

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

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36 **Abstract (200 words)**

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

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57

58 Introduction

59 The notion that maternal physiology, body composition, diet and lifestyle during pregnancy
60 have profound and enduring effects on offspring long-term health and disease risk into
61 adulthood has received strong evidential support across epidemiological, medical and basic
62 science fields¹⁻³. Thus, the ‘Developmental Origins of Health and Disease’ (DOHaD) concept
63 has emerged, proposing that poor developmental experience can provoke increased risk of
64 non-communicable disease in later life, particularly cardiovascular and metabolic
65 comorbidities such as hypertension, obesity and type-2 diabetes, atopic conditions and some
66 forms of cancer, as well as neurological impairment. A recent focus in DOHaD research has
67 been to probe **when** during pregnancy the conceptus is most vulnerable to such adverse
68 influences, thereby informing targeted protection and possible intervention. Increasing
69 evidence points to the importance of the time around conception (=periconceptual period).

70

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.

71 Periconceptual developmental conditioning

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

75 transition from parental to embryonic genomes⁴ and the onset of morphogenesis up to
76 implantation⁵. This represents a period of a few weeks, dependent upon mammalian species,
77 and is characterised by extensive change in morphology (emergence of distinct embryonic
78 and placental cell lineages); genomic re-organisation (epigenetic modifications such as DNA
79 methylation to regulate lineage-specific gene expression in the conceptus); and changes in
80 metabolism (setting homeostatic regulators for growth and energy supply). **See Figure 1 for**
81 **a resumé of key events**. It is however recognised that influences at every stage from earliest
82 childhood can shape preconception health and thereby influence eventual pregnancy and birth
83 outcomes.

84 Adverse developmental processes around the time of conception have been demonstrated in
85 human and animal models in response to diverse environmental situations. In vivo, the quality
86 of a mother's diet, both overnutrition and obesity⁶ or undernutrition⁷, and/or other aspects of
87 her physiological status including hyperglycemia/lipidemia⁸, may affect embryo potential with
88 consequences for offspring disease risk over the lifetime. Paternal lifestyle and phenotype can
89 similarly influence long-term offspring health, mediated either through the sperm or seminal
90 plasma⁹. Periconceptual parental influences may have particular and differing effects on
91 male and female offspring¹⁰. In addition, more babies are being born as a result of assisted
92 reproductive treatments (ART) some of which involve embryo culture and exposure to
93 potentially inappropriate environmental factors, which may alter offspring phenotype^{10,12}.
94 Long-term outcomes are consistent with the DOHaD concept, including cardiometabolic,
95 immunological and neurological non-communicable disorders.

96 To some the concept of 'periconceptual' origins of lifetime health may not be intuitive. Why
97 should this short window at the very start of development have such profound consequences
98 for the rest of our lives? Critically, the essential steps in reproduction over this period occur
99 when the few cells involved are fully exposed to environmental conditions, making them
100 **vulnerable** to disturbance of epigenetic mechanisms and an altered profile of embryonic gene
101 expression that persists through subsequent cell cycles and drives an altered developmental
102 programme. Metabolic and cellular homeostatic characteristics of the embryo, including
103 mitochondrial activity, can also change in response to nutrient availability. Conversely,
104 periconceptual sensitivity to environmental cues also raises the possibility that this window
105 is one of **opportunity**, providing the embryo with capacity to respond to prevailing conditions
106 and to optimise development to best suit survival and fitness⁷. Thus, periconceptual
107 developmental plasticity (induction of different phenotypes from a single genotype) may
108 facilitate setting of suitable growth and metabolic parameters to match the perceived

109 environment but which, if environmental conditions change, may become maladaptive and
110 lead to later disease³.

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

116

117 **Periconceptional developmental conditioning through maternal overnutrition** 118 **and obesity**

119 The global rise in maternal obesity is associated with reduced female fertility and heightened
120 risk of obesity in the offspring². Adverse effects of high maternal body mass index (BMI) on
121 the offspring may reflect elevated maternal glucose and insulin concentrations driving fetal
122 growth and adiposity, resulting in increased birth and childhood weight, but may also include
123 shared lifestyle factors within families⁶. Impaired offspring metabolism may also be associated
124 with increased risk of allergic and atopic conditions, revealing the complexity in phenotype².
125 Maternal obesity models in animals have confirmed the link with offspring cardiovascular and
126 metabolic disease risk^{6,13}.

127 Why might the periconceptional period be causal for obesity-related conditioning? Obese
128 women have higher circulating concentrations of inflammatory cytokines¹⁴, and of hormones
129 and metabolites which accumulate within the ovarian follicular fluid and can affect oocyte
130 maturation and potential adversely. Thus, maternal BMI is positively associated with increased
131 follicular fluid insulin, lactate, triglycerides, leptin and other metabolic regulators¹⁵. This rich
132 follicular fluid compromises the developmental competence of exposed animal oocytes in
133 experimental models, reducing embryo quality¹⁶. Moreover, oocytes from obese women are
134 smaller and produce blastocysts with increased triglycerides and reduced glucose
135 consumption, markers of poorer potential¹⁷.

136 In addition to metabolite overexposure, maternal obesity in mice induces defects in the
137 mitochondrial phenotype of eggs, including abnormal morphology and cristae structure¹⁸,
138 altered membrane potential and distribution¹⁸ and increased mitochondrial DNA content^{18,19},
139 all markers of disturbed mitochondrial function and energy homeostasis. Oocytes from obese

140 dams also exhibit increased oxidative stress and spindle abnormalities suggesting increased
141 risk of aneuploidy^{18,19}.

142 These mitochondrial defects in oocytes may derive from the elevated lipid content and inherent
143 insulin resistance caused by high maternal adiposity. Oocyte hyperlipidaemia in turn leads to
144 impaired metabolic regulation and endoplasmic reticulum stress **in mice**¹⁶, a condition where
145 proteins misfold during biosynthesis and which contributes to metabolic and cardiovascular
146 disease. Bovine and murine *in vitro* oocyte maturation models demonstrate that elevated fatty
147 acid concentrations perturb follicular physiology, reduce oocyte developmental competence,
148 including altered transcriptome and epigenome profiles in blastocysts, and lead to early
149 embryos with compromised metabolism and lower potential¹².

150 The combination of metabolic, mitochondrial and chromosomal alterations in oocytes and
151 embryos from obese mothers has important implications for subsequent development. In
152 mice, obese mothers have smaller fetuses and pups which develop overgrowth, adiposity and
153 glucose intolerance after birth²⁰. Transfer of mouse blastocysts from obese mothers to normal
154 recipients produces similarly growth-restricted fetuses with associated malformations despite
155 the absence of gestational maternal obesity¹⁸. Similarly, in sheep, female offspring from
156 embryos of obese natural mothers transferred to non-obese mothers exhibit increased
157 adiposity, with dysregulation in liver and muscle insulin signalling and hepatic fatty acid
158 oxidation²¹. These changes are associated with epigenetic perturbations in the liver, including
159 upregulation of microRNAs regulating insulin signalling²¹. Similarly, mouse embryos
160 transferred from diabetic mothers to control recipients exhibit fetal growth retardation and
161 congenital anomalies resembling natural diabetic pregnancies⁸; such structural changes are
162 in keeping with clinical practice, in which pre/periconceptual folic acid supplementation and
163 improved diabetes control reduce the incidence of anomalies.

164 The periconceptual effects of maternal obesity are also apparent in ART pregnancies.
165 Fertility declines with increasing BMI in women receiving donor oocytes, as in non-donated
166 pregnancies, suggesting reduced uterine receptivity²². However, in other studies, recipient
167 BMI had no effect on donor oocyte pregnancy success, whilst **donor** BMI was negatively
168 associated²³, indicating that pre-conception oocyte quality is influenced by maternal adiposity.

169

170 **Periconceptual developmental conditioning through maternal undernutrition**

171 **Human studies**

172 Poor nutrition in utero and low birth weight remain highly prevalent in low and middle income
173 countries and are associated with increased risks of chronic diseases in later life across
174 diverse human populations, particularly if followed by accelerated weight gain during
175 infancy^{1,3}. Similar human cardiometabolic and neurological consequences arise from maternal
176 exposure to famine, e.g. the Dutch Hunger Winter of 1944/45. In human studies it is difficult
177 to pinpoint gestational windows when heightened sensitivity to maternal undernutrition occurs,
178 but the Dutch famine analyses suggest a poorer prognosis for those offspring **conceived**
179 during the famine rather than experiencing it later in gestation²⁴. Similarly, individuals exposed
180 in utero, particularly during the first trimester, to the Chinese Great Famine (1959-61) have
181 increased risk of hypertension in adulthood²⁵. Exposure during the periconceptual period of
182 the Dutch famine is reported to cause epigenetic dysregulation resulting in reduced DNA
183 methylation of the imprinted growth-regulating IGF2 gene persisting into adulthood, along with
184 differential methylation in the regulatory regions of genes affecting growth and metabolism²⁴.

185 In another important human study, dramatic seasonal variation in maternal nutrient
186 consumption in The Gambia affected perinatal outcomes including birth weight, adult health
187 and mortality²⁶. By studying genomic regions where methylation patterns are highly correlated
188 across tissues derived from all three germ lines it has been possible to demonstrate that
189 maternal nutrition at conception alters the epigenome prior to gastrulation, with the effects
190 persisting, at minimum, well into childhood and adolescence²⁷. This periconceptual legacy
191 coincided with seasonal changes in maternal plasma methyl-donor biomarkers which, along
192 with BMI, are also predictive of childhood methylation patterns²⁸. So far, significant deviations
193 in the methylation patterns of loci predictive of immune function, tumour suppression²⁹ and
194 obesity³⁰ have been noted.

195 **Animal models**

196 Animal models have been essential for investigating mechanisms involved in the multistep
197 processes linking periconceptual maternal undernutrition with later-life disease risk. In
198 rodents, feeding a low protein diet (LPD) - specifically during the periconceptual period,
199 either exclusively during the final 3 days of oocyte maturation³¹ or the 3-4 day window of
200 preimplantation embryo development (Emb-LPD)^{32,33}, with normal nutrition at all other times -
201 is sufficient to induce an altered growth trajectory and cardiovascular, metabolic and neuro-
202 behavioural dysfunction in adulthood. Such targeted dietary models commonly show
203 hypertension in adult offspring, coupled with increased adiposity^{7,31-33}. Similar findings have
204 been reported in sheep³⁴.

205 Rodent and sheep models of maternal periconceptional undernutrition suggest that impaired
206 regulation of fetal development may underlie co-morbidities. For example, studies in sheep
207 have shown that the late gestation fetal cardiovascular response to hypoglycaemia is modified
208 by prior peri-implantation undernutrition³⁵. Moreover, peri-implantation and late gestation
209 maternal undernutrition affect fetal sheep skeletal muscle development differentially³⁶, and
210 maternal undernutrition in early gestation alters gestation length and fetal and postnatal
211 growth³⁷.

212 **Induction and response mechanisms**

213 The mouse embryonic period low protein diet (Emb-LPD) model has helped reveal how
214 periconceptional maternal undernutrition may initiate adverse effects during early
215 embryogenesis⁷. Emb-LPD reduces circulating maternal insulin and amino acid
216 concentrations, including reduced branched-chain amino acids (BCAAs) within the uterine
217 luminal fluid that bathes early embryos before implantation³⁸. BCAAs act as targets for embryo
218 nutrient sensors, enabling nutrient status to be sensed by blastocysts via the mammalian
219 target of rapamycin complex 1 (mTORC1) growth-regulating signalling pathway, inducing an
220 altered growth trajectory from before implantation³⁸ (see below), and shown by embryo
221 transfer to be induced within the blastocyst³³. Altered induction by Emb-LPD in mice activates
222 compensatory responses that are distinct between extra-embryonic (trophectoderm; primitive
223 endoderm) and embryonic (epiblast) lineages of the blastocyst (**Figure 1**). The Emb-LPD
224 trophoctoderm becomes more proliferative, adopts a more invasive migratory phenotype at
225 implantation, and activates increased endocytosis of maternal uterine luminal fluid proteins as
226 an alternative source of nutrients, leading to a placenta that is more efficient in nutrient transfer
227 to the fetus³⁸⁻⁴⁰. Similarly, the primitive endoderm activates compensatory responses to
228 enhance nutrient delivery via the yolk sac placenta, mediated through epigenetic
229 mechanisms^{40,41}.

230 In response to Emb-LPD, changes in embryonic lineages may help set the embryonic and
231 fetal growth trajectory to match prevailing nutrient availability. The embryonic lineages utilise
232 preimplantation nutrient sensing to regulate growth across somatic organs (e.g., liver and
233 kidney) through adaptations in the rate of ribosome biogenesis⁴². In essence, rRNA expression
234 is suppressed during periods of maternal dietary restriction but is increased, beyond that of
235 the control rate, when the dietary challenge is removed. This mechanism modulates the level
236 of DNA methylation at the rDNA promoter, thereby mediating RNA polymerase I interaction
237 with the promoter to regulate ribosome biogenesis and growth^{42,43}. Interestingly, rDNA has
238 also been found to be a genomic target for growth regulation in models of maternal high-fat or

239 obesogenic diets⁴³. This exquisite lifetime mechanism, activated in the preimplantation
240 embryo, is likely to be responsive to uterine luminal fluid nutrient concentrations and appears
241 to utilise a nutrient-sensing ribosome factor, Rrn3, to mediate the rDNA responses⁴². The
242 growth-regulating role of the embryonic lineages is critical since perinatal weight associates
243 with adult disease risk³³.

244

245

246 **Paternal origin of periconceptual developmental programming**

247 Whilst the connection between a mother's diet and the long-term health of her offspring has
248 been studied in detail, our understanding of how a father's diet impacts his offspring remains
249 limited. However, links are now emerging between paternal lifestyle, sperm quality and
250 impaired offspring health⁹. Here, both direct (sperm quality, epigenetic status, DNA integrity)
251 and indirect (seminal fluid composition) paternal mechanisms have been identified, with the
252 potential to affect **mouse** offspring development across multiple generations⁴⁴.

253 Mirroring female reproductive fitness, male fertility is closely linked to nutrition and body
254 composition. In humans and rodents, elevated BMI is associated with reduced sperm
255 motility⁴⁵, increased sperm abnormality⁴⁶, increased sperm reactive oxygen species levels,
256 reduced serum testosterone and increased oestradiol concentrations⁴⁷. Consumption of a
257 'Western-style' diet high in sugar, fat and processed food associates with reduced sperm
258 motility in men⁴⁸, while consumption of energy-dense diets in men and rodents is associated
259 with poor sperm motility, morphology and DNA integrity⁴⁹. Reduced sperm DNA integrity, as
260 occurs in obesity and diabetes, correlates with reduced **human** embryonic development and
261 decreased pregnancy rates⁵⁰. In men undergoing IVF treatment, obesity is associated with
262 reduced blastocyst development and live birth rates⁵¹. In rodents, paternal obesity induced by
263 high-fat diet increases sperm DNA damage⁵², reduces blastocyst development and
264 implantation rates⁵³ and causes sub-fertility in male and female offspring for up to two
265 generations⁵⁴. Interestingly, these negative effects on offspring development can be prevented
266 through paternal dietary and exercise interventions **in mice**⁵⁵, indicating that sperm-mediated
267 effects may be transient and even reversible. In rats, a paternal high-fat diet for 10 weeks
268 before mating affected female (but not male) offspring pancreatic β -cell function and increased
269 body weight, glucose intolerance and impaired insulin secretion⁵⁶. Offspring of male mice over-
270 nourished during neonatal life demonstrate glucose intolerance, fasting hyperglycaemia and
271 insulin resistance, mirroring the metabolic disturbance seen in their fathers⁵⁷.

272 Similar to the impacts of paternal obesity, paternal LPD in mice induces the expression of
273 genes involved in offspring hepatic lipid and cholesterol biosynthesis⁵⁸. Analysis of offspring
274 hepatic epigenetic status revealed genome-wide changes in DNA methylation, including the
275 key lipid regulator *PPARα*. In adulthood, offspring from male mice fed LPD have higher birth
276 weight, a reduced male:female offspring ratio, increased adult adiposity, hypotension, glucose
277 intolerance and elevated serum TNF-α levels⁵⁹. Furthermore, paternal LPD also affects
278 blastocyst *AMPK* gene expression, placental size, fetal growth and skeletal development⁶⁰.

279 As for maternal periconceptional nutrition models, epigenetic mechanisms are likely mediators
280 of effects of paternal phenotype and exposures on offspring development⁶¹. Changes in
281 patterns of sperm histone modifications (methylation, acetylation), DNA methylation and/or
282 RNA content are prime candidates for such paternal periconceptional programming. Sperm
283 from infertile men display significant changes in histone populations⁶², with enrichment of
284 active histone marks (i.e. H3K27me3) at key developmental and pluripotency genes in human
285 and mouse sperm⁶². Studies have also revealed that sperm-derived histones are transferred
286 into the oocyte and incorporate into zygotic chromatin following **human** fertilisation⁶³. However,
287 whether any of the 2-15% histones retained within the mammalian sperm contribute directly
288 to zygotic gene expression regulation is unknown. Human sperm also contain several
289 thousand coding RNA transcripts⁶⁴ and altered expression is linked with infertility⁶⁵. Recent
290 studies have shown that levels of sperm tRNA-derived small RNAs (tsRNAs) are altered by
291 paternal diet in mice⁶⁶. Interestingly, offspring generated by injecting zygotes with sperm
292 tsRNA taken from male mice fed a HFD showed impaired glucose tolerance and insulin
293 secretion⁶⁶. While such studies highlight the role of RNA populations in intergenerational
294 programming⁶⁷, the significance of these sperm-derived RNA molecules remains to be
295 elucidated.

296 Apart from sperm-specific mechanisms of developmental programming, seminal plasma
297 composition, (e.g. granulocyte-macrophage colony-stimulating factor) influences **mouse**
298 embryonic, placental and offspring development⁶⁸ and initiates maternal reproductive tract
299 immunological responses, essential in the establishment and maintenance of **human**
300 pregnancy⁶⁹. In mice, paternal seminal fluid impacts on the maternal uterine environment,
301 altering blastocyst development, placental size and adult offspring glucose tolerance,
302 adiposity and blood pressure⁷⁰.

303

304 **Defining the parental contribution to periconceptional developmental effects**

305 Shared maternal and paternal dietary and other lifestyle influences may potentially combine
306 for greater impact on periconceptional development. However, most research models to date
307 are uniparental in design and the combined effects of both parents are unknown. Whether the
308 impact of poor paternal diet on offspring development and wellbeing is of equivalent
309 significance to that of poor maternal diet is also unknown. As a first step, Box 2 and Figure 3
310 show a meta-analysis of our mouse maternal and paternal LPD diet models using published
311 data for offspring weight at birth, adult systolic blood pressure (SBP) and adult heart:body
312 weight ratio (a measure of heart capacity) including datasets covering maternal intervention
313 restricted to the periods of oocyte maturation, preimplantation development or the entirety of
314 gestation^{31,33,59}. The use of the same robust, statistical random effects regression analysis
315 across each of these studies strengthens our comparison of parental effects in the current
316 analysis. However, such rigorous statistical approaches are rarely adopted, especially in
317 animal model studies, and so we have restricted our analysis to data from these three studies
318 alone. Offspring birth weight was increased in response to maternal LPD during the terminal
319 stages of oocyte development (Egg-LPD) and during preimplantation development (Emb-LPD) (**Figure 3a**). Overall, the pooled estimate demonstrated parental
320 LPD increased offspring birth weight. Our second analysis explored the impact of parental
321 LPD on adult offspring SBP. Here, all maternal challenges resulted in offspring hypertension
322 (**Figure 3b**), while paternal LPD resulted in a trend towards lower blood pressure in the adult
323 offspring. Our final analysis examined the impact of parental diet on adult heart:body weight
324 ratio (**Figure 3c**). Only paternal LPD had a significant effect, reducing offspring heart:body
325 weight ratio. These new data demonstrate differential effects from paternal and maternal
326 periconceptional developmental exposures on offspring phenotype. It is essential that further
327 studies define the precise impacts and underlying mechanisms by which parental diet regimes
328 affect offspring development and wellbeing. Studies examining concurrent paternal and
329 maternal interventions on shared offspring outcomes are also warranted.
330

Box 2: Analysis of parental contribution effect

- Data for offspring phenotype were taken from Watkins et al 2008a³¹, 2008b³³ and 2014⁵⁹. 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 ($\Delta = \mu_1 - \mu_2$) 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; $P = 0.0035$).

332 **Periconceptual developmental programming and ART**

333 Direct evidence for human periconceptual effects comes from assisted reproductive
334 treatments (ART) in which mature gametes and the preimplantation embryo are exposed to
335 precisely timed in vitro manipulations. Several million apparently healthy ART children have
336 now been born worldwide, but relatively little is known about the possible impact of the
337 technology-associated exposures during conception and very early development on their
338 health status during childhood and later life. The spectrum of human demographic
339 confounders (including parental infertility), changes and improvements in ART techniques
340 over time, and the relative sample sizes used make analyses complex and the reported
341 outcomes need to be interpreted with caution. Nevertheless, it is well established that
342 singleton ART pregnancies have increased risk of low birth weight, congenital abnormalities
343 and higher mortality rate, although disentangling confounding by parental infertility is difficult⁷¹.
344 Human embryo culture media have changed over time and the predominant current practice
345 is to use commercially sourced media of proprietary (unspecified) composition (discussed
346 in¹²). Comparison of perinatal outcome following use of different commercial media, including
347 a multicentre randomised controlled trial, has indicated that birth weight is significantly
348 affected⁷², with effects on growth still manifest at age 2 years⁷³.

349 Compared with naturally conceived offspring, the cardiovascular phenotype of IVF children
350 and adolescents reveals increased risk of high blood pressure^{11,74}, vascular dysfunction with
351 abnormal blood flow and vessel thickness⁷⁵ and evidence of cardiovascular remodelling during
352 development *in utero* affecting heart shape and chamber size⁷⁴. Metabolic consequences
353 include increased fasting glucose and peripheral insulin resistance^{11,76}, raised plasma lipids,
354 and obesity⁷⁶. A systematic review found no difference in cognitive outcomes among children
355 conceived with conventional IVF and those conceived naturally, but did identify conflicting
356 findings that require clarification among studies of children conceived with intracytoplasmic
357 sperm injection⁷⁷.

358 Collectively, current evidence suggests that ART, like the in vivo nutritional models discussed
359 above, may alter the development and growth trajectory of human embryos, and increase the
360 risk of postnatal chronic cardiometabolic dysfunction. This legacy is unlikely to be due to
361 parental infertility in isolation since controls in some studies comprise those naturally
362 conceived offspring from sub-fertile parents^{11,75}. Moreover, ART animal models demonstrate
363 similar long-term consequences to human studies, despite normal parental fertility⁷⁸. Thus,
364 IVF embryo culture and transfer in mice results in offspring with altered growth trajectory,
365 relative hypertension, cardiovascular abnormalities and glucose/insulin dysfunction⁷⁸.

366 ART-associated adverse effects on long-term health appear to have an epigenetic origin
367 induced during the periconceptual period. ART children have an increased risk of rare
368 imprinting disorders associated with DNA methylation errors on imprinted genes⁷⁹ and
369 aberrant methylation of imprinted *H19* gene has been reported in human cultured embryos⁸⁰.
370 In mouse models, embryo culture may cause imprinted genes to lose their allele-specific
371 expression (particularly at the growth regulating *H19/IGF2* locus) together with aberrant
372 methylation patterning in embryos, placental and fetal tissues⁸¹. ART-induced aberrant
373 epigenetic profiles may also be propagated during human pregnancy in fetal and placental
374 tissues and persist into childhood affecting genes regulating growth such as the *IGF2/H19*
375 locus⁸². Media composition, particularly albumin or serum components or ammonium ion
376 accumulation from amino acid catabolism, may contribute to altered mouse epigenetic
377 status⁸³. Importantly, even a very limited culture period is sufficient to induce epigenetic
378 changes⁸¹. Embryo culture exposure also modifies expression and methylation of non-
379 imprinted genes in mice and alters expression of DNA methyltransferases⁸⁴. For example, in
380 mouse models ART affects the endothelial nitric oxide synthase (*eNOS*) gene implicated in
381 vascular dysfunction and modification of culture media composition may prevent this effect⁸⁵.
382 Although provocative, more studies in both animal models and humans are required in order
383 to replicate findings to date.

384

385 **Diversity and commonality in periconceptual effects**

386 The evidence reviewed above reveals that periconceptual experience can induce lifelong
387 changes in phenotype, affecting disease risk. Beyond these nutritional and ART conditions,
388 studies in rodents show broader examples of periconceptual effects, such as from maternal
389 stress⁸⁶. Moreover, maternal alcohol consumption exclusively around conception induced
390 metabolic dysfunction in rat adult offspring with evidence of epigenetic disturbance⁸⁷. In the
391 case of mouse maternal systemic inflammation at conception, whilst not affecting
392 cardiometabolic health, suppressed adult offspring innate immunity after challenge, possibly
393 to protect 'self' in a predicted pathogenic postnatal environment⁸⁸. In addition, mouse embryo
394 transfer experiments suggest that advanced maternal age may adversely affect offspring
395 cardiometabolic health⁸⁹, but the mechanisms underlying this age-associated effect are
396 unknown.

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

400 developmental plasticity, may confer advantage when the offspring are exposed to a similar
401 environment postnatally. During the periconceptual period there is rapid and radical
402 molecular, cellular and morphogenetic restructuring; the signalling pathways that control these
403 processes are sensitive to multiple molecules and other factors within the cellular environment
404 and may provide a mechanistic underpinning for this concept⁹⁰. However, as we have
405 described, the periconceptual setting of metabolic homeostasis may become maladaptive if
406 conditions change or if nutrient levels induce perturbations in metabolism, generating the
407 circumstances underlying adverse health risk. A consistent mechanism identified across
408 conditions and species has been epigenetic variation, a plausible pathway to 'biological
409 embedding' of early life exposures and transmission of phenotypic effects throughout life. This
410 has been demonstrated directly through manipulation of maternal one-carbon (1-C)
411 metabolism during early embryogenesis, potentially reducing the availability of methyl donor
412 groups necessary for DNA and histone methylation⁹¹, but such epigenetic changes are not
413 necessarily linked directly with changes in gene expression⁹². Thus, a periconceptual
414 maternal diet deficient in 1-C metabolite substrates and cofactors (vitamin B₁₂, folate,
415 methionine) in sheep modified offspring DNA methylation and led to adverse cardiometabolic
416 and immune dysfunction⁹³. Similarly, folate addition to rodent maternal LPD can rescue normal
417 expression and DNA methylation of metabolic regulators in offspring which underlie
418 cardiovascular dysfunction⁹⁴. A mouse paternal low folate diet altered sperm DNA methylation
419 profile, changed the placental transcriptome and resulted in offspring with craniofacial and
420 musculoskeletal malformations⁹⁵. Moreover, the negative impact of mouse paternal
421 undernutrition on sperm quality, testicular oxidative stress, fertility and offspring fat
422 accumulation and dyslipidaemia are reversed through vitamin and antioxidant
423 supplementation⁹⁶. As with ART, additional studies are warranted to define the critical
424 window(s) and pathways linking perinatal one-carbon metabolism, epigenetic variation and
425 programming of later offspring health.

426

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

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

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

436 This evidence calls for a major re-examination of public health policy to protect against future
437 disease risk through societal advice on, and greater provision of, preconception care⁹⁷ as also
438 promoted in the two accompanying reviews in this series (Stephenson et al, submitted; Barker
439 et al, submitted). Whilst a preconception focus on parental risk factors such as smoking and
440 excess alcohol intake is wise and well established, new drives to prepare nutritionally for
441 pregnancy are critical, including healthy body composition, physical activity and diet for both
442 parents⁹⁸. Further definition of the underlying epigenetic, cellular, metabolic and/or
443 physiological mechanisms and the exposures that drive them, is an important research
444 agenda that is pivotal to the characterisation of more specific recommendations for
445 preconception health.

446

447

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470

471 **Contributors**

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473 Godfrey. All authors provided input into the manuscript and approved the final version of the
474 manuscript.

475

476 **Figure legends**

477 **Figure 1. Biological events underpinning periconceptual conditioning**

478 The periconceptual period is one of extensive cellular change comprising the completion of
479 meiotic maturation of oocytes, differentiation of spermatozoa, fertilisation and resumption of
480 mitotic cell cycles in the zygote, marking the transition from parental to embryonic genomes⁴
481 and the onset of morphogenesis⁵. Periconceptual biology is indeed 'busy' – the
482 morphological and cellular changes occurring during the switch from parental to embryonic
483 generations leading to blastocyst formation are driven by pronounced sub-cellular and
484 molecular processes. These include global restructuring of the epigenome (mainly DNA
485 methylation and histone modifications that control gene expression), such that expression
486 from the new embryonic genome is distinct from the parental genomes⁹⁹. Epigenetic
487 reorganisation allows the embryo to first exhibit *totipotency*, a naïve cellular state conferring
488 the ability to construct both true embryonic (future fetal) cell lineages and the extra-
489 embryonic (placental) lineages that become evident in the blastocyst. Subsequently,
490 epigenetic modifications underpin embryo *pluripotency*, the capacity to generate all three
491 germ layers (ectoderm, mesoderm, endoderm) once gastrulation has taken place.
492 Morphogenesis of the blastocyst is followed by embryo hatching from the zona pellucida
493 coat and implantation mediated through adhesion of the outer trophoderm layer of the
494 blastocyst to the uterine endometrium and subsequent invasion and decidualisation.
495 Activation of the new embryonic genome before implantation not only permits de novo gene
496 expression distinct from parental genomes but also involves establishment of the embryo's
497 metabolism that matures over time¹⁰⁰.

498

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

502

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

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

508 with offspring from normal protein diet (NPD; 18% casein) fed parents. Analysis of 4 studies
509 involving female MF1 mice being fed LPD exclusively during the terminal stages of oocyte
510 maturation (3.5 days prior to mating; Egg-LPD), exclusively during preimplantation embryo
511 development (Emb-LPD) or throughout gestation (LPD). Forest plots also include offspring
512 data in response to a paternal low protein (Pat-LPD) fed to C57BL6 males prior to mating.
513 For Egg-NPD n = 189–80 from 19 litters; Egg-LPD n = 201-67 from 19 litters; NPD n = 131-
514 76 from 19 litters; LPD n = 116-85 from 19 litters; Emb-LPD n = 134-78 from 19 litters; Pat-
515 NPD n = 85-76 from 16 litters; Pat-LPD n = 73-62 from 16 litters. **A.** Plots present differences
516 between means (\pm 95% CI) of birth weight (grams) to study specific NPD group. Data
517 combining all LPD and all NPD treatment groups is used to determine the Pooled Estimate.
518 Heterogeneity (χ^2) between studies = 1.96 (3 df), I^2 = 33%. **B.** Plots present differences
519 between means (\pm 95% CI) of adult SBP (mmHg) to study specific NPD group. Data
520 combining all LPD and all NPD treatment groups is used to determine the Pooled Estimate.
521 Heterogeneity (χ^2) between studies = 1.05 (4 df), I^2 = 39%. **C.** Plots present differences
522 between means (\pm 95% CI) of heart:body weight ratio to study specific NPD group. Data
523 combining all LPD and all NPD treatment groups is used to determine the Pooled Estimate.
524 heterogeneity (χ^2) between studies = 1.86 (3 df), I^2 = 61%.

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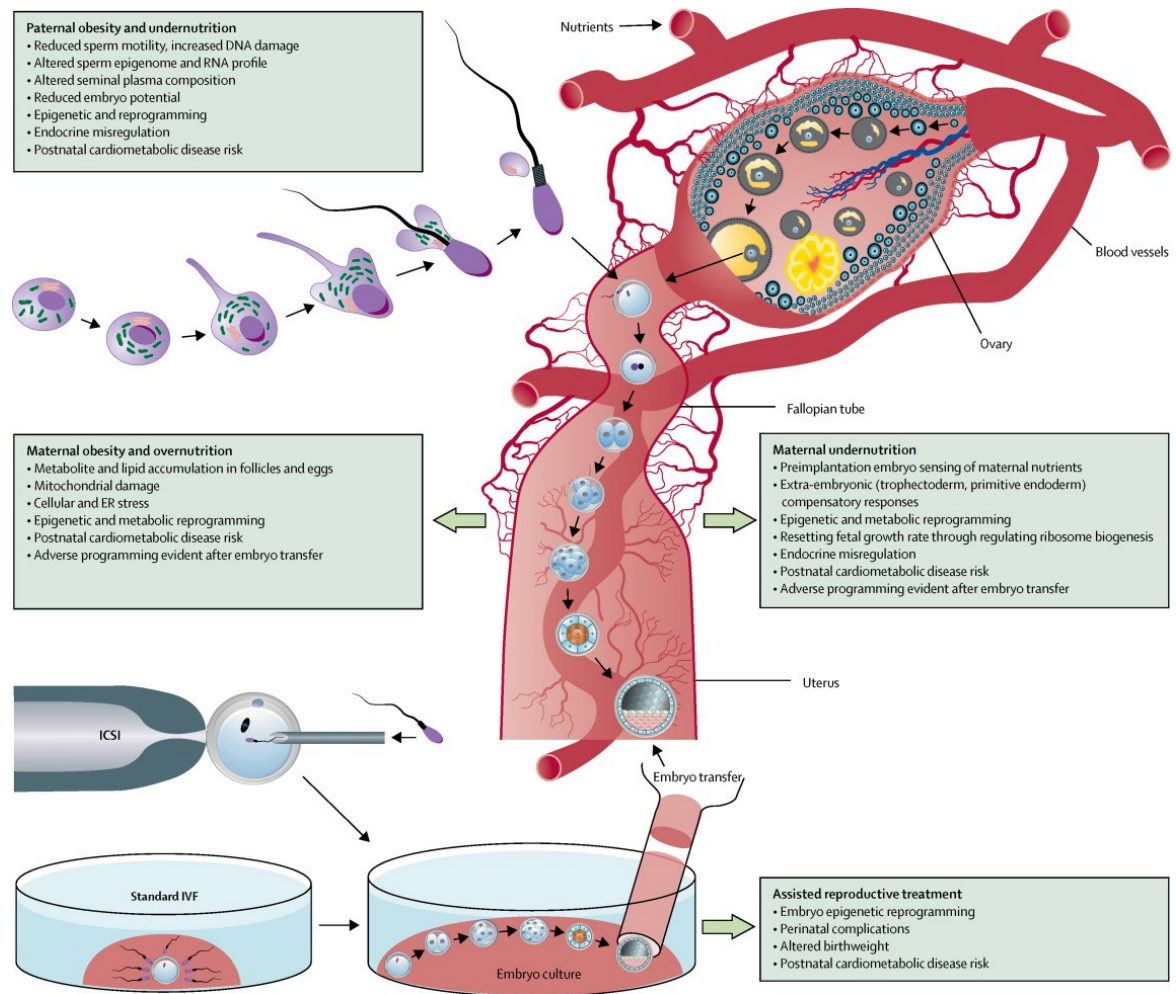
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823 **Figure 1**

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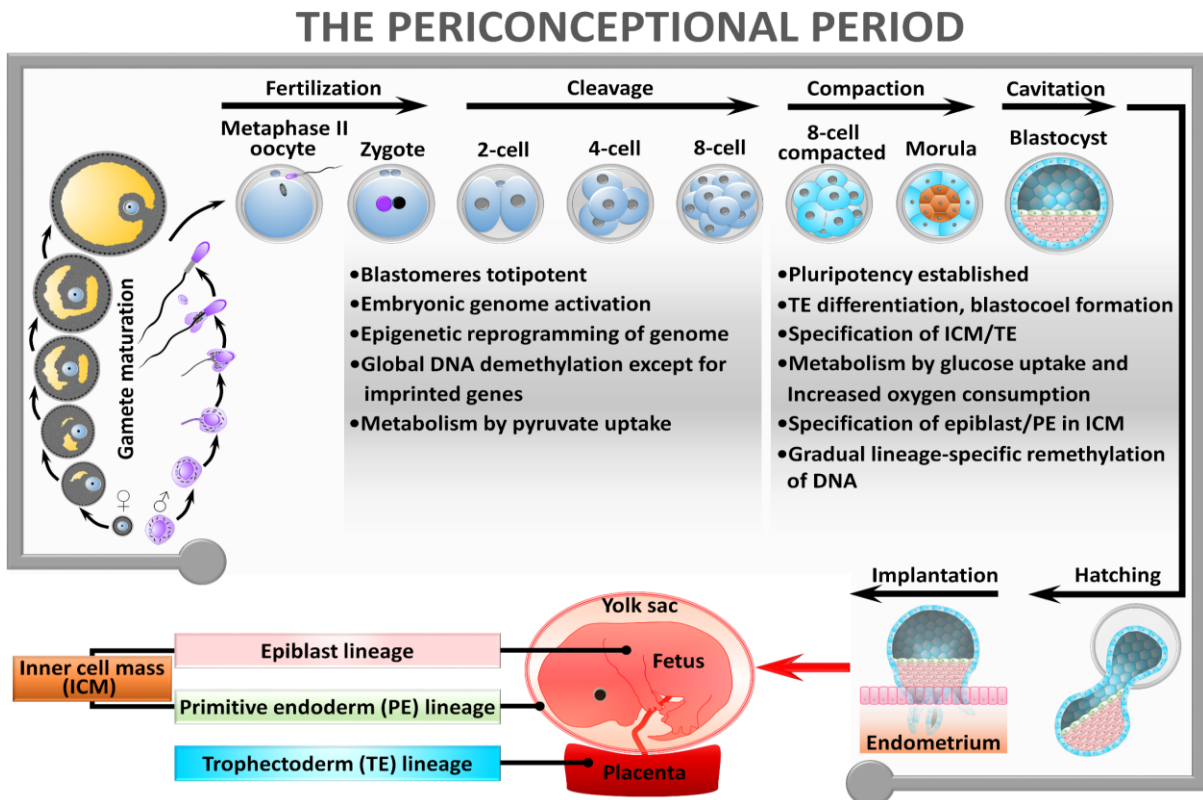
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842 Figure 3

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