Origins of lifetime health around the time of conception: causes and consequences

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Abstract (200 words)

Parental environmental factors including diet, body composition, metabolism and stress affect the health and chronic disease risk of people throughout their lives, as captured in the 'Developmental Origins of Health and Disease' (DOHaD) concept. Research across epidemiological, clinical and basic science fields has identified the period around conception as being critical in the processes mediating parental influences on the next generation's health. During this time, from the maturation of gametes through to early embryonic development, parental lifestyle can adversely influence long-term risks of offspring cardiovascular, metabolic, immune and neurological morbidities, often termed 'developmental programming'. We review 'periconceptional' induction of disease risk from four broad exposures: maternal overnutrition and obesity; maternal undernutrition; related paternal factors; and from the use of assisted reproductive treatment. Human studies and animal models demonstrate the underlying biological mechanisms, including epigenetic, cellular, physiological and metabolic processes. A novel meta-analysis of mouse paternal and maternal protein undernutrition indicate distinct parental periconceptional contributions to postnatal outcomes. We propose that the evidence for periconceptional effects on lifetime health is now so compelling that it calls for new guidance on parental preparation for pregnancy, beginning before conception, to protect the health of offspring.

Introduction

The notion that maternal physiology, body composition, diet and lifestyle during pregnancy have profound and enduring effects on offspring long-term health and disease risk into adulthood has received strong evidential support across epidemiological, medical and basic science fields[1-3]. Thus, the 'Developmental Origins of Health and Disease' (DOHaD) concept has emerged, proposing that poor developmental experience can provoke increased risk of non-communicable disease in later life, particularly cardiovascular and metabolic comorbidities such as hypertension, obesity and type-2 diabetes, atopic conditions and some forms of cancer, as well as neurological impairment. A recent focus in DOHaD research has been to probe ***when*** during pregnancy the conceptus is most vulnerable to such adverse influences, thereby informing targeted protection and possible intervention. Increasing evidence points to the importance of the time around conception (=**periconceptional period**).

Box 1: Key messages

Whilst evidence for developmental origins of later disease can be found throughout gestation and beyond, there is a growing consensus from both human and animal studies that a critical period is around conception and hence merits particular attention.

As we review, preconception maternal overnutrition and obesity, maternal undernutrition, related paternal factors, and assisted reproductive treatments all may change the phenotype and potential of gametes and early embryos, with enduring consequences across the lifespan.

Our new data reveal that suboptimal maternal and paternal nutrition around conception have similar effects on offspring weight, but differing effects on offspring blood pressure.

These critical influences on lifetime health occurring so early in development may reflect perturbations or adaptations in epigenetic, cellular, metabolic and/or physiological mechanisms. Defining these mechanisms and the exposures that drive them is critical to the characterisation of more specific recommendations for preconception health.

This emerging knowledge has significant societal and medical implications. In particular, it provides the basis for a new emphasis on preparation for pregnancy, before conception, to safeguard public health and as a means of disease prevention.

Periconceptional developmental conditioning

The **periconceptional period** has been variously defined, but for DOHaD processes the key events broadly cover the completion of meiotic maturation of oocytes, differentiation of spermatozoa, fertilisation and resumption of mitotic cell cycles in the zygote, marking the

transition from parental to embryonic genomes[4] and the onset of morphogenesis up to implantation[5]. This represents a period of a few weeks, dependent upon mammalian species, and is characterised by extensive change in morphology (emergence of distinct embryonic and placental cell lineages); genomic re-organisation (epigenetic modifications such as DNA methylation to regulate lineage-specific gene expression in the conceptus); and changes in metabolism (setting homeostatic regulators for growth and energy supply). **See Figure 1 for a resumé of key events**. It is however recognised that influences at every stage from earliest childhood can shape preconception health and thereby influence eventual pregnancy and birth outcomes.

Adverse developmental processes around the time of conception have been demonstrated in human and animal models in response to diverse environmental situations. In vivo, the quality of a mother's diet, both overnutrition and obesity[6] or undernutrition[7], and/or other aspects of her physiological status including hyperglycemia/lipidemia[8], may affect embryo potential with consequences for offspring disease risk over the lifetime. Paternal lifestyle and phenotype can similarly influence long-term offspring health, mediated either through the sperm or seminal plasma[9]. Periconceptional parental influences may have particular and differing effects on male and female offspring[10]. In addition, more babies are being born as a result of assisted reproductive treatments (ART) some of which involve embryo culture and exposure to potentially inappropriate environmental factors, which may alter offspring phenotype[10,12]. Long-term outcomes are consistent with the DOHaD concept, including cardiometabolic, immunological and neurological non-communicable disorders.

To some the concept of 'periconceptional' origins of lifetime health may not be intuitive. Why should this short window at the very start of development have such profound consequences for the rest of our lives? Critically, the essential steps in reproduction over this period occur when the few cells involved are fully exposed to environmental conditions, making them ***vulnerable*** to disturbance of epigenetic mechanisms and an altered profile of embryonic gene expression that persists through subsequent cell cycles and drives an altered developmental programme. Metabolic and cellular homeostatic characteristics of the embryo, including mitochondrial activity, can also change in response to nutrient availability. Conversely, periconceptional sensitivity to environmental cues also raises the possibility that this window is one of ***opportunity***, providing the embryo with capacity to respond to prevailing conditions and to optimise development to best suit survival and fitness[7]. Thus, periconceptional developmental plasticity (induction of different phenotypes from a single genotype) may facilitate setting of suitable growth and metabolic parameters to match the perceived

environment but which, if environmental conditions change, may become maladaptive and lead to later disease[3].

This article focuses on four broad periconceptional environmental exposures shown to induce adverse effects in humans and animal models (Figure 2), and discusses mechanistic causes and consequences. We also report new data on the relative contributions of maternal and paternal influences to long-term periconceptional influences in an established low protein diet model of parental undernutrition.

Periconceptional developmental conditioning through maternal overnutrition and obesity

The global rise in maternal obesity is associated with reduced female fertility and heightened risk of obesity in the offspring[2]. Adverse effects of high maternal body mass index (BMI) on the offspring may reflect elevated maternal glucose and insulin concentrations driving fetal growth and adiposity, resulting in increased birth and childhood weight, but may also include shared lifestyle factors within families[6]. Impaired offspring metabolism may also be associated with increased risk of allergic and atopic conditions, revealing the complexity in phenotype[2]. Maternal obesity models in animals have confirmed the link with offspring cardiovascular and metabolic disease risk[6,13].

Why might the periconceptional period be causal for obesity-related conditioning? Obese women have higher circulating concentrations of inflammatory cytokines[14], and of hormones and metabolites which accumulate within the ovarian follicular fluid and can affect oocyte maturation and potential adversely. Thus, maternal BMI is positively associated with increased follicular fluid insulin, lactate, triglycerides, leptin and other metabolic regulators[15]. This rich follicular fluid compromises the developmental competence of exposed animal oocytes in experimental models, reducing embryo quality[16]. Moreover, oocytes from obese women are smaller and produce blastocysts with increased triglycerides and reduced glucose consumption, markers of poorer potential[17].

In addition to metabolite overexposure, maternal obesity in mice induces defects in the mitochondrial phenotype of eggs, including abnormal morphology and cristae structure[18], altered membrane potential and distribution[18] and increased mitochondrial DNA content[18,19], all markers of disturbed mitochondrial function and energy homeostasis. Oocytes from obese

dams also exhibit increased oxidative stress and spindle abnormalities suggesting increased risk of aneuploidy[18,19].

These mitochondrial defects in oocytes may derive from the elevated lipid content and inherent insulin resistance caused by high maternal adiposity. Oocyte hyperlipidaemia in turn leads to impaired metabolic regulation and endoplasmic reticulum stress in mice[16], a condition where proteins misfold during biosynthesis and which contributes to metabolic and cardiovascular disease. Bovine and murine *in vitro* oocyte maturation models demonstrate that elevated fatty acid concentrations perturb follicular physiology, reduce oocyte developmental competence, including altered transcriptome and epigenome profiles in blastocysts, and lead to early embryos with compromised metabolism and lower potential[12].

The combination of metabolic, mitochondrial and chromosomal alterations in oocytes and embryos from obese mothers has important implications for subsequent development. In mice, obese mothers have smaller fetuses and pups which develop overgrowth, adiposity and glucose intolerance after birth[20]. Transfer of mouse blastocysts from obese mothers to normal recipients produces similarly growth-restricted fetuses with associated malformations despite the absence of gestational maternal obesity[18]. Similarly, in sheep, female offspring from embryos of obese natural mothers transferred to non-obese mothers exhibit increased adiposity, with dysregulation in liver and muscle insulin signalling and hepatic fatty acid oxidation[21] . These changes are associated with epigenetic perturbations in the liver, including upregulation of microRNAs regulating insulin signalling[21]. Similarly, mouse embryos transferred from diabetic mothers to control recipients exhibit fetal growth retardation and congenital anomalies resembling natural diabetic pregnancies[8]; such structural changes are in keeping with clinical practice, in which pre/periconceptional folic acid supplementation and improved diabetes control reduce the incidence of anomalies.

The periconceptional effects of maternal obesity are also apparent in ART pregnancies. Fertility declines with increasing BMI in women receiving donor oocytes, as in non-donated pregnancies, suggesting reduced uterine receptivity[22]. However, in other studies, recipient BMI had no effect on donor oocyte pregnancy success, whilst ***donor*** BMI was negatively associated[23], indicating that pre-conception oocyte quality is influenced by maternal adiposity.

Periconceptional developmental conditioning through maternal undernutrition

Human studies

Poor nutrition in utero and low birth weight remain highly prevalent in low and middle income countries and are associated with increased risks of chronic diseases in later life across diverse human populations, particularly if followed by accelerated weight gain during infancy[1,3]. Similar human cardiometabolic and neurological consequences arise from maternal exposure to famine, e.g. the Dutch Hunger Winter of 1944/45. In human studies it is difficult to pinpoint gestational windows when heightened sensitivity to maternal undernutrition occurs, but the Dutch famine analyses suggest a poorer prognosis for those offspring ***conceived*** during the famine rather than experiencing it later in gestation[24]. Similarly, individuals exposed in utero, particularly during the first trimester, to the Chinese Great Famine (1959-61) have increased risk of hypertension in adulthood[25]. Exposure during the periconceptional period of the Dutch famine is reported to cause epigenetic dysregulation resulting in reduced DNA methylation of the imprinted growth-regulating IGF2 gene persisting into adulthood, along with differential methylation in the regulatory regions of genes affecting growth and metabolism[24].

In another important human study, dramatic seasonal variation in maternal nutrient consumption in The Gambia affected perinatal outcomes including birth weight, adult health and mortality[26]. By studying genomic regions where methylation patterns are highly correlated across tissues derived from all three germ lines it has been possible to demonstrate that maternal nutrition at conception alters the epigenome prior to gastrulation, with the effects persisting, at minimum, well into childhood and adolescence[27]. This periconceptional legacy coincided with seasonal changes in maternal plasma methyl-donor biomarkers which, along with BMI, are also predictive of childhood methylation patterns[28]. So far, significant deviations in the methylation patterns of loci predictive of immune function, tumour suppression[29] and obesity[30] have been noted.

Animal models

Animal models have been essential for investigating mechanisms involved in the multistep processes linking periconceptional maternal undernutrition with later-life disease risk. In rodents, feeding a low protein diet (LPD) - specifically during the periconceptional period, either exclusively during the final 3 days of oocyte maturation[31] or the 3-4 day window of preimplantation embryo development (Emb-LPD)[32,33], with normal nutrition at all other times - is sufficient to induce an altered growth trajectory and cardiovascular, metabolic and neuro-behavioural dysfunction in adulthood. Such targeted dietary models commonly show hypertension in adult offspring, coupled with increased adiposity[7,31-33]. Similar findings have been reported in sheep[34].

Rodent and sheep models of maternal periconceptional undernutrition suggest that impaired regulation of fetal development may underlie co-morbidities. For example, studies in sheep have shown that the late gestation fetal cardiovascular response to hypoglycaemia is modified by prior peri-implantation undernutrition[35]. Moreover, peri-implantation and late gestation maternal undernutrition affect fetal sheep skeletal muscle development differentially[36], and maternal undernutrition in early gestation alters gestation length and fetal and postnatal growth[37].

Induction and response mechanisms

The mouse embryonic period low protein diet (Emb-LPD) model has helped reveal how periconceptional maternal undernutrition may initiate adverse effects during early embryogenesis[7]. Emb-LPD reduces circulating maternal insulin and amino acid concentrations, including reduced branched-chain amino acids (BCAAs) within the uterine luminal fluid that bathes early embryos before implantation[38]. BCAAs act as targets for embryo nutrient sensors, enabling nutrient status to be sensed by blastocysts via the mammalian target of rapamycin complex 1 (mTORC1) growth-regulating signalling pathway, inducing an altered growth trajectory from before implantation[38] (see below), and shown by embryo transfer to be induced within the blastocyst[33]. Altered induction by Emb-LPD in mice activates compensatory responses that are distinct between extra-embryonic (trophectoderm; primitive endoderm) and embryonic (epiblast) lineages of the blastocyst (**Figure 1**). The Emb-LPD trophectoderm becomes more proliferative, adopts a more invasive migratory phenotype at implantation, and activates increased endocytosis of maternal uterine luminal fluid proteins as an alternative source of nutrients, leading to a placenta that is more efficient in nutrient transfer to the fetus[38-40]. Similarly, the primitive endoderm activates compensatory responses to enhance nutrient delivery via the yolk sac placenta, mediated through epigenetic mechanisms[40,41].

In response to Emb-LPD, changes in embryonic lineages may help set the embryonic and fetal growth trajectory to match prevailing nutrient availability. The embryonic lineages utilise preimplantation nutrient sensing to regulate growth across somatic organs (e.g., liver and kidney) through adaptations in the rate of ribosome biogenesis[42]. In essence, rRNA expression is suppressed during periods of maternal dietary restriction but is increased, beyond that of the control rate, when the dietary challenge is removed. This mechanism modulates the level of DNA methylation at the rDNA promoter, thereby mediating RNA polymerase I interaction with the promoter to regulate ribosome biogenesis and growth[42,43]. Interestingly, rDNA has also been found to be a genomic target for growth regulation in models of maternal high-fat or

obesogenic diets[43]. This exquisite lifetime mechanism, activated in the preimplantation embryo, is likely to be responsive to uterine luminal fluid nutrient concentrations and appears to utilise a nutrient-sensing ribosome factor, Rrn3, to mediate the rDNA responses[42]. The growth-regulating role of the embryonic lineages is critical since perinatal weight associates with adult disease risk[33].

Paternal origin of periconceptional developmental programming

Whilst the connection between a mother's diet and the long-term health of her offspring has been studied in detail, our understanding of how a father's diet impacts his offspring remains limited. However, links are now emerging between paternal lifestyle, sperm quality and impaired offspring health[9]. Here, both direct (sperm quality, epigenetic status, DNA integrity) and indirect (seminal fluid composition) paternal mechanisms have been identified, with the potential to affect mouse offspring development across multiple generations[44].

Mirroring female reproductive fitness, male fertility is closely linked to nutrition and body composition. In humans and rodents, elevated BMI is associated with reduced sperm motility[45], increased sperm abnormality[46], increased sperm reactive oxygen species levels, reduced serum testosterone and increased oestradiol concentrations[47]. Consumption of a 'Western-style' diet high in sugar, fat and processed food associates with reduced sperm motility in men[48], while consumption of energy-dense diets in men and rodents is associated with poor sperm motility, morphology and DNA integrity[49]. Reduced sperm DNA integrity, as occurs in obesity and diabetes, correlates with reduced human embryonic development and decreased pregnancy rates[50]. In men undergoing IVF treatment, obesity is associated with reduced blastocyst development and live birth rates[51]. In rodents, paternal obesity induced by high-fat diet increases sperm DNA damage[52], reduces blastocyst development and implantation rates[53] and causes sub-fertility in male and female offspring for up to two generations[54]. Interestingly, these negative effects on offspring development can be prevented through paternal dietary and exercise interventions in mice[55], indicating that sperm-mediated effects may be transient and even reversible. In rats, a paternal high-fat diet for 10 weeks before mating affected female (but not male) offspring pancreatic β-cell function and increased body weight, glucose intolerance and impaired insulin secretion[56]. Offspring of male mice over-nourished during neonatal life demonstrate glucose intolerance, fasting hyperglycaemia and insulin resistance, mirroring the metabolic disturbance seen in their fathers[57].

Similar to the impacts of paternal obesity, paternal LPD in mice induces the expression of genes involved in offspring hepatic lipid and cholesterol biosynthesis[58]. Analysis of offspring hepatic epigenetic status revealed genome-wide changes in DNA methylation, including the key lipid regulator *PPARα*. In adulthood, offspring from male mice fed LPD have higher birth weight, a reduced male:female offspring ratio, increased adult adiposity, hypotension, glucose intolerance and elevated serum TNF-α levels[59]. Furthermore, paternal LPD also affects blastocyst *AMPK* gene expression, placental size, fetal growth and skeletal development[60].

As for maternal periconceptional nutrition models, epigenetic mechanisms are likely mediators of effects of paternal phenotype and exposures on offspring development[61]. Changes in patterns of sperm histone modifications (methylation, acetylation), DNA methylation and/or RNA content are prime candidates for such paternal periconceptional programming. Sperm from infertile men display significant changes in histone populations[62], with enrichment of active histone marks (i.e. H3K27me3) at key developmental and pluripotency genes in human and mouse sperm[62]. Studies have also revealed that sperm-derived histones are transferred into the oocyte and incorporate into zygotic chromatin following human fertilisation[63]. However, whether any of the 2-15% histones retained within the mammalian sperm contribute directly to zygotic gene expression regulation is unknown. Human sperm also contain several thousand coding RNA transcripts[64] and altered expression is linked with infertility[65]. Recent studies have shown that levels of sperm tRNA-derived small RNAs (tsRNAs) are altered by paternal diet in mice[66]. Interestingly, offspring generated by injecting zygotes with sperm tsRNA taken from male mice fed a HFD showed impaired glucose tolerance and insulin secretion[66]. While such studies highlight the role of RNA populations in intergenerational programming[67], the significance of these sperm-derived RNA molecules remains to be elucidated.

Apart from sperm-specific mechanisms of developmental programming, seminal plasma composition, (e.g. granulocyte-macrophage colony-stimulating factor) influences mouse embryonic, placental and offspring development[68] and initiates maternal reproductive tract immunological responses, essential in the establishment and maintenance of human pregnancy[69]. In mice, paternal seminal fluid impacts on the maternal uterine environment, altering blastocyst development, placental size and adult offspring glucose tolerance, adiposity and blood pressure[70].

Defining the parental contribution to periconceptional developmental effects

Shared maternal and paternal dietary and other lifestyle influences may potentially combine for greater impact on periconceptional development. However, most research models to date are uniparental in design and the combined effects of both parents are unknown. Whether the impact of poor paternal diet on offspring development and wellbeing is of equivalent significance to that of poor maternal diet is also unknown. As a first step, Box 2 and Figure 3 show a meta-analysis of our mouse maternal and paternal LPD diet models using published data for offspring weight at birth, adult systolic blood pressure (SBP) and adult heart:body weight ratio (a measure of heart capacity) including datasets covering maternal intervention restricted to the periods of oocyte maturation, preimplantation development or the entirety of gestation[31,33,59]. The use of the same robust, statistical random effects regression analysis across each of these studies strengthens our comparison of parental effects in the current analysis. However, such rigorous statistical approached are rarely adopted, especially in animal model studies, and so we have restricted our analysis to data from these three studies alone. Offspring birth weight was increased in response to maternal LPD during the terminal stages of oocyte development (Egg-LPD) and during preimplantation preimplantation development (Emb-LPD) (**Figure 3a**). Overall, the pooled estimate demonstrated parental LPD increased offspring birth weight. Our second analysis explored the impact of parental LPD on adult offspring SBP. Here, all maternal challenges resulted in offspring hypertension (**Figure 3b**), while paternal LPD resulted in a trend towards lower blood pressure in the adult offspring. Our final analysis examined the impact of parental diet on adult heart:body weight ratio (**Figure 3c**). Only paternal LPD had a significant effect, reducing offspring heart:body weight ratio. These new data demonstrate differential effects from paternal and maternal periconceptional developmental exposures on offspring phenotype. It is essential that further studies define the precise impacts and underlying mechanisms by which parental diet regimes affect offspring development and wellbeing. Studies examining concurrent paternal and maternal interventions on shared offspring outcomes are also warranted.

Box 2: Analysis of parental contribution effect

- Data for offspring phenotype were taken from Watkins et al 2008a[31], 2008b[33] and 2014[59]. Each study used the same NPD and LPD formulation fed to either female or male mice for distinct periconceptional durations.
- All three studies employed the same rigorous random effects regression analysis to account for the hierarchical nature of the studies in the statistical analyses.
- Raw data on individual offspring weight at birth, adult tail-cuff systolic blood pressure measurement and adult heart:body weight ratio for all groups were used for the analyses.
- Raw mean differences between experimental and study-specific control group (normalised to a value of 0) offspring were calculated ($\Delta = \mu1 - \mu2$) for birth weight, systolic blood pressure (SBP) and heart:body weight ratio parameters.
- Weight (%) refers to the individual contribution (by number of animals) of each study to the total Pooled Estimate. Heterogeneity (i.e. variation in outcomes between studies) was assessed using χ^2 test on Cochran's Q-statistic and by calculating I^2 (i.e. percentage of variation across studies attributed to heterogeneity rather than chance). As heterogeneity was significant for all analyses, pooled estimates were calculated by the random effects (Mantel-Haenszel) method.
- The largest effect on offspring birth weight was in response to maternal preimplantation (Emb-LPD) diet (raw mean difference: 0.18g, 95% CI 0.11 – 0.24; P<0.0001) (**Figure 3a**). Maternal LPD restricted to the terminal stages of oocyte maturation (Egg-LPD) also resulted in increased birth weight (raw mean difference: 0.09g, 95% CI 0.05 – 0.13; P<0.0001). However, maternal LPD throughout gestation had no impact (raw mean difference: 0.04g, P=0.26) on offspring birth weight (likely reflecting fetal growth regulation during gestation, discussed above), as did paternal LPD (raw mean difference 0.03g, P=0.09). Overall we observe a significant pooled estimate effect of parental LPD on offspring weight at birth (raw mean difference: 0.1g, 95% CI 0.07 – 0.13; P<0.0001) representing an increase in LPD offspring weight of 7.8%.
- Analysis of offspring SBP revealed all maternal LPD groups had elevated SBP (raw mean difference: Egg-LPD 6.92mmHg, 95% CI 4.95 – 8.90; P<0.0001; Emb-LPD 5.60mmHg, 95% CI 3.63 – 7.56; P<0.001; LPD 5.54mmHg, 95% CI 3.66 – 7.42; P<0.0001) (**Figure 3b**). In contrast, paternal LPD resulted in a trend towards the programming of lower offspring blood pressure (raw mean difference: -3.49mmHg, 95% CI -7.62 – 0.63; P=0.096). The differential parental effect on offspring SBP meant the pooled estimate showed no overall difference (raw mean difference: -0.36mmHg, 95% CI -1.75 – 1.02; P=0.61).
- Our final analysis examined the impact of parental diet on adult heart:body weight ratio. All groups displayed either a negative impact or no effect (**Figure 3c**). The largest size effect was observed in response to maternal Emb-LPD (raw mean difference: -0.05, 95% CI -0.1 – 0.01 P=0.073). Only the paternal LPD offspring heart:body weight ratio reached significance (raw mean difference: -0.03, 95% CI -0.07 – -0.01; P=0.038) (**Figure 3c**). Overall, the pooled effects indicated a reduction in adult heart:body weight ratio following parental, both maternal and paternal, LPD (raw mean difference: -0.03, 95% CI -0.05 – -0.01; P=0.0035).

Periconceptional developmental programming and ART

Direct evidence for human periconceptional effects comes from assisted reproductive treatments (ART) in which mature gametes and the preimplantation embryo are exposed to precisely timed in vitro manipulations. Several million apparently healthy ART children have now been born worldwide, but relatively little is known about the possible impact of the technology-associated exposures during conception and very early development on their health status during childhood and later life. The spectrum of human demographic confounders (including parental infertility), changes and improvements in ART techniques over time, and the relative sample sizes used make analyses complex and the reported outcomes need to be interpreted with caution. Nevertheless, it is well established that singleton ART pregnancies have increased risk of low birth weight, congenital abnormalities and higher mortality rate, although disentangling confounding by parental infertility is difficult[71]. Human embryo culture media have changed over time and the predominant current practice is to use commercially sourced media of proprietary (unspecified) composition (discussed in[12]). Comparison of perinatal outcome following use of different commercial media, including a multicentre randomised controlled trial, has indicated that birth weight is significantly affected[72], with effects on growth still manifest at age 2 years[73].

Compared with naturally conceived offspring, the cardiovascular phenotype of IVF children and adolescents reveals increased risk of high blood pressure[11,74], vascular dysfunction with abnormal blood flow and vessel thickness[75] and evidence of cardiovascular remodelling during development *in utero* affecting heart shape and chamber size[74]. Metabolic consequences include increased fasting glucose and peripheral insulin resistance[11,76], raised plasma lipids, and obesity[76]. A systematic review found no difference in cognitive outcomes among children conceived with conventional IVF and those conceived naturally, but did identify conflicting findings that require clarification among studies of children conceived with intracytoplasmic sperm injection[77].

Collectively, current evidence suggests that ART, like the in vivo nutritional models discussed above, may alter the development and growth trajectory of human embryos, and increase the risk of postnatal chronic cardiometabolic dysfunction. This legacy is unlikely to be due to parental infertility in isolation since controls in some studies comprise those naturally conceived offspring from sub-fertile parents[11,75]. Moreover, ART animal models demonstrate similar long-term consequences to human studies, despite normal parental fertility[78]. Thus, IVF embryo culture and transfer in mice results in offspring with altered growth trajectory, relative hypertension, cardiovascular abnormalities and glucose/insulin dysfunction[78].

ART-associated adverse effects on long-term health appear to have an epigenetic origin induced during the periconceptional period. ART children have an increased risk of rare imprinting disorders associated with DNA methylation errors on imprinted genes[79] and aberrant methylation of imprinted *H19* gene has been reported in human cultured embryos[80]. In mouse models, embryo culture may cause imprinted genes to lose their allele-specific expression (particularly at the growth regulating *H19/IGF2* locus) together with aberrant methylation patterning in embryos, placental and fetal tissues[81]. ART-induced aberrant epigenetic profiles may also be propagated during human pregnancy in fetal and placental tissues and persist into childhood affecting genes regulating growth such as the *IGF2/H19* locus[82]. Media composition, particularly albumin or serum components or ammonium ion accumulation from amino acid catabolism, may contribute to altered mouse epigenetic status[83]. Importantly, even a very limited culture period is sufficient to induce epigenetic changes[81]. Embryo culture exposure also modifies expression and methylation of non-imprinted genes in mice and alters expression of DNA methyltransferases[84]. For example, in mouse models ART affects the endothelial nitric oxide synthase (*eNOS*) gene implicated in vascular dysfunction and modification of culture media composition may prevent this effect[85]. Although provocative, more studies in both animal models and humans are required in order to replicate findings to date.

Diversity and commonality in periconceptional effects

The evidence reviewed above reveals that periconceptional experience can induce lifelong changes in phenotype, affecting disease risk. Beyond these nutritional and ART conditions, studies in rodents show broader examples of periconceptional effects, such as from maternal stress[86]. Moreover, maternal alcohol consumption exclusively around conception induced metabolic dysfunction in rat adult offspring with evidence of epigenetic disturbance[87]. In the case of mouse maternal systemic inflammation at conception, whilst not affecting cardiometabolic health, suppressed adult offspring innate immunity after challenge, possibly to protect 'self' in a predicted pathogenic postnatal environment[88]. In addition, mouse embryo transfer experiments suggest that advanced maternal age may adversely affect offspring cardiometabolic health[89], but the mechanisms underlying this age-associated effect are unknown.

The diversity of periconceptional induction conditions identified across mammalian species, coupled with clear evidence of both maternal and paternal pathways, implicates an early window when environmental exposures, combined with an inherent capacity for

developmental plasticity, may confer advantage when the offspring are exposed to a similar environment postnatally. During the periconceptional period there is rapid and radical molecular, cellular and morphogenetic restructuring; the signalling pathways that control these processes are sensitive to multiple molecules and other factors within the cellular environment and may provide a mechanistic underpinning for this concept[90]. However, as we have described, the periconceptional setting of metabolic homeostasis may become maladaptive if conditions change or if nutrient levels induce perturbations in metabolism, generating the circumstances underlying adverse health risk. A consistent mechanism identified across conditions and species has been epigenetic variation, a plausible pathway to 'biological embedding' of early life exposures and transmission of phenotypic effects throughout life. This has been demonstrated directly through manipulation of maternal one-carbon (1-C) metabolism during early embryogenesis, potentially reducing the availability of methyl donor groups necessary for DNA and histone methylation[91], but such epigenetic changes are not necessarily linked directly with changes in gene expression[92]. Thus, a periconceptional maternal diet deficient in 1-C metabolite substrates and cofactors (vitamin B_{12}, folate, methionine) in sheep modified offspring DNA methylation and led to adverse cardiometabolic and immune dysfunction[93]. Similarly, folate addition to rodent maternal LPD can rescue normal expression and DNA methylation of metabolic regulators in offspring which underlie cardiovascular dysfunction[94]. A mouse paternal low folate diet altered sperm DNA methylation profile, changed the placental transcriptome and resulted in offspring with craniofacial and musculoskeletal malformations[95]. Moreover, the negative impact of mouse paternal undernutrition on sperm quality, testicular oxidative stress, fertility and offspring fat accumulation and dyslipidaemia are reversed through vitamin and antioxidant supplementation[96]. As with ART, additional studies are warranted to define the critical window(s) and pathways linking perinatal one-carbon metabolism, epigenetic variation and programming of later offspring health.

Conclusion: Protecting health of the next generation and the way forward

We propose there is now sufficient evidence from human and animal research that the periconceptional period is a key window during which poor maternal and paternal physiology, body composition, metabolism and diet can induce increased risk of chronic disease in offspring, a lifetime legacy and major driver of health burden in the 21st century. The evidence that similar consequences can result from ART practices sharpens the focus on this window. Environmental factors may perturb gametes or early embryos, affecting homeostatic

mechanisms, or may induce adaptations to developmental environmental signals with consequences persisting into adulthood.

This evidence calls for a major re-examination of public health policy to protect against future disease risk through societal advice on, and greater provision of, preconception care[97] as also promoted in the two accompanying reviews in this series (Stephenson et al, submitted; Barker et al, submitted). Whilst a preconception focus on parental risk factors such as smoking and excess alcohol intake is wise and well established, new drives to prepare nutritionally for pregnancy are critical, including healthy body composition, physical activity and diet for both parents[98]. Further definition of the underlying epigenetic, cellular, metabolic and/or physiological mechanisms and the exposures that drive them, is an important research agenda that is pivotal to the characterisation of more specific recommendations for preconception health.

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Conflict of interest statement

KMG reports other from Nestle Nutrition Institute, grants from Abbott Nutrition and Nestec, outside the submitted work. The other authors have nothing to disclose.

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Figure legends

Figure 1. Biological events underpinning periconceptional conditioning

The periconceptional period is one of extensive cellular change comprising the completion of meiotic maturation of oocytes, differentiation of spermatozoa, fertilisation and resumption of mitotic cell cycles in the zygote, marking the transition from parental to embryonic genomes [4] and the onset of morphogenesis [5]. Periconceptional biology is indeed 'busy' – the morphological and cellular changes occurring during the switch from parental to embryonic generations leading to blastocyst formation are driven by pronounced sub-cellular and molecular processes. These include global restructuring of the epigenome (mainly DNA methylation and histone modifications that control gene expression), such that expression from the new embryonic genome is distinct from the parental genomes[99]. Epigenetic reorganisation allows the embryo to first exhibit *totipotency*, a naïve cellular state conferring the ability to construct both true embryonic (future fetal) cell lineages and the extra-embryonic (placental) lineages that become evident in the blastocyst. Subsequently, epigenetic modifications underpin embryo *pluripotency*, the capacity to generate all three germ layers (ectoderm, mesoderm, endoderm) once gastrulation has taken place. Morphogenesis of the blastocyst is followed by embryo hatching from the zona pellucida coat and implantation mediated through adhesion of the outer trophectoderm layer of the blastocyst to the uterine endometrium and subsequent invasion and decidualisation. Activation of the new embryonic genome before implantation not only permits de novo gene expression distinct from parental genomes but also involves establishment of the embryo's metabolism that matures over time[100].

Figure 2. Summary of periconceptional developmental conditioning from the four areas reviewed with main mechanisms highlighted in the progression of disease risk. ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization.

Figure 3. Defining the relative influence of maternal and paternal factors during periconceptional conditioning in mice following parental low protein diet (LPD; 9 % casein).

The effect of parental LPD on **(A)** offspring weight at birth, **(B)** adult offspring systolic blood pressure (SBP), and **(C)** adult offspring heart:body weight ratio are shown when compared

with offspring from normal protein diet (NPD; 18% casein) fed parents. Analysis of 4 studies involving female MF1 mice being fed LPD exclusively during the terminal stages of oocyte maturation (3.5 days prior to mating; Egg-LPD), exclusively during preimplantation embryo development (Emb-LPD) or throughout gestation (LPD). Forest plots also include offspring data in response to a paternal low protein (Pat-LPD) fed to C57BL6 males prior to mating. For Egg-NPD n = 189–80 from 19 litters; Egg-LPD n = 201-67 from 19 litters; NPD n = 131-76 from 19 litters; LPD n = 116-85 from 19 litters; Emb-LPD n = 134-78 from 19 litters; Pat-NPD n = 85-76 from 16 litters; Pat-LPD n = 73-62 from 16 litters. **A.** Plots present differences between means (± 95% CI) of birth weight (grams) to study specific NPD group. Data combining all LPD and all NPD treatment groups is used to determine the Pooled Estimate. Heterogeneity (χ^2) between studies = 1.96 (3 df), I^2 = 33%. **B.** Plots present differences between means (± 95% CI) of adult SBP (mmHg) to study specific NPD group. Data combining all LPD and all NPD treatment groups is used to determine the Pooled Estimate. Heterogeneity (χ^2) between studies = 1.05 (4 df), I^2 = 39%. **C.** Plots present differences between means (± 95% CI) of heart:body weight ratio to study specific NPD group. Data combining all LPD and all NPD treatment groups is used to determine the Pooled Estimate. heterogeneity (χ^2) between studies = 1.86 (3 df), I^2 = 61%.

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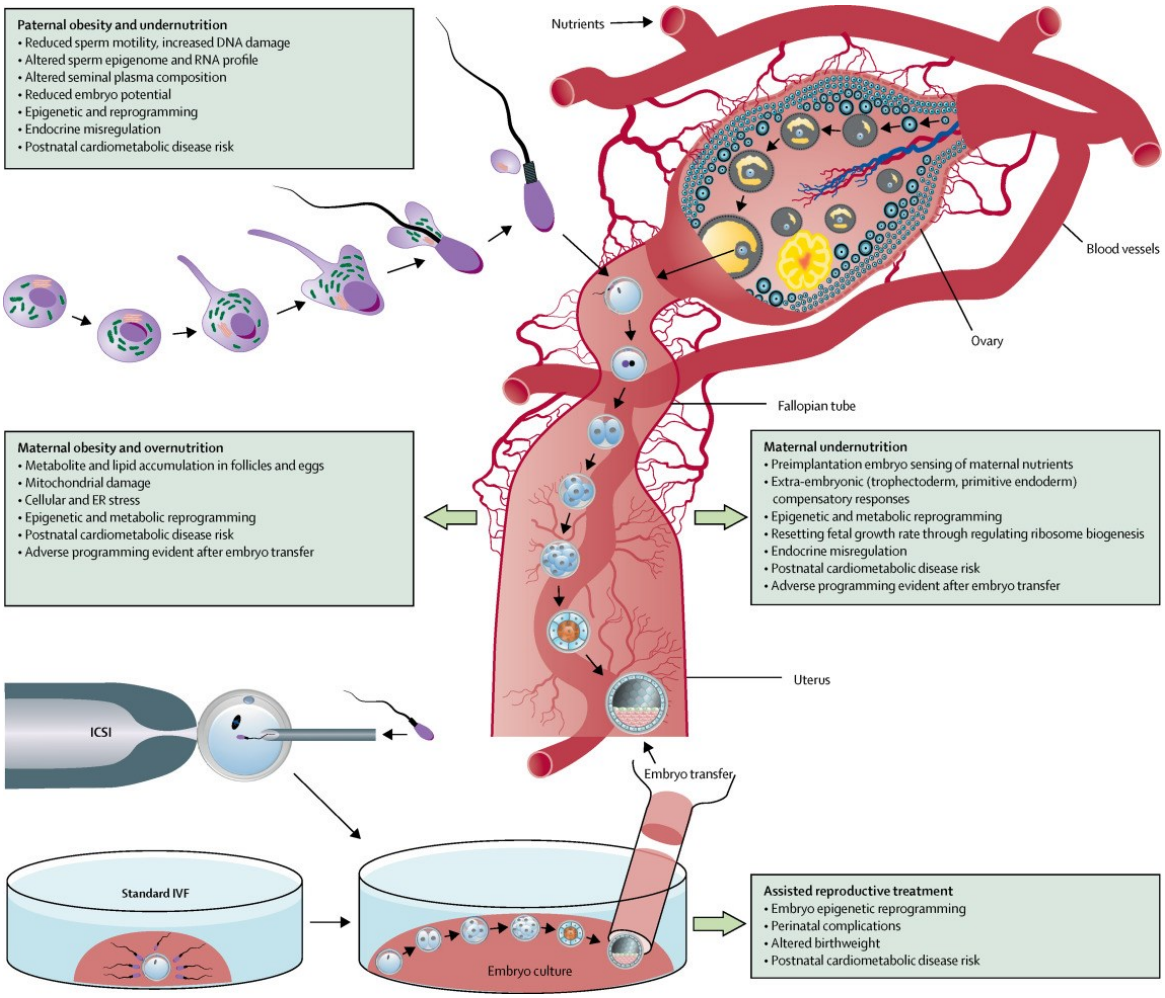
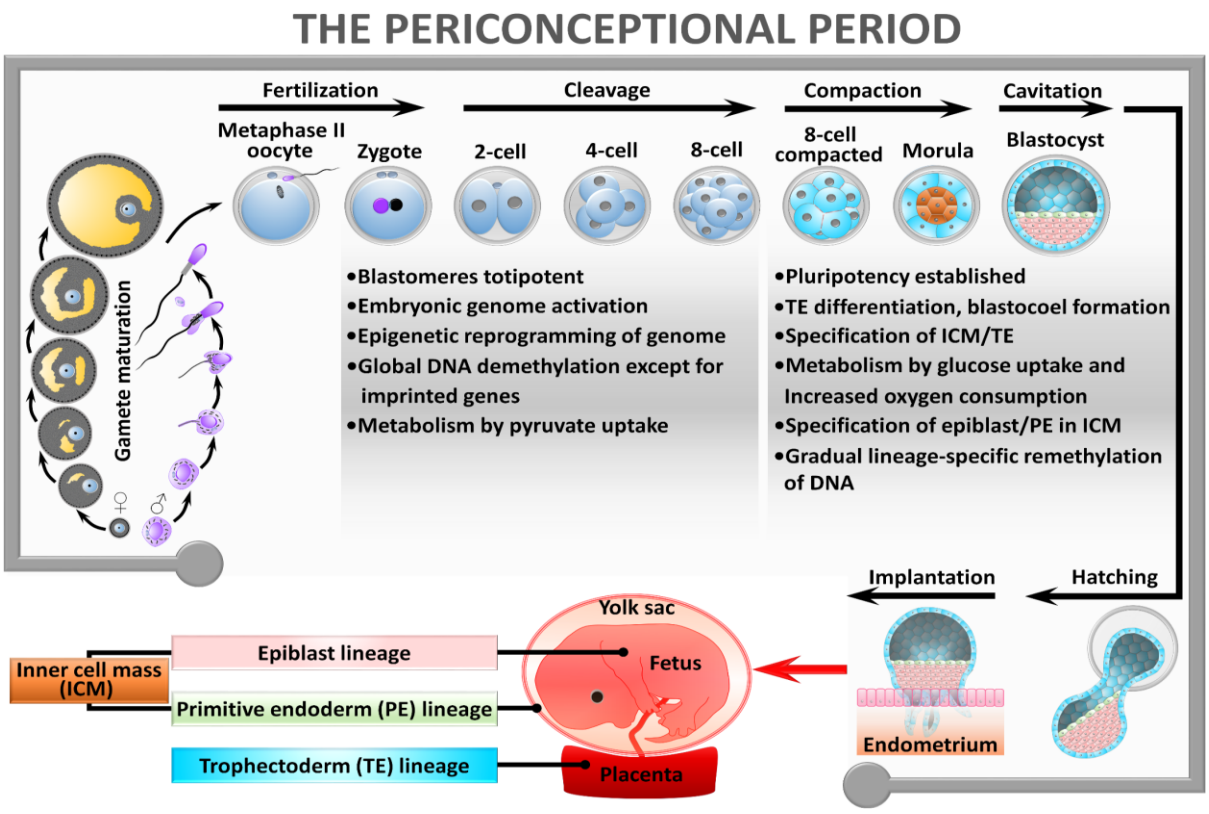
Figure 1


Paternal obesity and undernutrition
• Reduced sperm motility, increased DNA damage
• Altered sperm epigenome and RNA profile
• Altered seminal plasma composition
• Reduced embryo potential
• Epigenetic and reprogramming
• Endocrine misregulation
• Postnatal cardiometabolic disease risk
Nutrients
Blood vessels
Ovary
Fallopian tube
Maternal obesity and overnutrition
• Metabolite and lipid accumulation in follicles and eggs
• Mitochondrial damage
• Cellular and ER stress
• Epigenetic and metabolic reprogramming
• Postnatal cardiometabolic disease risk
• Adverse programming evident after embryo transfer
Maternal undernutrition
• Preimplantation embryo sensing of maternal nutrients
• Extra-embryonic (trophectoderm, primitive endoderm) compensatory responses
• Epigenetic and metabolic reprogramming
• Resetting fetal growth rate through regulating ribosome biogenesis
• Endocrine misregulation
• Postnatal cardiometabolic disease risk
• Adverse programming evident after embryo transfer
Uterus
ICSI
Embryo transfer
Standard IVF
Embryo culture
Assisted reproductive treatment
• Embryo epigenetic reprogramming
• Perinatal complications
• Altered birthweight
• Postnatal cardiometabolic disease risk

836 Figure 2



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842 Figure 3

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