Parental diet, pregnancy outcomes and offspring health: metabolic determinants in developing oocytes and embryos

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Abstract

The periconceptional period, embracing the terminal stages of oocyte growth and post-fertilisation development up to implantation, is sensitive to parental nutrition. Deficiencies or excesses in a range of macro- and micro-nutrients during this period can lead to impairments in fertility, fetal development and long-term offspring health. Obesity and genotype-related differences in regional adiposity are associated with impaired liver function and insulin resistance, and contribute to fatty acid-mediated impairments in sperm viability, oocyte and embryo quality; all of which are associated with endoplasmic reticulum stress and compromised fertility. Disturbances to maternal protein metabolism can elevate ammonium concentrations in reproductive tissues and disturb embryo and fetal development. Associated with this are disturbances to one-carbon metabolism which can lead to epigenetic modifications to DNA and associated proteins in offspring that are both insulin resistant and hypertensive. Many enzymes involved in epigenetic gene regulation utilise metabolic co-substrates (e.g. acetyl CoA and S-adenosyl methionine) to modify DNA and associated proteins, and so act as 'metabolic sensors' providing a link between parental nutritional status and gene regulation. Separate to their genomic contribution, sperm can also influence embryo development via direct interactions with the egg and by seminal plasma components which act on oviductal and uterine tissues.

Introduction

Epidemiological observations made by David Barker and colleagues were some of the first to identify the close relationship between reduced weight at birth, taken as a proxy of reduced in utero fetal development, and the increased prevalence for chronic ill-health in adult-hood (Barker 2007). Subsequent studies, repeated in separate human populations, revealed strong associations between altered patterns of fetal growth, a predisposition for childhood over-growth and adiposity, and increased risk of cardio-metabolic impairment in adulthood a (Forsen *et al.* 1997; Rich-Edwards *et al.* 1997; Dabelea *et al.* 1999; Cheung *et al.* 2000). However, it is also well established that parental diet can significantly influence the developmental competence of gametes and the preimplantation embryo, and that this too can have long-term implications for offspring wellbeing. Indeed, periconceptional development represents a continuum of stages during which, depending on the timing and nature of the parental nutritional insult, fetal development and offspring wellbeing can be affected differentially. Steegers-Theunissen *et al.* (2013) considered the periconceptional period for humans to span an interval from 14 weeks pre-ovulation, coincident with extensive ovarian follicular growth, to 10 weeks post-fertilisation, which culminates with closure of the secondary palate of the embryo.

Human and animal studies have demonstrated that all stages of gamete maturation and preimplantation embryo development are influenced directly by parental nutrition and hormonal status (Ashworth *et al.* 2009; Cetin *et al.* 2010; Martin *et al.* 2010; Wu *et al.* 2011) (Table 1). Deficiencies or excesses in a range of macro- and micro-nutrients are associated with significant impairments in reproductive performance, fertility, fetal development and long-term offspring health (Table 2). Indeed, the world today is faced with a dual burden of both under- and over-nutrition in human populations, with an estimated 3.5 million deaths globally attributed to undernutrition (Martin *et al.* 2010), whilst an estimated 3.3 billion people will become overweight or obese by 2030 (Kelly *et al.* 2008). Furthermore, in their report on the influence of maternal, fetal and child nutrition on the development of chronic disease in later life, one of the principal conclusions of the Scientific Advisory Committee on Nutrition (SACN) in the UK was that: “In the context of reproduction, the impact of energy dense diets of low micronutrient content on women and girls is of particular concern” (SACN, 2011).

However, the majority of studies examining parental periconceptional nutrition on embryo and offspring development have been conducted in animal models. In cattle and sheep, these concern enhancing reproductive performance and gamete quality, yield of product (i.e. milk or meat) or reducing the environmental impact of mass agriculture (Ashworth *et al.* 2009). However, these species are also important models in the validation of programming mechanisms reported in rodents, having greater similarities with human development (e.g. gestation length, timing of

embryonic genome activation, monotocous pregnancies). In contrast to the limited human data sets available, a sizable body of animal data has amassed, revealing the significance of parental periconceptional nutritional status and effects on reproductive performance and consequences for offspring development and health.

With the foregoing discussion in mind, the aim of the current article is to provide a comprehensive and contemporary overview of this subject, drawing on evidence from human and rodent studies where appropriate but, for the greater part, focussing on ruminant livestock species. The article seeks to identify novel areas worthy of further investigation, and emerging evidence of some of the underlying mechanisms.

Maternal nutrition

Obesity and periconceptional high-fat diets

It's estimated that between 26% and 29% of non-pregnant women, between 20-39 years, are overweight or obese (Hedley *et al.* 2004). Many studies have reported a negative effect of obesity on fertility (Table 1). Obese women show a reduced response to gonadotrophin stimulation, resulting in lower estradiol levels and fewer collected follicles, with equal or reduced live birth rates being reported post transfer (Esinler *et al.* 2008; Hill *et al.* 2011; Marquard *et al.* 2011). Follicular fluid bathing the cumulus–oocyte complex in obese women contains markedly increased levels of triglycerides and free fatty acids, potentially contributing to reduced oocyte quality (Robker *et al.* 2009). In addition to the direct effects of maternal obesity on follicular development, the risk of developing type II diabetes and polycycstic ovary syndrome (PCOS) in humans also increases in direct relationship to the duration and severity of maternal obesity. Increased central adiposity is associated with elevated insulin and androgen levels, endometrial hyperplasia and ovarian dysfunction (Kulie *et al.* 2011). Interestingly, in humans the impact of obesity on clinical pregnancy rates following IVF/ICSI appear to be weight and population sensitive with studies demonstrating reproductive impairments in Chinese women with a body-mass index (BMI) >25, but similar impairments were not observed in Caucasian women until a BMI >35 (Shah *et al.* 2011). Similarly, in women under 35, BMI has a negative impact on IVF outcome, but in women over 35 this impact is relatively minimal (Metwally *et al.* 2007; Sneed *et al.* 2008).

Accumulation of fat in non-adipose tissues (in particular liver and muscle) is linked to obesity and with peripheral insulin resistance in humans (Chen and Hess, 2008). However, accumulation of fat in visceral, as opposed to subcutaneous, adipose depots is also linked to insulin resistance and a broader range of medical disorders collectively referred to as Metabolic Syndrome (Gallagher *et al.*, 2009). Direct delivery of free-fatty acids (FFA) to the liver from visceral adipose tissues via the

hepatic portal vein is now believed to be the primary contributor to hepatic steatosis, hyperinsulinaemia and glucose intolerance (Wajchenberg, 2000). There is emerging evidence that this condition, so well characterised in humans, may also be prevalent in farm animal species most notably in high-yielding dairy cows. Some 30 years ago it was shown that British-Friesian cows contained a greater proportion of omental and peri-renal fat, and less subcutaneous fat, than a range of beef and beef x Friesian cows (Wright and Russel,1984). More recently, Sinclair (2010) advanced the hypothesis that the modern Holstein cow had inadvertently been bred to become increasingly insulin resistant. The regional re-distribution of body fat towards intra-abdominal depots (which are more resistant to insulin) contributes to greater lipolysis and release of FFA during negative energy balance. This proposition is supported in part by a retrospective analysis on plasma insulin concentrations which, in non-lactating cows, was two-fold (i.e. 40 vs 20 µIU/ml) greater than age and body condition-score matched Simmental x Holstein contemporaries. However, it is also supported by the observations of Chagas *et al.* (2009) who, as a consequence of conducting a series of glucose tolerance tests, found North-American Holsteins to be more insulin resistant than New Zealand Holstein Friesians cows. Most recently, Hostens *et al.* (2012) demonstrated that the fatty acid profile of FFA is closer to that of intra-abdominal fat than subcutaneous fat in post-partum dairy cows, and confirmed the higher catabolic activity of intra-abdominal fat during this period. In addition to impairments in liver function and health (Sinclair, 2010), such FFA are known to accumulate in ovarian follicular fluid and elicit toxic effects on bovine oocytes and pre-elongation embryos (e.g. Leroy *et al.,* 2005).

A rabbit model of maternal hyperlipidic induced obesity revealed significant over-expression of adipophillin, a gene associated with lipid accumulation, in blastocysts at the time of embryonic genome activation (Picone *et al.* 2011). Studies investigating the mechanisms underlying these impairments in lipid metabolism and mitochondrial function have identified endoplasmic reticulum (ER) stress response induced lipotoxicity as a central factor. Here, accumulation of intracellular triglyceride and FFA cause damage to the membranes of the mitochondria, ER and other organelles, resulting in the accumulation of intracellular ROS and the misfolding of ER proteins. As a consequence, protein degradation, caspase activation and the initiation of apoptosis occurs (Breckenridge *et al.* 2003; Malhotra and Kaufman 2007).

Dietary supplementation and reproductive performance

In mice, the feeding of high fat diets to females results in increased ovarian lipid accumulation, elevated levels of apoptosis within the ovary and cumulus-oocyte-complexes (COCs), reduced fertilisation rates and increased the production of mitochondrial reactive oxygen species (ROS) in blastocysts (Igosheva *et al.* 2010; Jungheim *et al.* 2010; Wu *et al.* 2010). The feeding of diets enriched in long-chain n-3 polyunsaturated fatty acids for four weeks prior to oocyte collection in

mice altered similarly mitrochondrial morphology and ROS levels, with reduced fertilisation capacity and ability to support development to the blastocyst stage (Wakefield *et al.* 2008).

Somewhat paradoxically perhaps, in light of the foregoing discussion, the feeding of lipid rich diets (i.e. diets supplemented with calcium soaps of palm oil fatty acids; 440 g/kg palmitic acid (C16:0), 400 g/kg oleic acid (C18:1, n-9), 95 g/kg linoleic acid (C18:2, n-6), 50 g/kg stearic acid (C18:0) and 15 g/kg myristic acid (C14:0)) to dairy cows in the absence of obesity has been shown to be beneficial, increasing follicular growth, oocyte and blastocyst cell number, and blastocyst yields (Fouladi-Nashta *et al.* 2007); although effects on pregnancy rates following transfer are not known. Similarly, whilst diets enriched in unsaturated fatty acids (i.e. either C18:2 n-6 or C18:3 n-3) fed to lactating dairy cows enhanced blastocyst cell number compared to diets rich in saturated fatty acids (Thangavelu *et al.* 2007), the number of transferrable embryos was unaltered and pregnancy rates were not established. In general, dietary inclusion levels of fat supplements in dairy cow diets are considerable lower than those offered in the aforementioned mouse studies, and they differ in fatty acid composition. Furthermore, the physiological status of lactating dairy cows, which are in negative energy balance and mobilising significant quantities of FFA which are enriched in saturated fatty acids, may negate to a certain extent any putative effects of dietary unsaturated fatty acids. There is a general lack of consensus on the overall benefits of feeding long-chain fatty acids to lactating dairy cows (Santos et al., 2008).

Interactions between level of feeding, maternal body condition and reproductive outcomes have also been identified in cattle. Whilst feeding virgin heifers of low body condition at 2x, relative to 1x, maintenance enhances blastocyst yields following OPU and IVF, the same level of feeding for heifers of moderate body condition reduces oocyte quality and embryo development (Adamiak et al. 2005). Effects in this study were cumulative and associated with prolonged exposure to hyperinsulinaemia (i.e. 48 µIU/ml) which reduces blastocyst yields (Figure 1). Similarly, in the study of Adamiak et al (2006), the effect of diet composition (i.e. low vs high starch, and low vs high dietary fat) on egg quality and embryo development was dependent on heifer body condition. Collectively, these observations highlight the complexity of interpreting effects of dietary interventions on oocyte quality and embryo development, and identify the need to conduct studies that assess effects on pregnancy establishment following embryo transfer.

In cattle and sheep increased dietary crude protein, or rumen degradable protein, is associated with elevated serum urea levels which impacts negatively on fertility (McEvoy *et al.* 1997). Elevated plasma urea concentrations, resulting from excess rumen degradable protein or dietary urea, can decrease uterine luminal pH (Elrod and Butler 1993; Meza-Herrera *et al.* 2010) and pregnancy rate in cows (Butler *et al.* 1996). In both cattle and sheep, the deleterious effects of urea on fertility are

likely to occur before day 4 of pregnancy (Fahey *et al.* 2001) or possibly during oocyte growth/maturation (Gath *et al.* 2012).

Indeed, high plasma concentrations of ammonium and urea during the antral stages of follicular development are associated with reduced embryo development following *in vitro* maturation, fertilisation and culture (Sinclair et al., 2000a). Both glucose and protein metabolism were increased in surviving embryos, each indicative of metabolic stress. Mean plasma ammonium concentrations in this study peaked at around 300 to 350 μM within two hours of feeding, and this suppressed appetite and the normal postprandial rise in insulin release (Sinclair et al., 2000b). Jugular vein infusion of either ammonium chloride or urea for several hours in beef heifers led to peak plasma ammonium and urea concentrations of around 800 μM and 14 mM respectively, with similar levels recorded in oviducal fluid (Kenny et al., 2002). However, with the exception of calcium, these treatments had no effect on oviductal glucose, lactate and electrolyte concentrations. These observations, together with those that have shown direct effects of ammonium exposure (75 to 350 μM) on mouse and human embryo metabolism and development during culture (Zander et al., 2006; Gardner et al., 2013), point to direct actions of this metabolite on the follicle-enclosed oocyte and pre-implantation embryo although, in the case of the former, effects may also be mediated through actions on granulosa and cumulus cells which, when pre-cultured in the presence of ammonium chloride, are less able to support oocyte and early embryo development (Rooke et al., 2004). Direct actions of ammonium can also lead to impaired fetal development as has been shown in both the mouse (Lane and Gardner, 2003) and sheep (Powell et al., 2006).

Nutrient deficient diets and offspring development

Mirroring the detrimental impacts of maternal overnutrition, human and animal models of periconceptional undernutrition have revealed similar sensitivity with regards to reproductive performance and the programming of offspring health. Analysis of epidemiological data examining the offspring of women exposed to the Dutch Winter famine showed that maternal nutrient restriction during the first trimester of pregnancy was linked to increased prevalence of coronary heart disease, raised lipids and obesity in offspring (Ravelli *et al.* 1999; Roseboom *et al.* 2000; Roseboom *et al.* 2001), whereas famine occurring during late gestation led to decreased glucose tolerance in adult life (Ravelli *et al.* 1998).

However, as with analysis into the impact of maternal overnutrition, the development of animal models, in particular the sheep, has become central in the elucidation of the physiological mechanisms underlying developmental programming. Over or under feeding of ewes for 8 weeks prior to conception reduced the number of cleaved oocytes following IVF as well as increasing

maternal serum insulin and estradiol levels respectively (Grazul-Bilska *et al*. 2012). The feeding of a half-maintenance diet for 2 weeks prior to oocyte collection revealed significant changes in transcript levels for genes associated with metabolic activity (Pisani *et al.* 2008). Maternal global undernutrition in the ewe and cow has been shown to both increase and reduce blastocyst development and trophectoderm cell number (Kakar *et al.* 2005; Borowczyk *et al.* 2006), stimulate uterine blood flow (Rumball *et al.* 2008) and increase placentome vascularity and placental growth factor expression (Vonnahme *et al.* 2007). These responses are suggestive of early adaptive changes ahead of placentation in order to maximise nutrient exchange and fetal development. Indeed, during late gestation within the undernourished ewe, compensatory responses are observed within the placenta, exerted through the mitogen-activated protein kinase/extracellular-signal-regulated kinase 1/2 (MAPK/ERK1/2) and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signalling pathways to increase vascular density (Zhu *et al.* 2007). As such, fetal growth whilst slower is maintained (Oliver *et al.* 2005). In the rodent, whilst maternal dietary restriction reduces placental weight at day 19 of gestation, glucose and system A amino acid transporter expression is upregulated in order to maintain fetal growth (Coan *et al*. 2010). Also in the fetus, significant changes in maturation of the HPA axis have been reported, coinciding with elevated levels of adrenocorticotrophic hormone and arterial blood pressure in twins (Edwards and McMillen 2002b; Edwards and McMillen 2002a), whilst premature hyperactivation of the fetal HPA has been associated with reduced fetal growth and premature delivery (McMillen *et al.* 2008). Stimulation of the fetal HPA axis may, in part, be driven through altered maternal cortisol and adrenocorticotrophic hormone levels in combination with altered placental 11β-hydroxysteroid dehydrogenase type 2 activities. These factors could result in higher transfer and exposure of the fetus to maternal glucocorticoids (Bloomfield *et al.* 2004; Jaquiery *et al.* 2006; Connor *et al.* 2009). Changes in fetal renal gene expression patterns (MacLaughlin *et al.* 2010), increased adrenal mass and elevated stress induced cortisol production (Zhang *et al.* 2010) as well as bed-dependent changes in vascular function (Torrens *et al.* 2009) may contribute additionally to the programming and changes in offspring cardiovascular responses observed in the maternal undernutrition sheep model. In cattle, low dietary protein in the first trimester of pregnancy followed by increased protein in the second trimester enhance placental development (Perry *et al.* 1999). Maternal nutrient intake during the first trimester affects offspring growth and adiposity in a sex specific manner with male fetuses exposed to a low level of nutrition being heavier throughout the post-weaning period. Females, however, only become heavier in adulthood (Micke *et al.* 2010). Analysis of offspring adipose tissue revealed differential expression of *IGF* and *LEP* genes dependent upon the depot analysed and the sex of offspring (Micke *et al.* 2011).

The specific sensitivity of the periconceptional period to maternal undernutrition has also been extensively demonstrated through the rodent maternal low protein diet (LPD) model. Initial observations in the rat demonstrated that an isocaloric LPD fed to dams exclusively during

preimplantation development (4 days following conception) reduced blastocyst cell number, altered perinatal and postnatal offspring growth and induced adult hypertension (Kwong *et al.* 2000). In an extension to these studies, using the same LPD, but fed to female mice exclusively during preimplantation development (3.5 days following conception), similar changes in offspring growth patterns and elevated systolic blood pressure, together with impaired vascular function and elevated patterns of offspring activity within an open field test were observed (Watkins *et al.* 2008a; Watkins *et al.* 2011). Interestingly, maternal LPD given during the terminal stages of oocyte maturation (3.5 days prior to conception) in the mouse did not alter offspring postnatal growth, but did affect adult systolic blood pressure, vascular function and behavioural phenotype (Watkins *et al.* 2008b). Reflecting changes induced through maternal obesity or high fat diets, maternal low protein diet has been shown to alter both mitochondrial localisation and membrane potential (Mitchell *et al.* 2009). Recently, (Eckert *et al.* 2012) demonstrated significant changes in amino acid, insulin and glucose levels within maternal serum and uterine fluids around the time of implantation in LPD fed mice. These changes in maternal metabolite levels coincided with altered levels of amino acids, particularly branched chain amino acids, within the blastocyst as well as altered phosphorylation of downstream effector molecules from the intracellular nutrient sensor and regulator mTORC1. It may, therefore, be the case that in response to LPD, maternal hyperglycaemia and depleted amino acid levels induces metabolic stress within the preimplantation embryo, initiating developmental programming within the fetus (Fleming *et al.* 2011).

The role of maternal periconceptional micronutrient status with regard to development of adult metabolic disorders has yet to be investigated fully. The role of many micronutrients as enzyme co-factors, in signal transduction and as antioxidants provides clear evidence that inadequate intake can affect short- and long-term development of the gametes, embryo and offspring. As the effects of micronutrients, trace elements and different vitamin supplements (i.e. B-vitamins, folate and methyl donors) have been reviewed in detail elsewhere (Andersen *et al.* 2006; Cetin *et al.* 2010; Laanpere *et al.* 2010) only a brief overview of this topic will be provided here.

In the mouse, maternal gestational restriction of copper, zinc, and vitamin E reduces offspring body weight and crown-to-rump length at birth as well as increasing systolic blood pressure and insulin levels post-weaning (Rosario *et al.* 2008). In addition, reduced placental 11β-hydroxysteroid dehydrogenase-2 activity was observed. As discussed above, fetal exposure to excess maternal glucocorticoids could modulate the activity of the HPA axis and fetal cardiovascular homeostasis. In sheep, the periconceptional feeding of diets deficient in B-vitamins (i.e. B12 and folate) and methionine results in offspring displaying hypertension, obesity, insulin resistance and global changes in liver methylation status, occurring to a greater extent within male offspring (Sinclair *et al.* 2007). A continuation of this study into the rat revealed similar phenotypic effects on male

offspring glucose homeostasis (Maloney *et al.* 2011). In the mouse, maternal dietary methyl donor supplementation for 2 weeks prior to conception negated the effect of bisphenol A on DNA hypomethylation, restoring the coat colour distribution in viable yellow agouti mouse offspring (Dolinoy *et al*. 2007).

Few human studies have explored the role of early micronutrient deficiencies with the development of DOHaD related disorders in offspring. Maternal preconceptional iron deficiency anemia has been associated with reduced fetal growth (Ronnenberg *et al.* 2004), whilst daily multiple micronutrient supplementation of pregnant women in Nepal results in modest reductions in offspring blood pressure at 2.5 years of age (Vaidya *et al.* 2008).

Molecular mechanisms of dietary effects in oocytes and embryos

Some of the cellular processes and molecular mechanisms influenced by maternal diet have been alluded to in earlier sections of this text. However, given that this article essentially focuses on long-term developmental consequences of parental nutrition during the periconceptional period, one's thoughts immediately turn to epigenetic mechanisms; a topic which has been extensively reviewed elsewhere in recent years (e.g. Bergman and Cedar, 2013), full details of which are beyond the scope of the current article.

DNA methylation, however, is the epigenetic mechanism most extensively studied, although it recognised that the establishment and erasure of DNA methylation marks within CpG dinucleotides is carefully orchestrated to coincide with covalent modifications to associated histone complexes in an inter-dependent manner during early development (Cedar and Bergman, 2009). Many enzymes involved in epigenetic gene regulation utilise co-substrates involved in cellular metabolism, and so provide a putative link between diet, cellular metabolism and gene regulation. Kaelin and McKnight (2013) considered a number of these enzymes and co-substrates including acetyl-CoA which in addition to its role in ATP production, energy metabolism and cellular biosynthesis, is also a substrate used by histone acetyl transferases (HATs) to modify histone tails. Oscillating intra-cellular levels of acetyl-CoA coincide with loss or gain of acetylation marks on a number of lysine residues on histones H3 and H4. Other 'metabolic sensors' include nicotinamide adenine dinucleotide (thought to be involved in histone deacetylation) and S-adenosyl methionine (SAM), involved in both histone and DNA methylation, and the focus of current research endeavours in the authors' lab.

DNA methyltransferases (DNMTs) utilse SAM, derived from the activation of methionine by ATP catalysed by the enzyme *methionine-adenosyl transferase* (MAT: EC.2.5.1.6). This enzyme is a key component of the linked folate-methionine cycles which generate SAM for use in a plethora of

reactions that include such critical processes as DNA synthesis (*vis-à-vis* purine and pyrimidine synthesis), and DNA and histone methylation. These cycles are expressed to a greater or lesser extent in all somatic cells within the ovary, the oocyte and in embryonic cells; although there are some species differences (Kwong et al., 2010). Specific dietary metabolites such as choline (betaine), methionine, folate and vitamin B_{12} (B12) act as intermediary components or cofactors for these cycles. Deficiencies in these and related micronutrients during the periconceptional period (embracing the terminal stages of oocyte growth and maturation, and post-fertilisation development to the blastocyst stage) in embryo donor ewes led to genome-wide epigenetic modifications to DNA methylation in offspring that become obese, insulin resistant and hypertensive (Sinclair et al., 2007). Other examples include the work of Anckaert et al. (2010), who cultured mouse pre-antral follicles for 12 days in standard control media (αMEM; Invitrogen), custom-made αMEM with methionine, folate, B12, B6 and choline removed, or custom-made αMEM with the aforementioned 1-C substrates and cofactors added back to match standard αMEM levels. Antral follicle development and oocyte maturation were both impaired under 1-C -deficient conditions. Furthermore, the methylation status of a differentially methylated region (DMR) within one (i.e., *Mest)* out of four imprinted genes assessed was significantly reduced relative to that for oocytes derived under standard culture conditions. These and other studies recently reviewed by Steegers-Theunissen *et al.* (2013) confirm a key role for maternal dietary-mediated epigenetic alterations to DNA and associated proteins in the long-term programming of fetal and offspring development and wellbeing.

Paternal nutrition

Sperm development and offspring health

Whilst our understanding of the developmental consequences of manipulating the maternal environment is well defined, the impact of paternal physiology and nutritional status around conception remains largely under-investigated. Spermatogenesis represents a complex series of events during which precursor spermatogonia undergo morphological, cytoplasmic and genomic reorganisation in order to generate the mature spermatozoa. As in the female, male reproduction is critically sensitive to nutritional status (Table 1). In rams, the daily rate of sperm production and the quality of the semen produced (i.e. sperm count and sperm motility) are decreased by undernutrition (Parker and Thwaites 1972; Robinson *et al.* 2006). In bulls up to the age of 2 years, levels of nutrition affect testicular development and sperm production (Vandemark *et al.* 1964; Gauthier and Berbigier 1982). Histological analysis reveals male nutrition to affect the diameter and proportion of the testes occupied by seminiferous tubules and seminiferous epithelium (Martin *et al.* 2010). In addition, deficiencies in vitamins, fatty acids, amino acids or exposure to heavy

metals can all negatively impact on male reproductive function (Martin *et al.* 1994; Robinson *et al.* 2006; Martin *et al.* 2010).

As with maternal obesity, studies in humans and mice have demonstrated significant associations between increasing male BMI and reduced sperm motility (Hammoud *et al.* 2009), increased incidences of sperm abnormality (Kort *et al.* 2006) and DNA fragmentation (Chavarro *et al.* 2011), and reduced pregnancy rates (Ghanayem *et al.* 2010). Subsequently, increased levels of sperm DNA fragmentation correlate with poor pre- and post-implantation development and decreased pregnancy rates (Bertolini *et al.* 2002; Seli *et al.* 2004; Bakos *et al.* 2008). In men, consumption of 'Western' type diets comprising processed meat, sweets, refined grains and snacks is associated with reduce sperm motility (Eslamian *et al.* 2012; Gaskins *et al.* 2012). Also, in men and male rodents, diabetes, or the consumption of high-energy diets, reduces sperm motility, increases sperm abnormality, DNA fragmentation (Agbaje *et al.* 2007), alters testis metabolism (Rato *et al.* 2013) and endocrine homeostasis (Tremblay *et al.* 1985) and impairs infertility rates (Bener *et al.* 2009).

Although the impact of male nutritional physiology as a key cause of impaired fertility is emerging in humans and animal models, the long-term effects of paternal nutrition on subsequent generations remains unclear (Table 2). In mice, paternal LPD programmes changes the expression of genes involved in offspring hepatic lipid and cholesterol biosynthesis (Carone *et al.* 2010), whilst repeated paternal fasting prior to mating significantly alters offspring serum glucose, IGF-1 and corticosterone levels (Anderson *et al.* 2006). A high fat diet in males increased sperm DNA damage (Bakos *et al.* 2011), reduced blastocyst development and implantation rates (Mitchell *et al.* 2011) and disrupted offspring pancreatic ß-cell function (Ng *et al.* 2010). Interestingly, offspring of males fed high fat diet also display impaired fertility (Fullston *et al.* 2012), suggestive of epigenetic transmission of paternal traits. In humans, paternal and grand-paternal dietary and smoking behaviours have been shown to influence offspring and grand-offspring phenotype and mortality risk (Pembrey *et al.* 2006). Recently, (Soubry *et al.* 2013) demonstrated a negative correlation between paternal obesity the DNA methylation status of the IGF2 differentially methylated region in offspring.

Diet and molecular mechanisms of sperm action

Appropriate DNA packaging and chromatin modifications are essential for spermatogenesis, resulting in highly compact, epigenetically modified and transcriptionally silent chromatin. Changes in the normal patterns of sperm DNA (methylation), histones (methylation, acetylation) or RNA content provide potential mechanisms through which altered paternal physiology could influence subsequent generations. Significant changes in DNA methylation (Aston *et al.* 2012) and histone

retention (Hammoud *et al.* 2011) patterns have been observed in sperm from infertile men, whilst varied degrees of infertility, including sterility, correlate with perturbations in histone methylation (Steilmann *et al.* 2011; Yap *et al.* 2011). Additional genomic factors such as haploinsufficiency of sperm protamines lowers sperm counts and induces DNA damage in mice (Cho *et al.* 2001; Perez-Crespo *et al.* 2008) whilst, in humans, altered protamine (P1:P2) ratio associate with reduced fertility rates (Aoki and Carrell 2003; Carrell *et al.* 2007). Currently histones, through their extensive capacity for epigenetic modifications and influence on chromatin structure, provide the best candidates for transmission of paternal programming effects into the offspring at fertilisation. Analysis of promoter sequences associated with active histone modifications (i.e. H3K27me3) in both human and mouse sperm reveal significant enrichment at key developmental and pluripotency genes (Brykczynska *et al.* 2010; Hammoud *et al.* 2011). Whilst it has yet to be determined whether any of the 2-15% of histones retained within the mammalian sperm contribute directly to zygotic gene expression regulation, studies have revealed that sperm derived histones are transferred into the oocyte and become incorporated within the zygotic chromatin (van der Heijden *et al.* 2006; van der Heijden *et al.* 2008).

Separate to their genomic contribution, sperm can also influence development through the initiation of oocyte calcium oscillations at the point of fertilisation. The egg-to-embryo transition is driven by a series of intracellular Ca^{2+} oscillations that sweep across the egg, initiated through sperm derived PLC-ζ (Swann *et al.* 2006). Manipulation of the number and amplitude of these Ca^{2+} oscillations has been shown to alter blastocyst cell number (Bos-Mikich *et al.* 1997) and fetal development (Ozil and Huneau 2001). Knockdown of PLC-ζ using RNAi in sperm has been shown to reduce the number of Ca^{2}+ transients at fertilization and affect litter size (Knott *et al.* 2005). Seminal plasma cytokines (i.e. granulocyte-macrophage colony-stimulating factor) also influence embryonic, placental and offspring development (Sjoblom *et al.* 2005) as well as initiating maternal reproductive tract immunological responses, essential in the establishment and maintenance of pregnancy (Sharkey *et al.* 2007; Stewart *et al.* 2009). Male mice fed a high fat diet showed accumulation of fatty-fluid filled cyst within the in seminal vesicle and prostate as well as degeneration of the seminiferous tubules within the testes (Gopal *et al.* 2010). At present, the impact of paternal nutrition on these additional programming mechanisms, and the long-term offspring cardiovascular and metabolic health risks remain unknown.

Conclusions

The concept that parental nutrition during the periconceptional period can have a lasting legacy influencing fertility, offspring health and wellbeing, is now firmly established for a wide-range of mammalian species including humans. Key insights into underlying mechanisms exist and the importance of paternal nutrition has recently come to light. A number of issues, however, remain. Other than for offspring welfare, the significance of these early 'programming' effects for traits of

commercial importance (e.g. offspring fertility, growth and general productivity) in large farmed animal species remains to be fully quantified relative to that determined by genetics and the environment of adult offspring. Full sequencing and annotation of genomes for domesticated species will facilitate the search for gene-regulatory networks that may be epigenetically altered. Focus then should be directed towards identifying how specific metabolites and metabolic co-substrates interact with chromatin to epigentically alter gene expression. Identifying key components (i.e. macro- and micro-nutrients) of parental diet will also advise on the nutritional management of embryo donors and recipients within breeding programmes for optimum fertility and improved pregnancy outcomes.

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Table 1. Examples of how parental periconceptional nutritional impacts on gametes and preimplantation embryos.

Sex	Treatment/manipulation	Species	Duration	Outcome	Reference
Female					
	Obesity/high fat diet	**Human**	Pre-conception	Increased follicular insulin, lactate, triglycerides and C-reactive protein levels	Robker et al. 2009
		Pig	Pre-conception	Lower ovulation rates and reduced viability of embryos	Gonzalez-Anova et al. 2011
		Rodent	From16 weeks prior to mating	Reduced rates of preimplantation development, elevated TE and reduced ICM cell number	Minge et al. 2008
		Rabbit	8 weeks prior to mating	Altered blastocyst transcript levels and reduced fetal growth	Picone et al. 2011
	Nutrient deficient diet	**Cow**	From 17 days prior to ovum pick up	Reduced feed intake increased blastocyst development and cell number	Nolan et al. 1998
		Cow	First trimester (0-99 days of gestation)	Low protein diet during the first trimester enhanced placental development	Perry et al. 1999
		Sheep	From 64 days prior to oocyte collection	Global undernutrition reduced rates of cleavage and blastocyst production	Borowczyk et al. 2006
		Sheep	From 18 days prior to 6 days post mating	Low maintenance diet increased blastocyst and trophectoderm cell number	Kakar et al. 2005
		R**odent**	Pre-implantation	Low protein diet reduced blastocyst and fetal H19 and Igf2 expression	Kwong et al. 2006
		Rodent	From 6 weeks prior to mating	Low protein diet impaired blastocyst mitochondrial metabolism, increased reactive oxygen species generation and reduced blastocyst viability	Igosheva et al. 2010
		Rodent	From 16 weeks prior to mating	Low protein diet increased ovarian apoptosis, reduced oocyte maturation and fetal growth	Jungheim et al. 2010
	Dietary supplementation	**Cow**	From 87 to 73 days prior to ovum pick up and IVF	Supplementation with n-3 fatty acids increased number of antral follicles and blastocysts of cleaved	Zachut et al. 2010
		Cow	From 39 days prior to blastocyst collection	Increased blastocyst cell number following dietary supplementation with flax or sunflower seed	Thangavelu et al. 2007
		Cow	OPU during 3 successive estrous cycles	Blastocyst yields: High level feeding beneficial for animals of low body condition, but detrimental for animals of moderate body condition	Adamiak et al. 2005
		Cow	From 25 days prior to 80 days post mating	Unsaturated fatty acids improved fertilization and embryo development	Cerri et al. 2009
		Cow	From 6 days prior to OPU	Urea supplementation reduced blastocyst yields	Ferreira et al. 2011

		Cow	From 18 days prior to oocyte collection	Cleavage and blastocyst production rates reduced from heifers fed a high plasma ammonia-generating diets	Sinclair et al. 2000a
		Sheep	From 30 days prior to 15 days post mating	High protein diet reduced fertility rates, embryo number and uterine pH.	Meza-Herrera et al. 2010
		Sheep	From 6 weeks prior to ovulation	n-6 PUFA reduced embryo development and transcript levels relative to control	Wonnacott et al. 2010
		Sheep	From 21 days prior to insemination	High maternal urea intake retards embryonic development and increases embryo mortality	McEvoy et al. 1997
		Pig	From 19 days prior to oocyte collection.	Increased oocyte maturation in high plane fed gilts	Ferguson et al. 2003
		Pig	From day 1 to 21 of estrus	Increased oocyte maturation and embryo survival in high fibre fed gilts	Ferguson et al. 2007
Male					
	Obesity/high fat diet	**Human**	Pre-conception	Negative relationship between BMI, sperm number and sperm chromatin integrity	Kort et al. 2006
		Human	Pre-conception	High BMI and central adiposity negatively affect sperm number and motility	Hammiche et al. 2012
		Human	Pre-conception	Positive correlation between BMI, seminal plasma adipokine levels and reduced sperm count	Thomas et al. 2013
		Rabbit	From 11 months prior to semen analysis	Reduced semen volume, sperm motility and increased sperm abnormality following hypercholesterolemic diet	Saez Lancellotti et al. 2010
		Rodent	From 15 to 45 weeks prior to semen analysis	Reduced sperm motility	Fernandez et al. 2011
		Rodent	Up to 13 weeks prior to mating	Reduced blastocyst development and implantation rates	Mitchell et al. 2011
		Rodent	10 weeks prior to mating	Reduced preimplantation development, altered blastocyst cell number and mitochondrial membrane potential	Binder et al. 2012.
		Rodent	16 weeks prior to semen analysis	Reduced sperm SIRT6 and increased H3K9 levels	Palmer et al. 2011
	Diabetes	**Human**	Pre-conception	Increased lipid peroxidation and reduced sperm count in diabetic men	La Vignera et al. 2011
		Rodent	Pre-conception	Reduced sperm concentration, motility and fertilisation, and preimplantation development rates.	Kim and Moley. 2008
	Dietary supplementation	**Human**	Pre-conception	Positive link between antioxidant intake and sperm number and motility	Eskenazi et al. 2005

Cow	Between 5 and 12 weeks prior to semen analysis	Improved sperm viability and motility following dietary docosahexaenoic acid supplementation	Gholami et al. 2010
Cow	6 months prior to semen analysis	Zn increased sperm number, motility and viability.	Kumar et al. 2006
Sheep	13 weeks prior to semen analysis	Fish oil increased sperm concentration, motility and docosahexaenoic acid levels	Samadian et al. 2010
Pig	4 months prior to semen analysis	Increased sperm concentration but reduced motility in boars fed organic selenium supplemented diet	Lopez et al. 2010
	6 months prior to semen analysis	Decreased sperm concentration, total sperm and motile sperm per ejaculate in boars fed fumonisin B1 supplemented diets	Gbore 2009
Pig	From 16 weeks prior to semen analysis	Increased sperm number and duration of ejaculation following omega-3 fatty acid supplementation	Estienne et al. 2008
Rodent	From 15 weeks prior to semen analysis and mating	Folic acid deficiency decreased cauda sperm numbers and increased sperm DNA fragmentation	Swayne et al. 2012
Stallion	From 90 days prior to semen analysis	Increased sperm motility and decreased abnormality rates following antioxidant supplementation	Contri et al. 2011

Table 2. Examples of how parental periconceptional nutritional impacts on pregnancy and offspring outcomes.

Sex	Treatment/manipulation	Species	Duration	Outcome	Reference
Female					
	Obesity/high fat diet	**Rabbit**	From 8 weeks prior to mating	Maternal hyperlipidic hypercholesterolemic diet alters blastocyst transcript levels and reduces fetal growth	Picone et al. 2011
	Nutrient deficient diet	**Sheep**	From 60 days prior to 30 days after mating	Changes in fetal hypothalamic-pituitary-adrenal axis development and maternal steroid levels	Bloomfield et al. 2004
		Sheep	From 60 days prior to 30 days after mating	Reduced fetal hypothalamic GR and POMC promoter DNA methylation and increased histone acetylation and methylation	Begum et al. 2012
		Sheep	From 60 days prior to 7 days after mating	Changes in fetal hypothalamic-pituitary-adrenal axis development in late gestation	Edwards and McMillen. 2002a
		Sheep	From 60 days prior to 7 days after mating	Increased blood pressure responses in twin fetuses	Edwards and McMillen. 2002b
		Sheep	From day 1 to 30 of gestation.	Increased pulse pressure, reduced rate pressure product, and altered baroreflex function in offspring at 1 year	Gardner et al. 2004
		Sheep	From 61 days prior to 30 days post mating	Maternal undernutrition induces preterm delivery through increased cortisol and prostaglandin levels	Kumarasamy et al. 2005
		Sheep	From 60 days prior to 30 days post mating	Increased uterine blood flow in periconceptional undernourished ewes	Rumball et al., 2008
		Sheep	From 60 days prior to 30 days after mating	Impaired adult offspring glucose tolerance	Todd et al. 2009
		Sheep	From 15 days prior to 15 days post mating	Maternal global nutrient restriction induces vascular dysfunction in adult offspring	Torrens et al. 2009
		Sheep	From 4 months prior to 1 week post mating	Maternal low or high maintenance feeding alters offspring hepatic insulin signalling and miRNA levels	Nicholas et al. 2013
		Sheep	From 30 days prior to 15 days post mating	Maternal high protein diet reduces fertility rates, embryo number and uterine pH	Meza-Herrera et al. 2010
		Sheep	From 8 weeks prior to 6 days following mating	Maternal methyl deficient diet Increases offspring body weight and adiposity, insulin-resistance, elevates blood pressure and alters immune responses to antigenic challenge	Sinclair et al. 2007
		Rodent	Pre-implantation	Maternal low protein diet increases systolic blood pressure in male offspring	Kwong et al. 2000
		Rodent	From 3.5 days prior to 3.5 following mating	Maternal low protein diet alters offspring birth weight, postnatal growth, cardiovascular regulation and behaviour	Watkins et al. 2008a, b
		Rodent	Pre-implantation development	Maternal low protein diet increases offspring postnatal adiposity and altered gene expression patterns	Watkins et al. 2011

		Rodent	From 3 weeks prior to 5 days following mating	Maternal methyl deficient diet alters glucose homeostasis in male offspring.	Maloney et al. 2011
	Dietary supplementation	**Human**	Pre-conception to 9.5 weeks of gestation	Maternal micro-nutrient supplementation induces sex specific reduction in cord blood methylation at IGF2R and GTL2-2 DMR's	Cooper et al. 2012
Male					
	Obesity/high fat diet	**Human**	Pre-conception	Reduced odds of live birth in couples undergoing ICSI	Colaci et al. 2012
		Human	Pre-conception	Paternal obesity correlates with hypomethylation of IGF2 DMR in offspring	Soubry et al. 2013
		Rodent	10 weeks prior to mating	Impaired beta-cell function, glucose intolerance and altered pancreatic gene expression in female offspring	Ng et al. 2010
	Nutrient deficient diet	**Rodent**	1 to 4 weeks prior to mating	Decreased offspring serum glucose, corticosterone and IGF-1 levels following paternal fast	Anderson et al. 2006
		Rodent	6-9 weeks prior to mating	Altered offspring cholesterol biosynthesis following paternal low protein diet	Carone et al. 2010
	Paternal and grandpaternal smoking and nutrition	**Human**	Pre-conception	Paternal smoking associated with BMI childhood in sons Grandfather's nutrition associated with grandson mortality risk.	Pembrey et al. 2006

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Figure 1. Regression coefficients for blastocysts of cleaved against plasma insulin concentrations determined at each of two oocyte recovery sessions within each of three successive estrous cycles from the study of Adamiak et al. (2005). Heifers were moderately fat at the beginning of the experimental period and were offered a high calorie diet at a level equivalent to twice their metabolisable energy requirements
910 for maintenance. Oocytes were matured, fertilised and cultured to the blastocyst stage *in vitro*. Mean plasma insulin concentration for these animals was 48 µIU/ml. Modified from Sinclair and Kwong (2010) with permission (Cambridge University Press).