently, oogonia enter meiosis and become arrested in the diplotene stage of meiosis I, at which point they are referred to as primary oocytes (Hunt & Hassold 2008, Pepling 2012). Concomitant with the oogonia-to-oocyte transition is breakdown of cysts and the freeing of individual oocytes to form primordial follicles (Pan et al. 2012). Breakdown of cysts and the establishment of the primordial follicle pool occur from postnatal (P) day 1 to P4 in rodents (Rajah et al. 1992, Pepling & Spradling 2001). During this time, there is a massive wave of germ cell death (atresia) via apoptosis and only 33% of oocytes survive to form primordial follicles (Pepling & Spradling 2001, Kezele et al. 2002). The primordial follicles begin to form near the ovarian core (medulla), and their assembly gradually shifts toward the surface (Rajah et al. 1992). Similarly, human primordial follicle assembly begins in medullary regions and radiates outwards into cortical regions (Sforza et al. 2003) with a wave of follicle atresia (Geber et al. 2012); however, this process begins well before birth at ~13 weeks postconception and continues until birth (Forabosco & Sforza 2007). Regardless of timing differences between species, the accumulated number of primordial follicles established early in life largely dictates the reproductive potential and lifespan of mammals, because once this pool is depleted, reproductive life ceases. Recent data have led to a challenge to this concept of a finite primordial follicle pool and on the basis of evidence which indicates that the mammalian ovary may have proliferative germ cells that could replenish the reserve (Johnson et al. 2004, 2005, Woods et al. 2012); however, this idea is still heavily debated (Tingen et al. 2009, Kerr et al. 2012, Zhang et al. 2012, 2013).

Follicle growth is regulated by a highly orchestrated neuroendocrine negative-feedback system characterized by hypothalamic release of gonadotropin-releasing hormone (GNRH), anterior pituitary release of gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)), and ovarian sex steroids (estradiol (E2) and progesterone) (Walker & Gore 2011). Hypothalamic GNRH stimulates the release of LH or FSH (depending on GNRH pulsatility) from the anterior pituitary (Popat et al. 2008, Tsutsumi & Webster 2009). These gonadotropins bind to their cognate receptors in the ovary, stimulating the production of sex steroids that aid in follicle growth and, importantly, feedback onto the hypothalamus and
anterior pituitary to regulate the production of GNRH and LH/FSH (Popat et al. 2008). It is not until puberty that GNRH pulsatility is sufficient to induce ovulation of late-antral or fully-grown follicles (Kawagoe & Hiroi 1983, Grumbach 2002, Russell & Robker 2007). It is currently not known what exact mechanism initiates GNRH pulsatility and, consequently, the onset of puberty. However, there is evidence that metabolic status conveyed to the hypothalamic kisspeptin system by leptin (Elias & Purohit 2013), ghrelin (Tena-Sempere 2013), and adiponectin (Martos-Moreno et al. 2010), amongst others, is critical. Nonetheless, once puberty is reached, regular menstrual (human) and estrous (rodent) cycles, governed by GNRH, LH/FSH, and sex steroids, result in follicle recruitment, development, and ovulation of an oocyte capable of being fertilized (Popat et al. 2008). This occurs at every cycle until the primordial follicle pool is depleted (Skinner 2005). Thus, one can imagine that events or insults occurring during critical hypothalamic–pituitary–gonad developmental windows may disrupt reproductive function and even impair fertility.

Notably, during fetal and early neonatal folliculogenesis, massive epigenetic remodeling occurs, including remethylation of the entire genome (Walker & Ho 2012). Thus in addition to the development and differentiation of germ and somatic cells being vulnerable to early-life insults, adversity may equally (or simultaneously) result in stable changes to the epigenotype of germ cells (Pan et al. 2012), having long-term implications for future generations (Fig. 1). Epigenetics refers to the study of changes in gene function, without alterations in the DNA sequence that are mitotically and/or meiotically heritable (Berger et al. 2009, Dupont et al. 2009). Factors contributing to altered epigenetic states include histone post-translational modifications, noncoding RNAs, transcription factors, and DNA methylation (Sarkies & Sale 2012). The latter largely occurs on cytosines at palindromic CpG dinucleotides (Bird & Wolffe 1999), and promoter CpG methylation is generally associated with a transcriptionally silent gene (Sarkies & Sale 2012). Interestingly, the oogonium/oocyte is subjected to massive fluctuations due to CpG methylation over the course of development. Specifically, PGCs during embryonic life undergo almost complete genomic demethylation upon arrival to the gonadal ridge (Lee et al. 2014). Postnatally, throughout folliculogenesis, the oocyte genome is remethylated as follicle growth progresses (Reik et al. 2001). Post-fertilization, another wave of demethylation occurs in both paternal (Oswald et al. 2000) and maternal (Wang et al. 2014) genomes. Despite massive erasure of the methylome in PGCs, some genomic sequences become resistant (Guibert et al. 2012), and oocyte methylation status is known to be a strong factor in the determination of pre-implantation embryo methylation status (Smallwood et al. 2011).

These two lines of evidence indicate that it is possible for DNA methylation status to get transmitted across
generations. Thus exposure of a pregnant female (F0 generation) to an insult will directly expose not only her offspring (F1 generation), but also the F1 offspring’s germ cells, which will later form the F2 generation (Fig. 2). Therefore, the first generation not directly exposed to an environmental insult in this scenario would be F3 generation offspring. If effects are observed in F3 generation offspring as a result of F0 exposure, it would indicate a germline-dependent transmission across generations, which has been termed transgenerational (Skinner 2008). Observation of effects only in F1 and/or F2 generation offspring would indicate germline-independent transmission across generations, which has been termed multigenerational (Skinner 2008). Moreover, epigenetic marks that are thought to reflect early-life nutritional status have been put forward as possible biomarkers of long-term disease risk (Heijmans et al. 2008, Godfrey et al. 2011, Dominguez-Salas et al. 2012, 2014, Khulan et al. 2012). Although the usefulness of these marks has not been thoroughly investigated, it is likely that technological advancement permitting the identification of specific epigenetic changes across the entire genome and linking of these marks with functional outcomes will improve our ability to understand how epigenetics modulates long-term health and disease risk.

**Human studies of reproductive programming: modulatory effects of early growth**

Early studies investigating the developmental origins of disease risk historically used birth weight (BW) as a proxy measure for intrauterine adversity (Barker et al. 1989, Barker 2006). Consequently, epidemiological investigations of populations born with low BW (LBW), due to intrauterine growth restriction (IUGR) and/or small-for-gestational age (SGA), showed significant associations between intrauterine growth, BW, and postnatal reproductive function (Cooper et al. 1996, van Weissenbruch & Delemarre-van de Waal 2006, Sloboda et al. 2007, van Weissenbruch 2007, Ibanez et al. 2011).

Adolescent girls and young adult women born SGA show reduced ovarian size and increased circulating gonadotropin levels, which are already detectable in infancy (Ibanez et al. 2000, 2002a). Whether disruption of ovarian development occurs as early as fetal life in humans is unclear. IUGR female fetuses show altered ovarian development characterized by reduced ovarian size and reduced proportions of primordial follicles (de Bruin et al. 1998), although in subsequent studies, IUGR fetuses did not show significant changes in volume or follicle number (de Bruin et al. 2001). A lack of change in fetal ovarian follicle numbers would indicate that the effects of IUGR on ovarian folliculogenesis may not be apparent until the post-pubertal time-point, although it is important to recall that the size of the ovary may not reflect follicle numbers and/or function.

SGA girls display exaggerated adrenarche, advanced menarche, low ovulation rates, and early-onset menopause (Cooper et al. 1996, Veening et al. 2004, Ibanez & de Zegher 2006). Furthermore, upon onset of puberty, SGA girls also display exaggerated adrenal androgen secretion, factors responsible for secondary sexual characteristics (pubic hair, deepening of voice, etc.), as well as hyperinsulinemia, which has been shown to be associated with hyperandrogenemia (Ibanez et al. 1999, 2004). These findings are indicative of an accelerated advancement in reproductive maturity and may be indicative of a reduced reproductive lifespan in SGA girls, compared with appropriate-for-gestational age (AGA) girls. Whether this advancement is associated with accelerated loss of ovarian follicles is unknown (although results from animal studies are indicative that this is the case, see below). Acknowledging this, it may be no surprise, therefore, that SGA girls are thought to be at an increased risk of experiencing premature infertility (Vikstrom et al. 2014), although these results are still contentious (Meas et al. 2010, Sadrzaadeh-Broer et al. 2011). Despite the fact that results from many studies have been indicative of an association between growth restriction in utero and reproductive abnormalities, SGA is not always associated with early menarche (Shim et al. 2013) or menopause (Treloar et al.
and in some circumstances, associations are mild (Hernandez et al. 2006, de Ferran et al. 2011). Recent results for a non-Western cohort of children have indicated that neither BW for gestational age or SGA status was associated with age at onset of puberty and instead, that SGA children were shorter at the onset of puberty, consistent with either a trade-off between linear growth and maturation or simply with less growth potential (Hui et al. 2012).

Other forms of reproductive dysfunction have also been associated with early-life adversity. Polycystic ovarian syndrome (PCOS) is one of the most common female endocrine disorders, affecting between 5 and 10% of adult reproductive age women (Aziz 2004, Hart et al. 2004). The symptoms include anovulation, excess androgen secretion, insulin resistance, obesity, and dyslipidemia (Hart et al. 2004). An association exists between abdominal fat deposition in both adolescent girls and adult women with the syndrome (Kirchengast & Huber 2001, Puder et al. 2005, Carmina et al. 2007, Hickey et al. 2009). It has been suggested that PCOS may arise through a gene–environment interaction (Franks 2008), and although PCOS is associated with a number of polymorphisms associated with androgen synthesis (Ferk et al. 2008, Shah et al. 2008), no clear genetic association has been established (Simoni et al. 2008).

Although both experimental and clinical data exist indicating that events in are associated with a later life PCOS phenotype, specific predisposing factors have not been clearly defined. IUGR followed by catch up growth during childhood increases the risk of precocious pubarche (Ibanez et al. 1998a), anovulation PCOS (Ibanez et al. 2002b, 2007), and characteristics of the metabolic syndrome in adolescence (Ibanez et al. 2006a). Insulin resistance has been suggested to be a central driver in these associations and treatment with insulin sensitizers in girls with precocious pubarche has been shown to significantly delay the onset of early menarche in this population (Ibanez et al. 2006b, 2008a).

Although it is clear that intrauterine factors play a significant role in the development of the female reproductive system and the risk of dysfunction later in life (de Zegher & Ibanez 2006, Ibanez et al. 2007, Melo et al. 2010, Franks & Berga 2011), the causal factors are not still clearly defined (Cresswell et al. 1997). In a small population of girls, SGA was associated with an increased risk of developing PCOS (Ibanez et al. 1998a,b, 2001, 2008b). However, this relationship may be different in babies that had grown normally babies. In a prospective study of normal adolescents, we have recently shown that BW was not associated with PCOS characteristics. These findings, however, need to be confirmed in other populations of unremarkable adolescents in equally large prospective studies. Recently, PCOS has also been associated with being large for gestational age (Mumm et al. 2013) as well as having no association with BW (Sadrzadeh et al. 2003). Interestingly, it has been proposed that two distinct birth pathways exist to development of PCOS, where high BW was associated with hyperandrogenism (as a single symptom), while low ponderal index was associated with the presence of all three key PCOS symptoms (menstrual dysfunction, hyperandrogenism, and polycystic ovaries) (Davies et al. 2012).

Animal studies of reproductive programming: modulatory effects of early growth

Few animal studies have investigated specifically the modulatory effects of growth on reproductive outcome in offspring. In these studies, IUGR is induced by uterine artery ligation, thus mimicking placental insufficiency (Wigglesworth 1974). Results of several studies have indicated that IUGR female offspring display delayed pubertal onset, while others mirror the results of human studies indicating advanced pubertal onset in female offspring born with a LBW (Engelbregt et al. 2000, 2002). Many animal models of IUGR use nutrient restriction and thus are discussed in detail below.

Maternal nutritional effects on reproductive function of offspring

Human data

Nutritional effects on reproduction are well established (Wade et al. 1996, Schneider 2004, Dupont et al. 2014). This reciprocal relationship between nutritional cues, energy intake, and metabolic indicators is not surprising as organisms must have adequate energy stores and resources for successful reproduction. There are a number of maternal conditions and/or pregnancy complications that restrict availability of nutrients to the fetus and decrease fetal growth (Sibley et al. 2005, Lager & Powell 2012), but a common theme is reduced nutrient supply. An adequate supply of nutrients is required to maintain a balance between the nutrient demands of the mother and those of the fetus (Bloomfield et al. 2013). The most common cause of growth restriction in term newborns is decreased fetal availability of nutrients and hormones (Godfrey 1998, Rosenberg 2008, Diderholm 2009). Numerous pregnancy complications can compromise availability of nutrients, including placental insufficiency.
and maternal nutrient restriction (Cetin & Alvino 2009, Diderholm 2009, Lausman et al. 2012) where impaired placental development and/or function is associated with inadequate fetal nutrient supply and LBW (Pardi et al. 2002, Cetin & Alvino 2009). Critically, it is now recognized that maternal obesity may also result in an environment of malnutrition as a proportion of obese mothers give birth to growth-restricted babies (Radulescu et al. 2013) and obesity is associated with compromised placental function (Pardi et al. 2002, Cetin & Alvino 2009, Diderholm 2009, Lausman et al. 2012, Hastie & Lappas 2014). In either case, maternal malnutrition alters maternal–fetal–placental nutrient exchange (Harding & Johnston 1995) and results in fetal adaptations that lead to increased disease risk.

How early-life nutritional cues affect PGC and reproductive development in offspring during critical prenatal windows however is unclear. Data collected from historical observations of humans have shed some lights on the effects that maternal nutrient challenges during pregnancy have on the reproductive performance and function of offspring. The Dutch Hunger Winter of 1944–1945 created a unique opportunity to study the relationship between prenatal famine exposure and adult health. Offspring born to mothers exposed to famine conditions during varying stages of pregnancy have been extensively followed up and numerous papers have been published outlining the relationship between maternal famine exposure and health outcomes of offspring (Painter et al. 2005, Heijmans et al. 2008, Roseboom et al. 2011) (and grand offspring) (Painter et al. 2008a, Veenendaal et al. 2013). Results of follow-up studies indicate that women born to mothers exposed to famine had more children earlier in life, compared with women born to control (not famine-exposed) mothers (Painter et al. 2008b), although the effect of famine on reproductive success was very small and has been disputed (Lumey & Stein 1997, 2009). Data collected on other historic cohorts indicate that women exposed to acute malnutrition during fetal life may experience negative effects on their reproductive systems, which could result in permanently impaired fecundity (Song 2013) as well as negative effects on metabolic function. There are results, however, indicating that not all offspring born under famine conditions develop postnatal metabolic complications as demonstrated in offspring of the Siege of Leningrad (Stanner et al. 1997, Stanner & Yudkin 2001), but effects on reproductive capacity and function in this cohort have not been thoroughly analyzed.

Data describing the relationship between in utero nutrient restriction and offspring age at menopause are limited. Famine during early childhood was reported to result in a decrease of 0.36 years in age at natural menopause (Elias et al. 2007). Consistent with this observation, malnourished women in developing countries have shorter reproductive lifespans, delayed or advanced onset of puberty, and early menopause (Osteria 1983, Riley 1994, Kirchengast & Winkler 1996, Lindstrom & Berhanu 1999). Steiner et al. (2010) reported a weak association between BW and age at menopause (HR 1.09; 95% CI 0.99, 1.20), but this relationship appears to be attenuated if one adjusts for gestational exposure to famine (Yarde et al. 2013), perhaps indicating that nutrient restriction plays a large role in the link between age at menopause and BW.

**Animal data**

Perinatal nutritional challenges have been shown to have long-term consequences for reproductive health (Guzman et al. 2006, Zambrano et al. 2006, Bernal et al. 2010, Sloboda et al. 2010, Aiken et al. 2013). Results from several animal models have indicated that pre- and/or postnatal nutrient restriction affects reproductive aging, as well as altering central regulation of reproductive hormones. The effects of maternal nutrient restriction on reproductive outcomes are sensitive to the timing of nutritional challenge during gestation as well as the type of nutrient challenge. Models involving total caloric restriction, protein restriction, or micronutrient manipulation all have shown that these challenges have reproductive effects on offspring, although the outcomes vary according to the model. This may reflect differential fetal and neonatal adaptive responses influencing reproductive developmental tempo depending on when the nutritional deficit occurred during gestation. Below we have summarized the literature on reproductive outcomes classified according to the major nutrient restriction models.

**Protein restriction**

Protein restriction during pregnancy and/or lactation in rats produces female offspring that display irregular cycles early in life, and decreased reproductive lifespan (Zambrano et al. 2005, Guzman et al. 2006, 2014). This is probably due to diminished ovarian reserves as adult offspring born to dams fed an isocaloric, protein-restricted diet throughout pregnancy had reduced numbers of primordial follicles, elevated levels of ovarian oxidative stress, and shortened ovarian telomere length compared with controls (Aiken & Ozanne 2014). The effects also vary according to developmental windows. Female offspring exposed to protein restriction during lactation alone have shown a decrease in circulating LH and an increase in systemic FSH levels at weaning, a
hormone profile that is distinct from those of offspring exposed to protein restriction during either pregnancy alone or pregnancy + lactation (Guzman et al. 2014). Furthermore, female offspring born to dams exposed to protein restriction during pregnancy or lactation alone exhibit a reduction in numbers of preantral and antral follicles at weaning, compared with offspring of mothers restricted during pregnancy and lactation (Guzman et al. 2014). In addition, exposure to protein restriction during pregnancy and lactation or lactation alone results in delayed onset of puberty (Guzman et al. 2006). After puberty, offspring born to lactationally protein restricted dams display increased numbers of preantral and small antral follicles but reduced numbers of primordial follicles, Graafian follicles, and corpora lutea (da Silva Faria et al. 2008) indicative of ovulatory dysfunction. These results indicate that a mismatch between in utero and postnatal nutrition produces an offspring reproductive phenotype that is distinct from those observed during the perinatal period and may negatively influence future reproductive success (da Silva Faria et al. 2008, 2010).

Total caloric restriction It is well established that total caloric restriction during pregnancy significantly affects ovarian development and function of offspring. In sheep, maternal nutrient restriction results in a negative effect on oocyte quality which results in lower oocyte cleavage after IVF and decreased morula and blastocyst formation (Grazul-Bilska et al. 2012). In rats, maternal caloric restriction throughout pregnancy results in LBW offspring that experience accelerated neonatal growth and early vaginal opening (VO), a marker of sexual maturation in rodents (Sloboda et al. 2009, Caron et al. 2012, Sanchez-Garrido et al. 2013). Early onset of puberty, however, is not always apparent in rodent models investigating caloric restriction (Chernoff et al. 2009), and in some cases puberty is delayed (Gerelsetseg et al. 2012). Nonetheless, in adulthood, offspring of dams fed calorically restricted diets have reduced numbers of primordial and antral follicles and elevated levels of ovarian oxidative stress, which has been associated with ovarian aging (Bernal et al. 2010). These offspring show a decrease in mRNA levels of the granulosa cell-specific estrogen receptor β in addition to a decrease in the oocyte-specific growth factor, GDF9 (Sloboda et al. 2009, Bernal et al. 2010), which is evidence of impaired folliculogenesis. Similarly, in cattle, maternal caloric restriction during the first trimester produces offspring with diminished ovarian reserve during adulthood, as demonstrated by reduced antral follicle count, decreased circulating anti-Müllerian hormone, and increased FSH in adulthood (Mossa et al. 2013). Furthermore, in sheep, offspring born to ewes fed calorie-restricted diets during the first two-thirds of pregnancy had reduced ovulation rates in adult life as determined by laparoscopic counts of corpora lutea (Rae et al. 2002). Together these observations are indicative of a shortened reproductive lifespan in offspring born to mothers on calorie-restricted diets. Premature ovarian aging is caused by early follicle loss or a reduction in the ovarian reserve; the finite pool of follicles that exist within the ovary. The follicular reserve is influenced by the rate of primordial follicle recruitment, follicle health, and reproductive cyclicity (Gleicher et al. 2011), all of which appear to be negatively affected in offspring born to calorie-restricted mothers. As outlined in the introduction, this reserve is vulnerable during the perinatal period when these nutritional insults occur, and it appears that changes in ovarian histogenesis induced by different levels of maternal nutrition contribute to the impaired reproductive phenotype of offspring (Rae et al. 2002, Mossa et al. 2013).

In sheep, maternal dietary restriction with or without micronutrient supplementation of selenium (Se) decreased cell proliferation in primordial, secondary and/or antral follicles, stromal cells, and blood vessels in fetal ovaries (Grazul-Bilska et al. 2009). Furthermore, maternal nutrient restriction at differing timepoints throughout pregnancy results in differential effects on ovarian development. Maternal nutrient restriction in sheep for the first 30 days of gestation reduced fetal germ-cell proliferation at day 65, but increased granulosa cell proliferation at day 110. In contrast, maternal underfeeding from 65 to 110 days or from 0 to 110 days altered the expression of genes that regulate apoptosis. Although the pathways may differ, both of these mechanisms probably contribute to the reduced number of ovarian primordial follicles that characterize this underfeeding model (Lea et al. 2006).

Maternal caloric restriction also modifies the development and the function of central reproductive control. In rats, maternal caloric restriction during the last week of pregnancy results in growth restriction and neonatal catch-up growth during lactation (Iwasa et al. 2010). The offspring were characterized by delayed VO (puberty) and decreased hypothalamic GNRH and Kiss1 mRNA expression prepubertally (Iwasa et al. 2010). Kiss1 encodes the peptide kisspeptin, which stimulates GNRH production (Matsui et al. 2004) and thought to be a key factor in the initiation of puberty (Seminara et al. 2003).

Although the female gonad develops during fetal life, with the establishment of PGCs, the breakdown of germ cell nests, and the establishment of primordial follicle
formation (being species-dependent), results from previous studies have indicated that nutritional challenge during the early-postnatal period influences reproductive function – even at the level of the follicles contained in the ovary. Delayed pubertal onset has been observed in rats (Castellano et al. 2011, Sanchez-Garrido et al. 2013) and mice (Caron et al. 2012) underfed during lactation using a model that modulates litter size and thus influences nutritional supply. In mice, this appears to be caused by a reduction in the number of axonal projections from the arcuate nucleus of the hypothalamus to the median preoptic nucleus (MPO) of the hypothalamus, which contains neurons that secrete GNRH (Caron et al. 2012). It was also observed that in these underfed mice that there was a reduction in the density of kisspeptin-immunoreactive fibers within the MPO, indicating that the kisspeptin input driving GNRH production is lacking (Caron et al. 2012). Importantly, this reduction in kisspeptin input to the MPO persisted into adulthood, potentially explaining the reduction in fertility observed in these offspring. Functionally, these alterations were associated with an inability of these offspring to produce an LH increase after ovariectomy at postnatal day 24, indicating impairment in central responsiveness to loss of negative feedback (Caron et al. 2012).

Micronutrient models Micronutrient deficiencies play a significant role in the modification of fetal metabolism, organ growth, and function (Allen 1994, 2005). Indeed, studies have identified significant improvement in BW outcome after maternal micronutrient supplementation, particularly in developing countries with inadequate nutrition or food insecurity (Haider & Bhutta 2006, Zagre et al. 2007, Haider et al. 2011). Most of the essential micronutrients needed for appropriate growth and development are absorbed through food consumption. Unfortunately, even in developed populations, pregnant women often do not maintain adequate nutritional intake during pregnancy. According to the Southampton Women’s Survey (SWS), 13% of pregnant women do not follow national nutritional guidelines for pregnancy, and only 2.9% of pregnant women take recommended supplements (Crozier et al. 2009, Inskip et al. 2009). Pregnant adolescents have been shown to be micronutrient deficient and at a high risk of giving birth to SGA babies (Baker et al. 2009). Carbon-1 metabolites such as vitamin B12 and folate play a critical role in DNA and histone methylation, influencing epigenetic regulation of gene expression and modifying signaling pathways that may underlie IUGR, and the modulation of developing organ systems including gonadal and brain development (Forges et al. 2007, Stover 2011, Dwarkanath et al. 2013, Gueant et al. 2013). Deficiencies in folate and/or vitamin B during pregnancy increase the risk of neural tube defects, cognitive or learning disabilities, and have long-term effects on hepatic regulation of metabolic function (Blaise et al. 2007, Stover 2011, Safi et al. 2012). The effects of prenatal intake of vitamin B12 on reproductive functions of female offspring are not known. Studies investigating on reproduction functions are localized to those that investigate the postnatal intake; in sheep, B12 and methionine restricted intake enhanced the number of estrogen-active antral follicles following FSH stimulation (Kanakkaparambil et al. 2009). In the future, studies investigating the role of micronutrient balance are likely to be combined with alterations in macronutrient balance as well, because human dietary patterns probably have simultaneous imbalances in both macro- and micronutrients.

Several studies have also focused on the effects of antioxidants during pregnancy on ovarian functions of offspring. A commonly studied antioxidant is melatonin, which is able to cross the placenta and has been found in the milk of lactating rodents (Klein 1972, Rowe & Kennaway 2002). Melatonin protects against follicular oxidative stress and slows the process of reproductive aging in the rat (Trentini et al. 1992, Tamura et al. 2012). Maternal administration of melatonin produces offspring that display delayed VO and lowered LH levels in a rodent model (Colmenero et al. 1991). Similarly, postnatal administration of melatonin in adult rats also decreases LH serum levels and delays the onset of the post-reproductive constant estrous-anovulatory state as observed in reproductive aging rats (Trentini et al. 1992). Other antioxidants, including vitamin C and E, have been found to be detrimental to the developing fetus when administered at high doses, at least in specialized high-risk pregnancies. Results from a clinical trial by Poston et al. (2006) indicated that prenatal supplementation of vitamin C and E increases the risk of producing LBW babies. In rats, maternal treatment with vitamin C in a hypoxic pregnancy attenuated hypoxia-induced maternal and placental oxidative stress (Richter et al. 2012), but the effects of vitamin C and E supplementation during pregnancy on reproduction of offspring have not yet been investigated.

Selenium is an essential trace element. It is an antioxidant found in trace amounts in many foods including nuts, cereals, fish, and eggs and is often associated with redox reactions and signalling pathways (Kurokawa & Berry 2013), and recently has been suggested...
to play a role in corpus luteum and/or placental function in pregnant cows (Kamada et al. 2014). Although essential in trace amounts, high levels of selenium result in toxicity (Gore et al. 2010). In zebrafish, elevated dietary selenium decreased fecundity, embryo survival, and overall reproductive success (Penglas et al. 2014). In sheep, maternal diet affected expression of connexin in the ovary, where selenium modulated the effects; connexin expression in the granulosa layer of antral follicles was decreased by high levels of selenium and increased in the granulosa layer of primary and theca of antral follicles (Grazul-Bilska et al. 2013).

**Nutrient excess**

Worldwide obesity has nearly doubled since 1980 with over 500 million people now considered as being obese (Frias & Grove 2012). Concomitant with the rise in obesity is an increase in the number of reproductive-aged women that are overweight or obese (Flegal et al. 2010, 2012). Maternal obesity is a major obstetric risk factor for adverse fetal, neonatal, and maternal outcomes (Leddy et al. 2008, Gaillard et al. 2013, Mission et al. 2013), in addition it has been associated with childhood obesity of offspring (Poston 2012) and an increased risk of those offspring developing the metabolic syndrome during adulthood (Rooney & Ozanne 2011, Frias & Grove 2012). Excessive gestational weight gain (Boynton-Jarrett et al. 2011, Deardorff et al. 2013) and a pre-pregnancy BMI of overweight/obese (Keim et al. 2009, Deardorff et al. 2013) are associated with early menarche in humans; however, childhood obesity, which is strongly associated with maternal obesity (Catalano et al. 2009), is also associated with early menarche (Kaplowitz 2008) making it difficult to ascertain independent effects. In contrast, results from rat models (Shrestha et al. 2011) have indicated that high-fat diet intake throughout pregnancy and lactation, inducing maternal obesity, advances the onset of puberty, and disrupts estrous cyclicity in female offspring (Connor et al. 2012). In particular, the offspring of obese mothers are more likely to display estrous cycles characterized by prolonged or persistent estrus, where 2 or more days spent in estrus may be indicative of premature ovarian aging (Connor et al. 2012). At the level of the ovary, it has recently been shown that gestating offspring born to mothers fed a high-fat/high-cholesterol diet have more atretic follicles (Leveille et al. 2014), which may indicate impaired fertility. Interestingly, in sheep, BWs above 5 kg are associated with a decrease in fecundity even after controlling for subcutaneous adiposity (Gardner et al. 2009), indicating that fetal macrosomia, which is associated with maternal obesity independently (Mission et al. 2013), affects future reproductive success. Similar to models of caloric restriction, these offspring phenotypes, as a result of maternal nutritional excess, are probably due in part to impaired ovarian histogenesis, as demonstrated by a decrease in primordial follicles in fetal ovaries at gestation day 103 in gestating ewes (Da Silva et al. 2003) allowed to feed ad libitum.

Exposure of pups to lactating obese dams also appears to affect reproductive development. Long Evans Tokushima (LETO) rats fostered to obese Otsuka Long Evans Tokushima fatty (OLETF) dams have more frequent cycles characterized by two or more days spent in estrus during young adulthood (Schroeder et al. 2013). However, onset of puberty was not different in LETO rats reared by OLETF nor did VO occur earlier in rats (Sanchez-Garrido et al. 2013) or mice (Caron et al. 2012) overfed during lactation as a result of reduction in litter size. This is in contrast to the results indicating that prenatal exposure to an obesogenic environment induced by a high-fat (Connor et al. 2012) or an n-6 polysaturated fatty acid-rich maternal diet (Hilakivi-Clarke et al. 1997) results in advanced onset of puberty.

Although early-postnatal overfeeding as a result of reduction of litter size does not advance the onset of puberty in female rats, lactationally overfed mice display significant reductions in the number of arcuate neuronal projections to the MPO of the hypothalamus (Caron et al. 2012), probably contributing to a disruption in adult estrous cyclicity and a decrease in the fertility index (Caron et al. 2012) demonstrating that neural alterations have functional reproductive implications. In mice, neonatal overnutrition as a result of litter manipulation (Liu et al. 2013a,b) or lactational maternal high-fat feeding (Vogt et al. 2014) results in adult-onset obesity and insulin resistance (Liu et al. 2013a), which have adverse effects on oocyte and zygote quality (Minge et al. 2008, Igosheva et al. 2010, Junghelm et al. 2010, Luzzo et al. 2012, Machtinger et al. 2012). Moreover, female offspring born to high-fat-fed dams produce oocytes with differentially methylated promoter regions of key metabolic genes (Ge et al. 2014). It is not known if these epigenetic changes result in transgenerational effects; however, it has been recently shown in mice that high-fat diet-induced maternal obesity causes metabolic effects in F2 generation offspring via maternal inheritance (King et al. 2013). However, It is unclear if these effects are germine-dependent or independent, which could be due to differential maternal adaptations to pregnancy in F1 offspring.
In addition to the increased availability and consumption of low-cost, hypercaloric food contributing to the obesity epidemic, high fructose consumption, through both beverages and food, has been identified as another important conducive factor in the progression of obesity (Sloboda et al. 2014). The long-term female reproductive outcomes of early-life exposure to maternal high fructose intake currently remain unclear. Interestingly, in rats, maternal fructose intake (10% in the drinking water) before and during pregnancy increases the male-to-female sex ratio of litters (Gray et al. 2013). This is in accordance with the Trivers–Willard hypothesis, which states that females in a better body condition produce a greater proportion of male offspring (Trivers & Willard 1973, Rosenfeld & Roberts 2004). Future studies are required to fully investigate the effects of high-carbohydrate, high-sugar, and high-fat diets, so that animal models can reflect the shifting change in our access to high-energy, low nutritional value food.

It is worth noting that offspring of obese mothers that have increased rates of obesity may suffer a double effect on their reproductive function. Adipose tissue has the capacity for aromatization – where excess adipose tissue can contribute to increases in the conversion of testosterone to E2 (Deslypere et al. 1985). The pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) increases the levels of expression of the aromatase gene (Zhao et al. 1996), and as obesity has been classified as a pro-inflammatory environment, it is possible that TNFα contributes to increased capacity for aromatization of adipocytes. Thus, it has been suggested that in the cases of obesity, high levels of circulating E2 due to adipocyte-induced testosterone aromatization may impair hypothalamic-pituitary-gonadal function, driving an increase in central negative feedback at the levels of the hypothalamic and the pituitary (Gosman et al. 2006). This results in impairments in ovulation and in oocyte development. Thus, in offspring of obese mothers, where the intrauterine environment results in ovarian modifications, there is probably a pre–postnatal interaction on ovarian dysfunction.

### Conclusion

Disease risk is established well before birth. Obesity and type 2 diabetes, once thought to be lifestyle-mediated, are...
now known to be influenced by fetal adaptations to in utero conditions, including poor prenatal nutrition. Critically, these disease effects span multiple generations, but how this occurs is unclear. Germ cells (oocytes) in the growing fetal ovary are similarly vulnerable to prenatal events and are likely to contribute to this transgenerational disease risk. As the gametes that will eventually give rise to grand-offspring form during fetal life, it is possible that the link between poor prenatal nutrition and postnatal disease lies in the ovary – involving the developing germ cells and their function. There is now established evidence demonstrating that poor prenatal conditions result in fetal growth restriction, LBW, postnatal reproductive dysfunction, poor pregnancy outcomes, and may even contribute to early aging and menopause (Fig. 3). A steady rise in the rate of LBW and thus increased risk of reproductive deficits and subfertility may be a reality for forthcoming generations. However, the underlying mechanisms are still poorly understood. Thus, it is now essential to integrate evidence from large prospective human studies using targeted experimental animal models that will uncover these mechanisms and begin to indicate potential interventions. This information, derived from an integrated approach, should strive to inform both public health and education policy.

Declaration of interest

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

Funding

D M S is funded by a Canada Research Chair in Perinatal Programming.

References


Pengzhe S, Hamme K & Ellingsen S 2014 Selenium and mercury have a synergistic negative effect on fish reproduction. Aquatic Toxicology 149 16–24. (doi:10.1016/j.aquatox.2014.01.020)


Clinical Endocrinology and Metabolism 92 46–50. (doi:10.1210/jc.2006-1378)


Woods DC, Telfer EE & Tilly JL 2012 Oocyte family trees: old branches or new stems? PLoS Genetics 8 e1002848. (doi:10.1371/journal.pgen.1002848)


de Zegher F & Ibanez L 2006 Prenatal growth restraint followed by catch-up of weight: a hyperinsulinemic pathway to polycystic ovary syndrome. Fertility and Sterility 86 (Suppl 1) S4–S5. (doi:10.1016/j.fertnstert.2006.03.013)


Received in final form 13 October 2014
Accepted 27 October 2014
Accepted Preprint published online 27 October 2014