Sex differences in the intergenerational inheritance of metabolic traits

Ionel Sandovici[1,2,3,4], Denise S. Fernandez-Twinn[1,4], Antonia Hufnagel[1,4], Miguel Constância[1,2,3,✉] and Susan E. Ozanne[1,3,✉]

[1]Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK.

[2]Department of Obstetrics and Gynaecology and National Institute for Health Research Cambridge Biomedical Research Centre, Cambridge, UK.

[3]Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK.

[4]These authors contributed equally.

✉e-mail: jmasmc2@cam.ac.uk; seo10@cam.ac.uk

Abstract

Strong evidence suggests that early life exposures to suboptimal environmental factors, including those *in utero,* influence our long-term metabolic health. This has been termed developmental programming. Mounting evidence suggests that the growth and metabolism of male and female fetuses differ. Therefore, sexual dimorphism in response to pre-conception or early life exposures could contribute to known sex-differences in susceptibility to poor metabolic health in adulthood. However, until recently, many studies, especially those in animal models, focussed on a single sex, or, often in the case of studies performed during intrauterine development, did not report the sex of the animal at all. In this review we (a) summarise the evidence that male and females respond differently to a suboptimal pre-conceptional or *in utero* environment, (b) explore the potential biological mechanisms that underlie these differences and (c) review the consequences of these differences for long-term metabolic health, including that of subsequent generations.

Main

Epidemiological data suggest that there is sex disparity in the prevalence of metabolic disorders[1]. For example, type 2 diabetes prevalence is higher in men (IDF Diabetes Atlas, 6th edition), whereas obesity prevalence is higher in women[2]. These may be determined at sexual maturity, where differences in sex hormones could interact differentially with metabolic pathways[1]**.** However**,** growing evidence suggests that differences in metabolic disease risk are established much earlier in life. Although genetic and current environmental factors (including diet and physical activity) influence our metabolic health, our risk of becoming obese and developing type 2 diabetes is also primed by exposure to adverse conditions early in life including *in utero*. This phenomenon, termed "developmental programming" or the Developmental Origins of Health and Disease (DOHaD) has been widely documented following a range of suboptimal *in utero* exposures including those that are nutritional, hormonal, immunological or pollutant and toxicants such as alcohol or drugs[3]. In addition to *in utero* exposures, there is evidence that a variety of maternal and paternal pre-conceptional exposures and stimuli, affecting oocyte[4] and sperm[5,6], can impact on male and female offspring health differentially (Fig. 1). In both gametes, there is evidence that changes in epigenetic marks may mediate these outcomes[7,8]. Although under-explored, there is therefore growing evidence for a dichotomy in the incidence, timing and/or severity of metabolic disorders resulting from suboptimal prenatal exposures dependent on offspring sex, with males seemingly at higher risk of disease[9]. The origins of this disparity remain poorly understood and hence here we review the evidence for sex differences in intergenerational inheritance (Box 1) of metabolic traits, the underlying biological mechanisms, and highlight the implications of this when exploring strategies for maximizing population health.

Evidence for dimorphic vulnerabilities during development

Epidemiological studies

Maternal factors that program the health of the offspring *in utero* include maternal stress, smoking, infections and under- and over-nutrition – all manifesting in increased offspring risk of cardiovascular and metabolic diseases in later life. Early studies focussed mainly on the effect of undernutrition during pregnancy, with epidemiological studies assessing the effect of famine during pregnancy[10]. In 1991, Hales, Barker and colleagues surveyed the birth records of men born in Hertfordshire between 1920 and 1930, and showed that low weight at birth was associated with an increased risk of impaired glucose tolerance in later life[11].

Since these initial studies, the relationship between birthweight and metabolic diseases has been reproduced in populations worldwide[12], with potential sex-specific effects being reported as early as 1999[13]. Pregnant women exposed to undernutrition during the Dutch Famine delivered babies with low birthweight compared to those born the year before or after the famine. Female offspring exposed *in utero* to the famine had increased BMI (body mass index) and waist circumference at 50 years of age, an association not found in male offspring.

With the rise in obesity prevalence in women of reproductive age, worldwide, recent research has also highlighted the effects of maternal obesity and gestational diabetes, some of which are sexually dimorphic. Pre-conceptional maternal BMI in the Rotterdam Preconception Cohort influenced first trimester embryonic growth of female embryos only, with female embryos of underweight mothers showing accelerated growth and development, whereas those of obese mothers grew more slowly[14]. However, in studies of long-term outcomes following development in an obesogenic environment, male offspring are often more affected. A study assessing food quality during pregnancy via the Health Eating Index (HEI)-2010, showed that in male offspring, glucose, insulin and adiponectin levels at age 4-7 were increased if the HEI of the mother's diet was high, indicating an unhealthy diet. However, this association was not present in girls at the same age[15]. Overall, human observations show that female offspring are more likely to develop adiposity in response to maternal

undernutrition[16]. In contrast, maternal overnutrition is more likely to increase adiposity risk in the male offspring[17].

In recent years, paternal health at the time of conception has been demonstrated to affect embryo development, fetal growth and long-term offspring health[18], with some effects sexually dimorphic (e.g. smoking in the father at a young age was associated with increased BMI at 9 years of age in their sons, but not their daughters[19]). The examples presented so far show intergenerational inheritance, with traits passed on from one generation to the next. Data regarding sexual dimorphism with transgenerational transmission is limited.

Sex dimorphic effects of ART on metabolism

Assisted reproductive technologies (ART) have been shown recently to influence short- and long-term offspring health. In Western countries, more than 1% of live childbirths are conceived by ART[20]. One common ART is *in vitro* fertilization (IVF), in which oocytes are retrieved from the woman after superovulation, fertilized *in vitro*, cultured to blastocyst stage, then transferred to the recipient womb. Babies conceived via ART generally have lower birthweight compared to naturally conceived babies[21]. Separating the different IVF steps showed that all procedures (embryo transfer, superovulation and embryo culture) independently and cumulatively increased placental abnormalities[22], with embryo culture being a major contributor[23]. Abnormalities included impaired vascularisation, which may explain lower birthweight.

While data surrounding the effects of ART on offspring health are scarce, long-term studies identified an increased, sex-dependent risk of developing metabolic diseases[24]. Belva et al.[25] showed that girls conceived via intracytoplasmic sperm injection (ICSI) had increased adiposity at the age of 14. A similar increase in adiposity was observed only in boys with advanced pubertal development, highlighting a delayed onset of these adverse outcomes in boys compared to girls[25]. Similar findings were reported in mice: impaired glucose tolerance was observed only in female offspring conceived by IVF and ICSI[26].

Effect of sex on the outcome of complicated pregnancies

It has long been known that the human male fetus is more vulnerable to a suboptimal environment *in utero* than females[27]. Male fetuses have a higher growth rate compared to female fetuses and a larger placenta to support growth, at the expense of a lower capacity to buffer adverse *in utero* environments[27,28]. This is supported by meta-analyses of human pregnancy studies showing that boys are more likely to be delivered preterm and have an increased perinatal mortality[29].

Sexual dimorphism likely also plays a role in the adaptation of maternal physiology to pregnancy. Increased uterine artery resistance, indicative of impaired adaptation of the maternal uterine vasculature, is frequently associated with a male fetus[30]. Furthermore, pregnancy complications, such as gestational diabetes and late onset pre-eclampsia are more frequent in pregnancies with a male fetus[31].

Evidence for sexual dimorphism in animal studies

Animal models are invaluable for elucidating mechanisms underlying sexual dimorphism in response to a suboptimal *in utero* environment. Rodent models have been particularly helpful due to their high litter size, and the benefit of both male and female offspring being exposed to the same maternal milieu *in utero*[32]. Mouse studies have shown sexually dimorphic programming effects on fetal development, with both maternal and paternal diet affecting female placental gene expression more than male[33,34]. Conversely, long-term follow up studies highlight significantly more long-term effects of maternal high-fat feeding on male mouse offspring metabolism[35]. Table 1 summarizes a large cross-section of the evidence from human and animal studies supporting sexual dimorphic and programming effects on metabolism.

Mechanisms leading to intergenerational sex differences

Sex-specific effects on intergenerational programming of metabolic traits are the outcome of mechanisms that operate at different time-windows throughout organismal development, as well as at several levels of biological complexity, from molecules to subcellular organelles, organs, or even the entire organism, with significant crosstalk between these processes.

Effects mediated by sex chromosomes and sex hormones

Although the primary role of sex chromosomes is to determine sexual differentiation of gonads and sex steroid hormones, they also lead to sex differences that are independent and precede the actions of sex steroid hormones.

1. Sex differences due to effects of sex chromosomes

Recent evidence obtained in bulls suggests that X and Y-chromosome bearing spermatozoa, although similar morphologically, have subtly different proteome content. X spermatozoa have higher levels of GAPDH and LDHA, both involved in glucose metabolism, while Y spermatozoa have higher expression of GSTM3 which is implicated in glutathione metabolism[36] (Fig. 2a). At fertilization, the combination of the sex chromosomes establishes biological sex, typically XX for females and XY for males. In females, most (but not all) X-linked genes, undergo X chromosome inactivation (XCI)[37]. Sex-chromosome determined effects, arising prior to the formation of gonads, include differences in the dosage of genes escaping XCI[38] (Fig. 2b), skewed XCI (leading to a larger number of cells expressing either the paternal or maternal copy of a given gene subject to XCI)[39] and the expression of chromosome Y-linked genes in men[37].

To distinguish the contributions of gonadal sex versus sex chromosomes in adiposity risk, Link et al. exposed their mouse models of XX and XY mice with ovaries and XX and XY mice with testes to a high-fat diet[65]. They found that an XX chromosome complement accelerates dietary fat-induced weight gain in mice with either male or female gonads. They then demonstrated that the dosage of the *Kdm5c* gene, an X-chromosome gene encoding a histone demethylase that escapes X-

inactivation, is a determinant of the X chromosome effect on adiposity, by showing that hemizygous *Kdm5c*$^{+/-}$ mice accumulate less fat mass after high-fat feeding, compared to *Kdm5c*$^{+/+}$ mice[40].

2. Sex differences due to effects of sex hormones

After the gonads are formed and functional, sex hormones act as an additional mechanism by which sex-dependent effects on metabolic traits occur (Fig. 2c and recently reviewed[1,41]). Sexual dimorphism in key metabolic organs, leading to lower cardio-metabolic disease risk in premenopausal women compared to men of the same age is well documented[1,41]. A significant fraction of all autosomal genes shows sex-biased gene expression, although in humans these effects are mostly small[42]. These patterns vary greatly across human tissues, with a larger proportion of sex-differentially expressed (DE) genes being active in tissues with key roles in metabolism, such as skeletal muscle, liver and adipose tissue[43,44] (Fig. 2d).

In mice, some of these tissue-specific sex-related gene expression differences are substantial and particularly relevant for dimorphic metabolic traits, either under metabolic resting state, or when exposed to nutritional stressors. For example, the preferential use of glycolysis or fatty acid β-oxidation for energy production in rodent skeletal muscle was linked to the differential expression of *Pfkfb3* (male enriched) and *Pdk4* (female enriched)[45]. *Lyplal1*, a gene expressed at significantly higher levels in female adipose tissue, suppresses adiposity gain in response to a high-fat/high-sugar diet[46]. In contrast, *Lnc2*, encoding lipocalin 2, is expressed at significantly lower levels in female adipose tissue. In total *Lcn2* knockouts, adenoviral expression of *Lcn2* in white adipocytes leads to metabolic disturbances only in females[47]. In many species, sex-biased hepatic gene expression is controlled by pituitary GH secretion patterns, which is in turn regulated by the hypothalamus in a sex-dependent manner[48]. In livers of mice, GWAS of chromatin states revealed distinct mechanisms of sex-dependent gene regulation, with prominent roles attributed to FOXA pioneer factors, proposed to confer sex-dependent chromatin opening, and STAT5, which regulates sex-biased genes by binding to distal enhancers in a sex-biased chromatin state[49,50].

179 In addition to sex differences in gene expression, sex-biased alternative mRNA splicing plays a role in
180 key metabolic tissues such as liver[51] and skeletal muscle[52] (Fig. 2d). Even though only approximately a
181 third of all genes with sex-DE and sex-biased splicing associate with hormone response elements[53],
182 sex hormones may potentially impose another layer of regulation (Fig. 2d), via secondary effects.
183 Another link between sex hormones and sexually dimorphic metabolism is the gut microbiome,
184 which differs between males and females. In mice, male castration changes gut microbiome to a
185 female-like profile[54]. Moreover, glutamine levels and glutamine/glutamate (Gln/Glu) ratios are lower
186 in male mice and regulated by androgen and partially by the gut microbiome (Fig. 2e), which may
187 contribute to sex differences in glucose metabolism[54]. This study[54] provided evidence for a lower
188 Gln/Glu ratio in men compared to females, correlated with worsened glucose metabolism. Gut
189 microbiota also influence bile acid metabolism and the enterohepatic recirculation of estrogen and
190 androgens, providing additional mechanisms for regulating metabolism[55]. The intergenerational
191 effects of maternal high-fat diet feeding in mice suggests that programmed sex-specific changes in
192 offspring gut microbiota are coincident with weight gain, liver steatosis and a pro-inflammatory state
193 in males[56].
194
195 **Sex differences related to epigenetic mechanisms**
196 Evidence suggests that epigenetic mechanisms play a key role in programming metabolic traits
197 throughout development (Fig. 3). In each generation, the epigenome undergoes two major waves of
198 reprogramming, one after fertilization and the other one during germline development[57].
199 Reprogramming is defined here as the erasure of epigenetic marks at the genome-wide level, which
200 leads to extensive chromatin remodelling. However, some of the epigenetic marks can resist these
201 reprogramming events, providing a mechanism for the establishment of sex-related differences in
202 the intergenerational inheritance of metabolic traits. In this section, we first present the major
203 features of epigenetic reprogramming, and then review current evidence linking these events to sex
204 differences in metabolic traits.

1. Marks escaping post-fertilization reprogramming

The genome of mature oocytes is relatively DNA hypomethylated compared to that of somatic cells, with a notable exception for active gene bodies, which are highly methylated[58], and 22% of their genome is occupied by broad H3K4me3 domains, inversely correlated with DNA methylation[59]. Sperm DNA is hypermethylated and protamines replace the histones, condensing it into a genetically inactive state, outliers from these rules being promoters, putative intra- and intergenic enhancers, regions harbouring imprinted genes and clusters of microRNAs, which retain histones and associate with lower levels of DNA methylation[60-62]. Some of the epigenetic marks acquired during gametogenesis are stable for a short time after fertilization and play key roles in the early stages of embryo development[63]. In the case of DNA methylation, one class of genes that resist post-fertilization reprogramming are imprinted genes, a small set of 150-200 genes that exhibit parent-of-origin expression[64] (Box 2). The expression of these genes depends on germline-derived differentially methylated regions (DMRs), protected against demethylation post-fertilization by local binding of Zfp57 and Zfp445, two KRAB-zinc finger proteins[64].

In the case of histone marks, the broad H3K4me3 domains brought in through the oocytes, modulate the maternal-to-zygotic transition[59], while inheritance of oocyte-specific H3K27me3 and H2AK119ub1 is responsible for the transitory paternal allele-specific expression of dozens of genes, in a DNA-methylation-independent manner, in preimplantation embryos[65,66]. Although H3K27me3-dependent imprinting is largely lost in the embryonic cell lineage, at least five genes maintain their imprinted expression in the extra-embryonic cell lineage through the somatic acquisition of repressive DNA methylation on the maternal allele[65]. Additionally, oocyte-derived DNA methylation plays a major role in regulating early stages of trophoblast development[67]. Genes marked by H3K4me3, but not H3K27me3, in sperm are more likely to be expressed in the 4-cell stage embryos[60].

Histone variants play critical roles during zygotic genome activation. In the mouse, the histone variant H3.3 is present in both male and female gametes. Sperm-derived H3.3 is removed shortly after fertilization from the zygote via the second polar body[68]. Oocyte-derived H3.3 is then

incorporated preferentially into the male pro-nucleus before genome activation[69]. H3.3-mediated paternal chromatin remodelling is essential for the development of preimplantation embryos and the activation of the paternal genome during embryogenesis[68]. The histone variants TH2A and TH2B, which are highly expressed in oocytes, also contribute to the activation of the paternal genome after fertilization[70]. The oocyte-specific linker histone H1foo, acting as a maternal factor, mediates the nuclear deposition of H3 variants between the one- and two-cell stage and contributes to rapid changes in the higher-order chromatin organization[71]. Because histone variants exhibit distinct posttranslational modifications (the barcode hypothesis[72] proposed for H3 variants) that can be modulated by environmental factors such as parental diet, they may provide a route for sex-dependent intergenerational inheritance, including those of metabolic traits.

Small RNAs, such as microRNAs and Transfer RNA-derived Fragments (tRFs), acquired via vesicular transport during the epididymal transit of sperm are additional key regulators of the preimplantation gene expression program[73,74]. 5'-tRNA halves induced by high-fat diet in mouse sperm have been proposed to promote intergenerational inheritance of metabolic disorders by affecting the expression of genes involved in glucose metabolism, oxidative stress, and autophagy[75]. tRFs are highly expressed in mouse stem cells (both TSC – trophoblast stem cells – and ESC – embryonic stem cells) and play key roles in the epigenetic silencing of long terminal repeat (LTR)-retrotransposons and IAP and MusD/ETn, two of the most active endogenous retrovirus (ERV) families[76].

2. Sex-specific marks gained during differentiation

At implantation, the developing embryo reaches pluripotency, a stage followed by a progressive restriction of cellular plasticity, which is accompanied by a gradual acquisition of epigenetic marks in a lineage and cell-type-specific manner[63], termed here Epigenetic Programming. There is growing evidence for sexual dimorphism in the way epigenetic marks are acquired during cell differentiation[77]. Prior to the formation of the gonads, sex-related epigenetic dissimilarities may be related to differences in the gene dosage of components of the epigenetic machinery. Examples include the lysine demethylases *Kdm6a* and *Kdm5c*, two X-linked genes that escape XCI, and the Y-

linked *Kdm5d* and *Kdm6c*[78] (see section: Sex differences due to effects of sex chromosomes, and Fig. 2b). In addition, the inactivated X-chromosome can act as a sink for heterochromatic factors such as HP1, thereby reducing the availability of factors required for gene silencing at other heterochromatin loci, leading to differences in the expression of hundreds of autosomal genes, in a sex-dependent manner[79].

After the formation of the gonads, the sex hormones act as epigenetic modifiers. The transcription of many genes encoding epigenetic writers, readers and erasers is regulated by sex hormones, including the histone methyltransferase *Ezh2*/*Kmt6*, which contains an estrogen-response element[80]. Additionally, recruitment of some epigenetic modifiers to their target genes is dependent on sex hormones, for example the histone H3K9 demethylase Jhmd2a, which is recruited to loci bound by the androgen-receptor[81]. The interplay between the epigenetic mechanisms illustrated above and an altered *in-utero* environment has the potential to exacerbate programmed sex-related differences in the epigenome, thus contributing to sexual dimorphism in the intergenerational programming of metabolic diseases[9].

3. Sex-specific marks acquired during gametogenesis

A major wave of epigenetic reprogramming also occurs during germline development. The epigenetic information is first erased in the primordial germ cell (PGCs), which form in the peri-gastrulation-stage embryos, and then re-established during germline development, according to the sex of the embryo[82]. The erasure of DNA methylation marks is incomplete at sequences belonging to several classes of retrotransposons, such as IAPs in mouse[83] and SINE–VNTR–*Alu* in human[84], providing a potential route for intergenerational epigenetic inheritance. Following DNA demethylation, the gonadal PGCs undergo remodelling of repressive histone modifications (H3K27me3 and H3K9me3) resulting in a sex-specific signature in mice. While loss of H3K27me3 is more pronounced in male PGCs between E10.5 and E13.5, this mark is relatively protected in female PGCs by the action of the histone methyltransferase Ezh2. On the other hand, there is a dramatic increase in H3K9me3 peaks in male PGCs between E10.5 and E13.5[85].

283 The re-establishment of germ cell-specific epigenetic marks occurs at different developmental stages
284 in the two sexes, ultimately leading to the establishment of sperm and oocyte-specific epigenomes.
285 Acquisition of DNA methylation is dependent on Dnmt3a and Dnmt3l in both germlines[86]. In the
286 female germline, this process takes place after birth, during the oocyte growth phase[86], led by the
287 Setd2-dependent deposition of H3K36me3[87]. In contrast, DNA methylation is acquired prior to birth
288 in the male germline, guided by broad deposits of H3K36me2 added by the lysine methyltransferase
289 Nsd1[88], and maintained through many mitotic cell divisions before entering meiosis[86]. The different
290 developmental windows and mechanisms for the acquisition of epigenetic marks in the two
291 germlines, provides additional routes for sex-specific effects on the intergenerational programming
292 of metabolic traits.

293 4. Sex-specific impact of environment on epigenetic marks

294 Overall, because epigenetic marks are often environmentally responsive[89], an altered milieu during
295 gametogenesis has the potential to induce epigenetic alterations that may persist post-fertilization,
296 in a manner that is dependent on the parental sex. Supporting this concept, 16 weeks of HFD (high-
297 fat diet) feeding preceding conception in female mice induces global hypomethylation across the
298 zygote genome, mediated by deficiency of the demethylation-protecting factor Stella/Dppa3 in
299 oocytes, leading to developmental defects[90].

300 In male mice, exposure to a calorie-restricted diet leads to hypomethylated regions in the sperm,
301 which are resistant to early embryo methylation reprogramming[91]. Additionally, post-natal paternal
302 folate deficiency alters sperm H3K4me3 levels at promoters and putative enhancers regions of genes
303 involved in early pre- and post-implantation embryogenesis, with a modest, but significant
304 correlation between sperm H3K4me3 alterations and differentially expressed genes in the 8-cell
305 embryos[62]. Furthermore, paternal toxicant exposure[92] and paternal obesity[93] are associated with
306 epigenetic changes in sperm at loci associated with altered placental gene expression, suggesting
307 that sperm-inherited factors affect early placental development, which then leads to postnatal
308 metabolic phenotypes. A rat study showed that female, but not male offspring of HFD-fed sires,

become glucose intolerant and resistant to HFD-induced weight gain in adult life, a phenotype associated with altered expression of several miRNAs, piRNAs and tRFs in the sperm of F0 fathers[5]. Furthermore, paternal exercise in mice negated the detrimental effects of a paternal HFD in offspring, with subtle differences in the fat mass accrual between the two sexes[94]. Paternal exercise almost completely negated the effect of HFD on specific sperm tRFs, such as the highly abundant fragments of tRNA-Gly-GCC, tRNA-Gly-CCC, and tRNA-His-GTG[94]. One of the tRFs highlighted in the above study, tRF-Gly-GCC, is known to repress genes associated with the endogenous retroelement MERVL, which are thought to affect placenta size and function[95].

In humans, a randomized, double-blinded controlled trial of peri-conceptional maternal micronutrient supplementation, identified hundreds of loci with sex-related DNA methylation differences in cord blood DNA samples, some of which persisted in infant life[96]. *Ex vivo*, there is evidence for more pronounced DNA demethylation in female cells during the derivation of induced pluripotent stem cells (iPSCs)[97], a process dependent on the reactivation of the inactive X chromosome[98]. Further studies are required, to establish whether environmentally-induced epigenetic alterations imposed on the zygote via oocyte or sperm affect one sex more than the other, leading to sex-specific intergenerational programming.

Sex differences related to mitochondrial function

Mitochondria play vital roles in the normal function of eukaryotic cells, from ATP production, to core metabolic processes, intracellular calcium homeostasis, thermogenesis and apoptosis[99]. In this section, we highlight the interconnections between mitochondria and the other mechanisms presented above (e.g. sex hormones and epigenetic changes), discuss key aspects of mitochondrial inheritance and review the evidence suggesting that male and female mitochondria respond differently to environmental factors, leading to sexually dimorphic intergenerational inheritance of metabolic diseases.

1. Key features of mitochondria function and inheritance

335 Metabolites generated in the mitochondria via the tricarboxylic acid (TCA) cycle are essential co-
336 factors of the epigenetic machinery, such as acetyl-CoA, used as substrate for histone acetylation,
337 NAD+ used by the family of Sirtuin histone deacetylases, and α-ketoglutarate used by the TET1/3
338 dioxygenase enzymes, involved in DNA demethylation, and the JmjC domain-containing histone
339 demethylases[100]. Mitochondria are also the site for the initial step in the production of all steroid
340 hormones, including the sex hormones, via the conversion of cholesterol to pregnenolone[101] (Fig. 4a).
341 At fertilization, mitochondrial content differs significantly between the two gametes, with only
342 several copies in the sperm and thousands in the mature oocytes. Owing to these marked
343 differences, and the tagging of paternal mitochondria via ubiquitylation for post-fertilization
344 destruction[102], inheritance of mitochondrial DNA (mtDNA) is virtually exclusively via the maternal
345 lineage[103]. Mitochondria content increases significantly during oocyte maturation and is critical for
346 oocyte quality[104]. Concomitant with the increase in copy numbers, mtDNA undergoes a genetic
347 bottleneck during oogenesis, with only a small fraction of maternal mtDNA molecules ultimately
348 represented in each mature egg[105] (Fig. 4b). The process of strict maternal inheritance of mtDNA
349 renders the mitochondrial genome susceptible to accumulating mutations that are harmful when
350 inherited by males, but are otherwise neutral or even beneficial to females, thus contributing to sex
351 differences in disease susceptibility in the offspring (the so-called "mother's curse effect"[106]).
352 Although most of the evidence supporting this evolutionary theory stems from studies performed in
353 plants and the fruit fly *D. melanogaster*[107], some evidence suggests it may apply to mammals,
354 including humans. In mice, infertility was observed in males carrying a pathogenic 4,696-bp deletion
355 (ΔmtDNA) in 70-80% of their mitochondria[108], while females with similarly large deletions escaped
356 infertility[109]. In humans, mitochondrial disease curtails the reproductive success of men to 65% of the
357 general population[110], while women remain unaffected[111].

358 2. Sex differences in metabolism related to mitochondria

359 The links between mitochondria, the epigenetic machinery via cellular metabolism, and sex hormone
360 production, together with the matrilineal inheritance of mitochondria and the higher propensity of

mtDNA mutations to be more harmful to males, as highlighted above, contribute to sex differences in metabolic traits. There is growing evidence for sexual dimorphism in mitochondria function, both at baseline and under stress, in metabolic organs such as fat[112], skeletal muscle[113], liver[114] and the endocrine pancreas[115], explained, at least in part, by effects of sex hormones on these organelles[46]. Findings from a large collection of ~100 genetically diverse inbred mouse strains – the hybrid mouse diversity panel (HMDP) – showed that adipose mtDNA copy number is significantly higher in females[116]. This trait was mapped to a SNP (single-nucleotide polymorphism) located on chr17 (rs48062344), in a linkage disequilibrium block that contains two other SNPs predicted to overlap estrogen receptor binding sites. Only females harbouring TT genotypes at rs48062344 had higher adipose expression of OXPHOS genes than CC genotypes[116]. Trans-eQTL analyses linked these effects to *Ndufv2*, a gene more highly expressed in white adipose tissue of female mice across the HMDP and that encodes a core subunit of mitochondrial complex I, promoting mitochondrial biogenesis and ROS production. Overexpression of *Ndufv2* limited to the adipose tissue in mice fed HFD demonstrated that *Ndufv2* regulates adipose mass and insulin sensitivity in a sex-by-strain dependent manner, with its gain of function being more beneficial in TT rather than CC females[116] (Fig. 4c).

In a study that exposed female and male C57BL/6J (B6) and DBA/2J (D2) mice to calorie-restricted (CR) and standard diets, many sex- and strain-dependent differences in hepatic mitochondrial morphology and function were observed[117]. For example, mitochondrial size increased in B6 and D2 males and in B6 females fed the 40% CR diet, however, no increase was observed in D2 females fed the same diet. Additionally, the study identified significant increases in respiratory chain complexes I, II, and III in CR-fed D2 females, whereas B6 males and females fed 40% CR showed higher complex I and II levels, respectively[117].

3. Mitochondria and sex differences in metabolic disease

There is emerging evidence for higher risk of metabolic dysfunction in male offspring of mothers exposed to inadequate environments during pregnancy, related to sex differences in mitochondria

function, as discussed in the examples below (see section: Hyperlipidaemia and fatty liver). Mitochondrial dysfunction resulting from maternal exposures has been reported both pre- and post-natally. Maternal obesity in mice led to increased components of the mitochondrial electron transport chain (complex II and ATP synthase) in male placentae only[118]. Male fetuses of rat mothers fed iron-deficient diets showed reduced hepatic complex IV respiration and increased cytosolic superoxide, whereas female fetuses did not exhibit any hepatic alteration in oxidant levels or mitochondrial function[114].

In rats, maternal low-protein diet led to increased reactive oxygen species production, a higher expression of mitochondrial subunits of the electron transport chain NADH-ubiquinone oxireductase subunit 4L and an overexpression of PPRAG (peroxisome proliferator-activated receptor-gamma) and UCP2 (uncoupling protein-2) in the pancreatic islets of male offspring only in young adult life (3 months of age)[119]. Similarly, maternal obesity in mice induced compromised mitochondrial respiration in pancreatic islets of young adult male offspring, characterised by decreased ATP synthesis-driven respiration and increased uncoupled respiration[115].

In mice, a decrease in mitochondrial-linked complex II-III was observed in skeletal muscle of young (3-month-old) adult male offspring of obese dams, which was not observed in females at this age[120]. Taken together, current data suggests that mitochondrial dysfunction due to an altered maternal environment is stronger in the male offspring, both during fetal and young adult life, which may contribute to their increased risk of metabolic disease. However, it is unclear if this apparent sexual dimorphism persists with age (see section: Ageing trajectories and metabolic health-span).

Sex differences related to placental function

1. Placenta dimorphism in normal and abnormal pregnancies

In both human and rodents, mean placental weight at term is greater in male than in female conceptuses[121]. Moreover, male placentae function near their maximum capacity, while female placentae maintain a significantly higher reserve[27] (Fig. 5). The ontogeny of these morphological and

functional differences is difficult to study in normal human pregnancies. However, in rodent species total placental volume is larger in males than in females from mid-gestation to term[122]. Additionally, the junctional zone (Jz, the endocrine layer of the rodent placenta) is larger in males than in females, while the reverse is true for the labyrinthine zone (Lz, the site of nutrient and oxygen exchange)[123]. Placental metabolism also differs by fetal sex, with higher usage of glutamine for mitochondrial respiration in male placentae[124], higher levels of polyamine metabolites in female placentae[125], and lower capacity of female placentae to prevent excessive fetal exposure to increased levels of maternal glucocorticoids, related to a lower activity of placental 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2)[126].

The morphological and functional dimorphism of male and female placentae are associated with widespread differences in placental transcriptome throughout gestation[125,127,128], as well as with sex-related differences in DNA methylation patterns[129]. In turn, the sex-related differences in the placental transcriptome and epigenome are due to effects of sex chromosomes before the development of the fetal gonads, followed by a combination of both sex chromosomes and sex hormones in later gestation[130]. The strategies used for XCI in different eutherian mammals differ, with selective inactivation of the paternal X chromosome in the female mouse placenta and a weakly skewed paternal X chromosome inactivation in the human placenta[131]. Despite these differences, some key similarities between human and mice may be evolutionary conserved. One of the prime candidates for a placental mediator of sex-dependent responses to maternal environment is the gene encoding O-linked N-acetylglucosamine transferase (*OGT*), which escapes XCI and has higher expression in female placentae in both species and acts as a nutrient sensor[132,133] (Fig. 2b). OGT establishes sex differences in placental H3K27me3, which is higher in females than in males (Fig. 5) and is thought to control the sex-dependent expression differences of over a third of the 4,500 genes that are differentially expressed between male and female trophoblast cells on embryonic day (E)12.5[132].

Given the complexity in sex-related differences in morphology, function and gene expression patterns, it is not surprising that the placenta shows dimorphic responses to both maternal[118,134-136] and paternal[34,137] diets, which may contribute to the intergenerational programming of metabolic traits in a sex-dependent manner[32]. In rodents, a multitude of mechanisms that affect placenta function, leading to altered patterns of fetal growth and sex-specific programming of metabolic disorders, have been described. These range from altered expression of imprinted genes with known roles in placenta development and function (Box 2), altered function of placenta transporters[137], reduced cytotrophoblast cell proliferation[136], placenta inflammation[136,137], oxidative stress[118] and mitochondria dysfunction[118].

2. Fetal sex-dependent maternal adaptations to pregnancy

The sex of the conceptus can also influence maternal adaptations to pregnancy in a sexually-dimorphic manner. Prime candidates for mediating the impact of the conceptus sex on the mother are the maternal-fetal immune interactions within the pregnant uterus, which affect its vascularization[138], placental-derived hormones[139,140] and metabolites[125], as well as fetus-derived hormones transferred into the maternal circulation[141]. So far, the analysis of such mechanisms in a sex-specific manner is the exception rather than the rule. One notable example is the spermine metabolite N1,N12-diacetylspermine (DiAcSpm), produced by placenta and higher in the serum of women pregnant with a female fetus[125] (Fig. 5). Progeny of both sexes within litters complicate studying sex-related influences on maternal adaptations to pregnancy in rodents. CRISPR-Cas9 strategies that produce male- or female-only litters[142] will facilitate future study of mechanisms that mediate sex-specific maternal adaptations to pregnancy and consequences for offspring.

Long-term consequences of sex-related metabolic differences

Adiposity, body weight regulation and obesity

463 Gross anatomical differences in anthropometry, are apparent between women and men and change
464 with age. Approximately 100 genetic variants have been identified that explain differences in BMI or
465 waist-to-hip ratio. To establish if some of these genetic variants associate with either age and/or sex,
466 Winkler et al.[143] carried out a large genome-wide interaction meta-analysis of 114 human studies
467 within the Genetic Investigation of Anthropometric Traits (GIANT) Consortium. They tested the
468 association of ~2.8M SNPs with BMI and BMI-adjusted waist-hip ratios in four groups (men ≤50y,
469 men >50y, women ≤50y, women >50y). They identified 44 loci associated with waist-hip ratio that
470 were differentially expressed in the two sexes and some of these differences diminished with
471 advancing age and under the influence of menopausal hormonal changes (Fig. 6). This study was thus
472 instrumental in providing insight into the biology that underlies body shape and weight change with
473 age. In the context of programming, maternal obesity differentially impacted body fat of girls and
474 boys such that boys born to mothers with a higher BMI had higher body fat from ages 2-6 years
475 compared to boys born to normal-weight and overweight mothers, while girls' body composition was
476 unaffected in the first 6 years of life[17].
477 Studies in a model of maternal obesity in C57BL/6J mice revealed sex-dependent body weight
478 regulation, with male offspring of obese mothers gaining more weight than controls, while body
479 weight trajectories of female offspring were unaffected[144]. In the same study, maternal obesity also
480 affected adipose tissue expansion and distribution, such that only male offspring of obese mothers
481 increased their visceral and total fat mass, while their subcutaneous fat depots were reduced. This
482 phenotype of increased adiposity (WAT – white adipose tissue – fat mass relative to body weight)
483 was also observed in a separate study of young (10 weeks) male offspring of mothers fed a HFD[145].
484 Furthermore, adipocyte size was significantly increased in older males (17 weeks) but not in females.
485 Sex differences were also found in WAT metabolism and inflammation, while differentiation was
486 altered in both a sex- and time-specific manner.
487 In contrast, others have shown that, in pregnant mice fed a diet high in sugar[146] or high in both sugar
488 and fat[147], female offspring are more vulnerable to increased body weight and adiposity and

impaired glucose homeostasis[148,149]. More of such studies are needed to dissect not only the effects of nutritional programming on specific WAT depots, but at a more basic level to determine the sex-specific transcriptomes of different adipose tissue depots and their contributions to metabolic homeostasis. One such study by Almeida et al. found a role for the endocannabinoid system in the developmental origins of metabolic disease[150]. Specifically, across different adipose tissue depots, they showed that maternal HFD decreased CB1 and CB2 proteins in the subcutaneous adipose tissue of male pups only, while increased visceral and decreased subcutaneous CB1 was observed in female pups. CB1 was increased in brown adipose tissue, regardless of sex[150]. In addition, maternal HFD differentially altered estrogen receptor abundance across the adipose depots in male and female pups[150]. Thus, both endocannabinoid and estrogen signalling play important roles in lipogenesis, adipogenesis and thermogenesis.

Recently, work in our lab showed that metformin treatment to pregnant obese mice throughout gestation had a positive effect on the mother, improving her glucose homeostasis and reducing her overall fat mass[151,152]. However, the effects on adipocyte biology in the offspring were sexually-dimorphic, with increased WAT deposition with exaggerated adipocyte hyperplasia and increased macrophage infiltration being observed only in young male offspring of the metformin-treated group[152]. This highlights the sex-specific transmission also of interventions applied to the mother *in utero*.

Insulin resistance, beta-cell function and type 2 diabetes

Programming studies in animal models generally show an earlier and greater impact on male offspring glucose tolerance. For example, in a rat model of maternal protein restriction, 17-month-old male offspring display insulin resistance and impaired glucose tolerance[153], whereas female offspring display insulin resistance only at an advanced age of 21 months and even then, remain glucose tolerant[154]. Similar observations have been made in a mouse model of maternal obesity, with only male offspring displaying impaired glucose tolerance by 6 months of age[155]. Consistent with

these observations, pancreatic islets from female offspring of obese dams appear to be adapted to deal with a nutritionally rich environment, an effect not observed in males[115]. However, programming effects induced by paternal overnutrition leads to impaired glucose tolerance especially in the daughters and granddaughters, rather than the male offspring[5].

Hyperlipidaemia and fatty liver

A recent study investigating the effects of maternal obesity on lipid distribution in offspring[144] reported that, while maternal obesity did not have any significant effects on circulating triglycerides (TGs), males had significantly higher baseline circulating TGs than females, mainly in the low-density lipoprotein fractions. Serum cholesterol levels were also higher in male offspring at baseline and increased only in males under the influence of maternal obesity. In the liver, total lipid mass was increased only as an effect of maternal obesity and not sex, however, the composition of hepatic fatty acids, triglycerides and phospholipids were different in the two sexes, and this was linked to sex-specific transcriptional and post-transcriptional regulation of genes involved in hepatic lipid metabolism[144]. In a rat model of maternal obesity, male offspring presented greater physiological and histological Non-Alcoholic Fatty Liver Disease (NAFLD) characteristics than females[156].

In humans, the liver is reported to be one of the metabolic organs showing the highest degree of sexual dimorphism. This is reflected by a difference in the prevalence of NAFLD, which is twice as high in men as it is in women. While reasons for this difference are unclear, it has been proposed that differences in adipose tissue distribution, serum lipids or the protective action of hormone/estrogens might play a role[157]. The expression of genes for fatty acid and cholesterol synthesis are closely linked to oscillations in estrogen and its receptor[158]. Thus, estrogen might also play a contributing factor in slowing the progression of NAFLD in females, for example by reducing the severity of oxidative stress, suppressing hepatic mitochondrial function and inhibiting stellate cell activation and fibrogenesis[159,160] (Fig. 6).

Ageing trajectories and metabolic health-span

It is well established that the prevalence of diseases, such as type 2 diabetes increases with age. Furthermore, it is recognised that in most species, including humans, males age more rapidly than females and thus have both reduced lifespans and health-spans[161] (Fig. 6). Telomeres (hexameric repeat sequences at the end of chromosomes) are thought to be indicators of biological age and have been associated with age and age-associated diseases[162]. Consistent with these differences in lifespan, telomeres are shorter in males compared to females[163]. Additionally, studies based on epigenetic biomarkers of aging (the "epigenetic clock") have shown that men have higher epigenetic ageing rates than women in a variety of tissues[164], with clear evidence that androgens are the main driver of male-accelerated epigenetic ageing demonstrated by castration in sheep[165].

Accelerated cellular ageing is one of the key mechanisms underlying developmental programming and is thought to be mediated through various factors, including increased oxidative stress and increased telomere shortening[166]. Additionally, it has been shown that young male adults that were born pre-term have shorter telomeres than those born at term, an effect not observed in females[167]. Furthermore, maternal risk factors of adverse pregnancy and birth outcomes are associated with the offspring epigenetic clock of gestational age at birth[168] and recent evidence shows that the child sex is one of the strongest predictive factors of epigenetic age deviations at birth in pregnancies exposed to an altered maternal environment[169]. Therefore, the impact of *in utero* exposures on the ageing process that is already enhanced in males could explain the greater vulnerability of male offspring to programmed poor metabolic health (Fig. 6).

Conclusions and future perspectives

In recent years the scientific community has called for increased recognition and consideration of sex differences when designing and reporting research studies[170,171]. “Accounting for sex” has therefore become a justified “requirement” with a growing body of evidence supporting sexual dimorphism in

567 many key cellular processes encompassing multiple organs and cell types, beyond the well-
568 established sex-specific traits in the gonads. In addition, the presented statistical analyses are often
569 underpowered to confirm sex-specific effects[172]. Many studies analyse males and females separately
570 and conclude sexual dimorphism when a significant effect is observed in one sex only. To formally
571 demonstrate sex differences, it is essential to incorporate sex as a covariate in statistical models[173].
572 Our knowledge of the underlying developmental mechanisms that establish sex differences in shared
573 cell types, tissues and organs therefore remain poorly understood. The full implications for normal
574 development, disease susceptibility and progression can only be fully understood when substantial
575 work includes data at all levels between sexes, including genetic-driven molecular mechanisms,
576 'signalling-to-chromatin' and whole-body physiology. While the focus of this paper was to highlight
577 the impact and mechanisms of action of environmental exposures prior to conception and during
578 pregnancy on sexually dimorphic metabolic traits, including generational effects, the review of the
579 current data identifies major knowledge gaps in specific areas and raises new important research
580 directions.
581 Understanding the mechanisms underlying DOHaD requires major research efforts to define how
582 genetic and epigenetic traits are influenced by the environment and if responses are modified by
583 offspring sex. As discussed in this review, Genome Wide Association Studies (GWAS) that investigate
584 correlation between genetic variants (SNPs) and phenotypic traits, have identified sex differences in
585 autosomal genetic architecture for metabolic-related genes (e.g. fat deposition, body size and shape,
586 disease). However, most GWAS to date are not sex-stratified or sex-specific (the latter defines SNPs
587 associated with traits in one sex only, despite being present in the genome of both). Because of these
588 limitations, a modest number of genotype and sex interactions have been identified. This highlights
589 the need to perform both sex-specific GWAS studies in large cohorts to determine the impact of sex
590 on all SNP/phenotype associations, as well as sex-stratified epigenome scans (EWAS).
591 Fundamental to our overall understanding of metabolic programming, but highly challenging, given
592 the modest number of genotype-sex interactions, is the characterization of genotype- and

epigenotype-interacting environmental factors. Large human cohorts, and well-powered animal studies, will be required to provide insights into sex and environmental effects for all variant genes, coupled with gene expression studies. Recent proteome-genome maps identified genetic associations for ~3800 plasma proteins stratified by age or sex and established the value of cis-protein variants for annotation of causal disease genes at GWAS loci[174]. This study highlighted the need for systematic efforts for both molecular QTLs and disease GWAS to better understand the mechanisms underlying sex differences.

The observation that cells from females and males are inherently different could either be due to intrinsic differences in genotype (i.e. sex chromosomes) or result from changes in the surrounding metabolic milieu (e.g. sex hormones or other hormones or metabolites). Many studies fail to report the sex of the cells for *in vitro* experiments. Single-cell technologies will facilitate understanding the intracellular mechanisms that contribute to cellular sex differences, including epigenetic states and gene expression. In the context of metabolic traits and effects of early life programming on later disease, single-cell technologies will be instrumental in defining sex differences in cell signalling pathways in normal development but also in response to environmental and life history changes. It will be important to use a variety of programming models across the developmental spectrum in both sexes and interrogate cell-type responses to identify key signalling systems, including transcription factors and epigenetic effects that may lead to sex differences and responses. The use of genetically engineered mouse models, stratified by sex, will also be key to delineate a) sexually dimorphic traits linked causally to gene function and b) causal genes of sex differences that occur in specific environments or specific disease states. An important mouse resource already exists to help achieve this – the International Mouse Phenotyping Consortium – where male and female knockout mutants go through a phenotyping pipeline that spans developmental, cellular, physiological, behavioural and morphological traits. The analysis of such well phenotyped models are providing invaluable data on the underlying sex differences in genetic architecture for specific traits[175,176].

A major point of interest when discussing metabolic traits in the context of sex differences, relates to the possibility that such traits have a genetic-environmental component that can be inherited across multiple generations. Substantial data has been accumulated, both in human and model systems, to suggest that environmentally-induced changes can be passed on through paternal and/or maternal germlines, through mechanisms involving epigenetic marks (DNA methylation; histone modifications), small RNAs (see section: Sex differences related to epigenetic mechanisms) and mitochondrial DNA (see section: Sex differences related to mitochondrial function). In mammals, despite extensive epigenetic reprogramming events occurring at two developmental time points, single-copy loci related to metabolic pathways seem to resist the erasure of epigenetic marks in the germline. Comprehensive data regarding the extent to which the male and female germlines differ in responses to environmental exposures and the impact on the early female and male embryo development during pre-conception and post-conception metabolic programming is still required.

Many studies attributing a role to epigenetic mechanisms in intergenerational inheritance of metabolic traits are correlation-based and not mechanistically informed. Importantly, significant inconsistencies between studies are observed, ascribed to differences in study design e.g. dietary composition, duration of dietary manipulation, genetic background and tissue analysed. Likewise, there are multiple instances, in which the epigenetic changes observed do not align with the expected outcomes in gene expression patterns.

Emerging data raises the possibility that parent-of-origin specific effects of common genetic variants may have an important modulatory role in several traits, including growth and metabolism. Parent-of-origin effects occur when the phenotypic effect of an allele depends on whether it is inherited in offspring from the mother or father. Recent GWAS have reported parent-of-origin effects on fetal growth, which were independent of genomic imprinting[177]. It is likely that this mode of regulation, i.e. parent-of-origin effects, is more widespread in the genome that previously thought. The best characterized parent-of-origin effects are those related to genomic imprinting, that show parent-of-

origin dependent gene expression. Clearly, parental-origin effects may also be caused by other factors present in the oocyte or sperm that modulate metabolic traits in offspring.

Imprinted genes have evolved to play key roles in growth, nutrient acquisition, and metabolism; many imprinted genes are expressed in the placenta and play a crucial role in the process of placentation. Surprisingly, the extent to which imprinted gene expression is altered by cellular sex is virtually unknown. Evolutionary questions regarding the reproductive strategies of the two sexes are also pertinent in the context of specific organ sexual dimorphism. For example, extensive liver sexual dimorphism is thought to be associated with female reproductive functions. Females are exposed to higher evolutionary pressures than males to optimise the coupling of energy metabolism with reproduction.

The existing knowledge reviewed in this article highlight the importance of considering sex in the design and interpretation of studies related to DOHaD. Furthermore, studying sex differences in the context of stem cell behaviour and homeostasis is likely to be of importance for regenerative medicine and tissue engineering. Examples published to date include studies performed in muscle-derived stem cells, with higher muscular regeneration in a mouse model of DMD when derived from female donors, human embryonic stem cell-derived pancreatic progenitors, with improved maturation and glucose responsiveness when implanted into females, and organoids, which showed sex differences in the ability to form the self-organized 3D structures[220]. In the future, studying sex differences in DoHaD, could unlock sex-specific targeted drug treatments and/or nutritional interventions.

Table 1: Cross-sectional evidence of sexual dimorphism in metabolism

Overview of sexual dimorphism in the function of key metabolic organs in human and sexual dimorphic intergenerational programming effects observed in male (non-italic) and female (*italic*) offspring in human and animal models (species are indicated between parentheses).

Metabolic organs and sexual dimorphism	Programming factor	Effect in the metabolic organ of the offspring	Disease in the offspring

(human observations)			
Fat - *Subcutaneous rather than visceral fat storage (hips and thighs)*[60] - Hypertrophic abdominal adipose tissue expansion[60] - *lower adipose tissue inflammation*[60]	Maternal undernutrition	- *Indexes of fat mass distribution in adulthood (BMI, ratio of waist to mid-thigh circumferences) increased (human)*[16]	- *Obesity (human)*[16]
	Maternal overnutrition	- Increased adipocyte size at P70 (mouse)[145] - *Increased gonadal fat pad weights (mouse)*[178] - adipose inflammation (mouse)[179]	- Obesity (human)[17] - Increased Adiposity (mouse)[180]
	Paternal overnutrition	- *Increased gonadal and retroperitoneal fat pad weights in F1 (mouse)*[146] - *Increased proportion of body fat and impaired insulin sensitivity in F2 (mouse)*[146]	- *Obesity in F1 and F2 (mouse)*[146]
Liver - Higher de novo lipogenesis[147]	Maternal overnutrition	- Increased fat deposition (mouse)[56]	- NAFLD (mouse)[156]
Muscle - *Higher skeletal muscle insulin sensitivity*[60] - reduced lipid storage capacity[60]	Maternal high-fat diet (HFD)	- Exacerbated increase in oxidative phosphorylation (OXPHOS) and electron transfer system (ETS) capacity in gastrocnemius muscle upon weaning on HFD versus control diet (mouse)[181]	- Increased fasting glucose and increased lean body mass (mouse)[181]
Endocrine pancreas - *6% more ß-cells*[182] - *Greater insulin secretion capacity*[1]	Maternal overnutrition	- *Increased insulin secretion, mitochondrial respiration and reduced apoptosis (mouse)*[115]	- Impaired glucose tolerance (mouse)[155]
	Paternal overnutrition		- *Impaired glucose tolerance (mouse)*[5]
Hypothalamus - Less anorexigenic POMC neurons[9]	Maternal overnutrition	- Hypothalamic inflammation/ gliosis (mouse)[183]	- Obesity at older Age (mouse)[183]
	Maternal undernutrition	- *Reduced anorexogenic peptides (pig)*[184]	- *Excessive weight in adult life (pig)*[184]

Box 1. Transmission of non-genetic information across generations

Transmission of non-genetic information across generations has been observed in both plant and animal species and provides a route through which traits resulting from environmental exposures

672 can be perpetuated in the offspring[185]. Although this phenomenon had been described as early as the
673 18th century by naturalists such as Lamarck, it has not been easily accepted, because it contradicts
674 Darwin's theory of natural selection or the hypothesis that germ cells cannot be modified by somatic
675 signals (the Weismann barrier)[186].
676 *Intergenerational transmission* (also known as "parental effects") refers most commonly to the
677 effects of a parent's environment (F0) on their offspring (F1). In mammals, exposures during
678 pregnancy (the primary trigger) can directly affect the germline of the developing F1 embryo, thus
679 transmitting effects to the F2 generation.
680 *Transgenerational transmission* describes persistent effects in the absence of any direct exposure to
681 the triggering environment (F2 and beyond in males and non-pregnant females, and F3 and further
682 generations when the initial exposure is in pregnant females). Transgenerational effects are likely to
683 be mediated by epigenetic mechanisms that resist the epigenetic reprogramming waves during
684 germline development and post-fertilization, although confounding mechanisms[187], such as cryptic
685 genetic variation, behavioural effects (e.g. maternal nurturing) and microbiota effects should be
686 carefully accounted for.
687 *Parent-of-origin transmission* refers to genetic effects in which the phenotypic effect in the offspring
688 depends on whether the allele was inherited maternally or paternally. Although parent-of-origin
689 transmission has many causes, the best characterized phenomenon in mammals is genomic
690 imprinting, in which epigenetic mechanisms acting during germline development lead to the
691 exclusive expression of one of the two alleles, in a parent-of-origin specific manner[188].
692
693 **Box 2. The role of imprinted genes in mediating sex-related differences of metabolic traits**
694 In mammals, the phenomenon of genomic imprinting was discovered through pronuclear transfer
695 experiments of fertilized mouse eggs, which demonstrated that both a maternal and a paternal set of
696 chromosomes are required for early development[189,190]. These studies also demonstrated the key
697 role played by the paternal genome in placenta and paved the way to the identification of many

imprinted genes with key roles in placenta development and function, some of which are exclusively imprinted in this organ[191,192]. Altered DNA methylation and expression of imprinted genes in placenta, with sex-related differences, has been observed in several studies in mice exposed to altered maternal diets[135,193] or paternal alcohol exposure[194]. Recently, genetic manipulation at the *Igf2-H19* imprinted locus in mice, restricted to placental Jz, identified altered expression of genes encoding placental hormones, in a sex-dependent manner[195,196]. Given the key role of placenta in programming adult disease[197], these sex-specific alterations of imprinted genes in placenta are likely to contribute to the increased risk for metabolic disease in adult life, in a sexually dimorphic manner.

A role for imprinted genes in mediating sex-related differences of metabolic traits extends beyond the placenta. Regulatory variants at the imprinted *KLF14* gene were recently associated with increased risk for T2D in females, via sex-specific effects on adipocyte size and body composition[198]. In mice, adipocyte-specific deletion of *Klf14* led to increased and decreased fat mass in females and males, respectively, while adipocyte-specific overexpression resulted in lower total body fat in females fed a HFD, with no effect in males[199]. *Trim28*/*Kap1* is a regulator of genomic imprinting, being required for the maintenance of DNA methylation at germline imprints during the wave of post-fertilisation epigenetic reprogramming[200]. Liver-specific ablation of this gene leads to male-predominant hepatosteatosis[201]. Additionally, deletion of *Trim28* in adipocytes promotes increased adiposity, while glucose tolerance is preserved, with these effects being stronger in females[202]. Paternally expressed *Pw1*/*Peg3* is another gene that promotes sexual dimorphism in metabolism, with male mice carrying a paternally inherited *Pw1*/*Peg3* mutant allele showing reduced masculinization of growth and metabolism, increased adiposity, insulin resistance, and fatty liver[203].

Box 3. Outstanding questions

- How does sex impact on SNP and phenotypic trait association identified through GWAS?
- How widespread are sexually dimorphic epigenetic signatures in different tissues in response to different life course exposures?

- Which factors within a cell's microenvironment mediate sexually dimorphic changes in its metabolism and function?
- How do the male and female germlines differ in their response to a suboptimal *in utero* environment?
- How is imprinted gene expression altered by cellular sex?

References

1. Tramunt, B. *et al.* Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia* **63**, 453–461 (2020).
2. World Health Organization. Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (2021).
3. Fernandez-Twinn, D. S., Hjort, L., Novakovic, B., Ozanne, S. E. & Saffery, R. Intrauterine programming of obesity and type 2 diabetes. *Diabetologia* **62**, 1789–1801 (2019).
4. Wu, L. L. *et al.* Mitochondrial dysfunction in oocytes of obese mothers: Transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development* **142**, 681–691 (2015).
5. de Castro Barbosa, T. *et al.* High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. *Mol. Metab.* **5**, 184–197 (2015).
6. Chang, R. C., Wang, H., Bedi, Y. & Golding, M. C. Preconception paternal alcohol exposure exerts sex-specific effects on offspring growth and long-term metabolic programming. *Epigenetics Chromatin* **12**, 9 (2019).
7. Donkin, I. & Barrès, R. Sperm epigenetics and influence of environmental factors. *Mol. Metab.* **14**, 1–11 (2018).
8. Ge, Z.-J. *et al.* Maternal Diabetes Causes Alterations of DNA Methylation Statuses of

749 Some Imprinted Genes in Murine Oocytes. *Biol. Reprod.* **88**, 117 (2013).
750 9. Dearden, L., Bouret, S. G. & Ozanne, S. E. Sex and gender differences in developmental
751 programming of metabolism. *Mol. Metab.* **15**, 8–19 (2018).
752 10. 16. Barker, D. J. P. The origins of the developmental origins theory. *J. Intern. Med.* **261**,
753 412–417 (2007).
754 11. 17. Hales, C. N. *et al.* Fetal and infant growth and impaired glucose tolerance at age 64.
755 *BMJ* **303**, 1019–1022 (1991).
756 12. 18. Gluckman, P. D., Hanson, M. A., Phil, D., Cooper, C. & Thornburg, K. L. Effect of In
757 Utero and Early-Life Conditions on Adult Health and Disease. *N. Engl. J. Med.* **359**, 61–73
758 (2008).
759 13. Ravelli, A. C. J., Van Der Meulen, J. H. P., Osmond, C., Barker, D. J. P. & Bleker, O. P.
760 Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am. J. Clin.*
761 *Nutr.* **70**, 811–816 (1999).
762 14. Van Duijn, L., Rousian, M., Laven, J. S. E. & Steegers-Theunissen, R. P. M.
763 Periconceptional maternal body mass index and the impact on post-implantation (sex-
764 specific) embryonic growth and morphological development. *Int. J. Obes. (Lond).* 45,
765 2369–2376 (2021).
766 15. Francis, E. C., Dabelea, D., Shankar, K., Perng, W. & Francis EllenFrancis, E. C. Maternal
767 diet quality during pregnancy is associated with biomarkers of metabolic risk among
768 male offspring. *Diabetologia* **64**, 2478–2490 (2021).
769 16. Stein, A. D. *et al.* Anthropometric measures in middle age after exposure to famine
770 during gestation: evidence from the Dutch famine. *Am. J. Clin. Nutr.* **85**, 869–876 (2007).
771 17. Andres, A. *et al.* Longitudinal body composition of children born to mothers with normal
772 weight, overweight, and obesity. *Obesity (Silver Spring)* **23**, 1252–1258 (2015).

18. Watkins, A. J., Rubini, E., Hosier, E. D. & Morgan, H. L. Paternal programming of offspring health. *Early Hum. Dev.* **150**, 105185 (2020).
19. Pembrey, M. E. *et al.* Sex-specific, male-line transgenerational responses in humans. *Eur. J. Hum. Genet.* **14**, 159–166 (2006).
20. Kocourkova, J., Burcin, B. & Kucera, T. Demographic relevancy of increased use of assisted reproduction in European countries. *Reprod. Health* **11**, 37 (2014).
21. Schieve, L.A. *et al.* Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N. Engl. J. Med.* **346**, 731–737 (2002).
22. de Waal, E. *et al.* The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model. *Hum. Mol. Genet.* **24**, 6975–6985 (2015).
23. Vrooman, L.A. *et al.* Assisted reproductive technologies induce temporally specific placental defects and the preeclampsia risk marker sFLT1 in mouse. *Development* **147**, dev186551 (2020).
24. Heber, M.F. & Ptak GE. The effects of assisted reproduction technologies on metabolic health and disease. *Biol. Reprod.* **104**, 734–744 (2021).
25. Belva, F. *et al.* Pubertal development in ICSI children. *Hum. Reprod.* **27**, 1156–1161 (2012).
26. Donjacour, A., Liu, X., Lin, W., Simbulan, R. & Rinaudo, P.F. In vitro fertilization affects growth and glucose metabolism in a sex-specific manner in an outbred mouse model. *Biol. Reprod.* **90**, 80 (2014).
27. Eriksson, J. G., Kajantie, E., Osmond, C., Thornburg, K. & Barker, D. J. P. Boys Live Dangerously in the Womb. *Am. J. Hum. Biol.* **22**, 330–335 (2010).
28. Desoye, G. & Wells, J. C. K. Pregnancies in Diabetes and Obesity: The Capacity-Load

797 Model of Placental Adaptation. *Diabetes* **70**, 823–830 (2021).
798 29. Vatten, L. J. & Skjærven, R. Offspring sex and pregnancy outcome by length of gestation.
799 *Early Hum. Dev.* **76**, 47–54 (2004).
800 30. Teulings, N. E. W. D. *et al.* Independent influences of maternal obesity and fetal sex on
801 maternal cardiovascular adaptation to pregnancy: a prospective cohort study. *Int. J.*
802 *Obes. (London)* **44**, 2246–2255 (2020).
803 31. Broere-Brown, Z. A. *et al.* Fetal sex and maternal pregnancy outcomes: a systematic
804 review and meta-analysis. *Biol. Sex Differ.* **11**, 26 (2020).
805 32. Aiken, C. E. & Ozanne, S. E. Sex differences in developmental programming models.
806 *Reproduction* 145, R1–R13 (2013).
807 33. Mao, J. *et al.* Contrasting effects of different maternal diets on sexually dimorphic gene
808 expression in the murine placenta. *Proc. Natl. Acad. Sci. U.S.A.* 107, 5557–5562 (2010).
809 **This paper uncovered pronounced sexual dimorphism in gene expression patterns in**
810 **placentae of litters exposed to abnormal maternal diets.**
811 34. Binder, N. K. *et al.* Paternal obesity in a rodent model affects placental gene expression
812 in a sex-specific manner. *Reproduction* **149**, 435–444 (2015).
813 35. Lecoutre, S. *et al.* Depot- and sex-specific effects of maternal obesity in offspring's
814 adipose tissue. *J. Endocrinol.* **230**, 39–53 (2016).
815 36. Rahman, M. S. & Pang, M.-G. New Biological Insights on X and Y Chromosome-Bearing
816 Spermatozoa. *Front. Cell Dev. Biol.* **7**, 388 (2020).
817 37. Khramtsova, E. A., Davis, L. K. & Stranger, B. E. The role of sex in the genomics of human
818 complex traits. *Nat. Rev. Genet.* **20**, 173–190 (2019).
819 38. Deng, X., Berletch, J. B., Nguyen, D. K. & Disteche, C. M. X chromosome regulation:
820 diverse patterns in development, tissues and disease. *Nat. Rev. Genet.* **15**, 367–378

(2014).

39. Migeon, B. R. *Females are Mosaic: X Inactivation and Sex Differences in Disease*. (Oxford Univ. Press, Oxford, 2007).

40. Link, J.C. et al. X chromosome dosage of histone demethylase KDM5C determines sex differences in adiposity. *J. Clin. Invest.* **130**, 5688–5702 (2020). **This study demonstrated that the dosage differences of the X-linked escapee gene *Kdm5c* contribute to the male/female differences in adipocyte biology.**

41. Goossens, G. H., Jocken, J. W. E. & Blaak, E. E. Sexual dimorphism in cardiometabolic health: the role of adipose tissue, muscle and liver. *Nat. Rev. Endocrinol.* **17**, 47–66 (2021).

42. Aguet, F. *et al.* The impact of sex on gene expression across human tissues. *Science* **369**, 3066 (2020). **This study provided a survey of sex differences in the human transcriptome and its genetic regulation across 44 human tissues.**

43. Gershoni, M. & Pietrokovski, S. The landscape of sex-differential transcriptome and its consequent selection in human adults. *BMC Biol.* **15**, 7 (2017).

44. Lopes-Ramos, C. M. *et al.* Sex Differences in Gene Expression and Regulatory Networks across 29 Human Tissues. *Cell Rep* **31**, 107795 (2020).

45. Christianto, A. *et al.* Sex differences in metabolic pathways are regulated by Pfkfb3 and Pdk4 expression in rodent muscle. *Commun. Biol.* **4**, 1264 (2021).

46. Norheim, F. *et al.* Gene-by-Sex Interactions in Mitochondrial Functions and Cardio-Metabolic Traits. *Cell Metab.* **29**, 932–949 (2019).

47. Chella Krishnan, K. *et al.* Sex-specific metabolic functions of adipose Lipocalin-2. *Mol. Metab.* **30**, 30–47 (2019).

48. Waxman, D.J. & O'Connor, C. Growth hormone regulation of sex-dependent liver gene

845 expression. *Mol. Endocrinol.* **20**, 2613–2629 (2006).
846 49. Sugathan, A. & Waxman, D.J. Genome-wide analysis of chromatin states reveals distinct
847 mechanisms of sex-dependent gene regulation in male and female mouse liver. *Mol. Cell.*
848 *Biol.* **33**, 3594–3610 (2013).
849 50. Hao, P. & Waxman, D.J. STAT5 Regulation of Sex-Dependent Hepatic CpG Methylation at
850 Distal Regulatory Elements Mapping to Sex-Biased Genes. *Mol. Cell. Biol.* **41**, e00166–
851 e00220 (2021).
852 51. Blekhman, R., Marioni, J. C., Zumbo, P., Stephens, M. & Gilad, Y. Sex-specific and lineage-
853 specific alternative splicing in primates. *Genome Res.* **20**, 180–189 (2010). **This paper**
854 **identified genes that exhibit sex-specific alternative splicing in primates, with evidence**
855 **for possible contribution to human evolution.**
856 52. Lindholm, M. E. *et al.* The human skeletal muscle transcriptome: sex differences,
857 alternative splicing, and tissue homogeneity assessed with RNA sequencing. *FASEB J.* **28**,
858 4571–4581 (2014).
859 53. Mayne, B. T. *et al.* Large Scale Gene Expression Meta-Analysis Reveals Tissue-Specific,
860 Sex-Biased Gene Expression in Humans. *Front. Genet.* **7**, 183 (2016).
861 54. Gao, A. *et al.* Sexual dimorphism in glucose metabolism is shaped by androgen-driven gut
862 microbiome. *Nat. Commun.* **12**, 7080 (2021). **This study identified an important**
863 **contribution of androgen to the differences in glucose metabolism between the two**
864 **sexes, through the modulation of the gut microbiome.**
865 55. Cross, T.L., Kasahara, K. & Rey, F.E. Sexual dimorphism of cardiometabolic dysfunction:
866 Gut microbiome in the play? *Mol. Metab.* **15**, 70–81 (2018).
867 56. Wankhade, U.D. *et al.* Maternal High-Fat Diet Programs Offspring Liver Steatosis in a
868 Sexually Dimorphic Manner in Association with Changes in Gut Microbial Ecology in

Mice. *Sci. Rep.* **8**, 16502 (2018).

57. Reik, W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447, 425–432 (2007).
58. Smallwood, S. A. *et al.* Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat. Genet. 2011 438* **43**, 811–814 (2011).
59. Dahl, J. A. *et al.* Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* **537**, 548–552 (2016).
60. Hammoud, S. S. *et al.* Distinctive Chromatin in Human Sperm Packages Genes for Embryo Development. *Nature* **460**, 473–478 (2009).
61. Molaro, A. *et al.* Sperm methylation profiles reveal features of epigenetic inheritance and evolution in primates. *Cell* **146**, 1029–1041 (2011).
62. Lismer, A. *et al.* Histone H3 lysine 4 trimethylation in sperm is transmitted to the embryo and associated with diet-induced phenotypes in the offspring. *Dev. Cell* **56**, 671–686 (2021).
63. Hemberger, M., Dean, W. & Reik, W. Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. *Nat. Rev. Mol. Cell. Biol* **10**, 526–537 (2009).
64. Tucci, V., Isles, A. R., Kelsey, G. & Ferguson-Smith, A. C. Genomic Imprinting and Physiological Processes in Mammals. *Cell* **176**, 952–965 (2019).
65. Inoue, A., Jiang, L., Lu, F., Suzuki, T. & Zhang, Y. Maternal H3K27me3 controls DNA methylation-independent imprinting. *Nature* **547**, 419–424 (2017).
66. Mei, H. *et al.* H2AK119ub1 guides maternal inheritance and zygotic deposition of H3K27me3 in mouse embryos. *Nat. Genet.* **53**, 539–550 (2021).
67. Branco, M. R. *et al.* Maternal DNA Methylation Regulates Early Trophoblast Development. *Dev. Cell* **36**, 152–163 (2016).

68. Kong, Q. *et al.* Histone variant H3.3-mediated chromatin remodeling is essential for paternal genome activation in mouse preimplantation embryos. *J. Biol. Chem.* **293**, 3829–3838 (2018).
69. Torres-Padilla, M.E., Bannister, A.J., Hurd, P.J., Kouzarides, T. & Zernicka-Goetz, M. Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos. *Int. J. Dev. Biol.* **50**, 455–461 (2006).
70. Shinagawa, T. *et al.* Histone variants enriched in oocytes enhance reprogramming to induced pluripotent stem cells. *Cell Stem Cell* **14**, 217–227 (2014).
71. Funaya, S., Ooga, M., Suzuki, M.G. & Aoki, F. Linker histone H1FOO regulates the chromatin structure in mouse zygotes. *FEBS Lett.* **592**, 2414–2424 (2018).
72. Hake, S.B. & Allis, C.D. Histone H3 variants and their potential role in indexing mammalian genomes: the "H3 barcode hypothesis". *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6428–6435 (2006).
73. Conine, C. C., Sun, F., Song, L., Rivera-Pérez, J. A. & Rando, O. J. Small RNAs Gained during Epididymal Transit of Sperm Are Essential for Embryonic Development in Mice. *Dev. Cell* **46**, 470–480 (2018).
74. Sharma, U. *et al.* Small RNAs Are Trafficked from the Epididymis to Developing Mammalian Sperm. *Dev. Cell* **46**, 481–494 (2017).
75. Chen, Q. *et al.* Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* **351**, 397–400 (2016).
76. Schorn, A.J., Gutbrod, M.J., LeBlanc, C. & Martienssen, R. LTR-Retrotransposon Control by tRNA-Derived Small RNAs. *Cell* **170**, 61–71 (2017).
77. Engel, N. Sex Differences in Early Embryogenesis: Inter-Chromosomal Regulation Sets the Stage for Sex-Biased Gene Networks: The dialogue between the sex chromosomes and

autosomes imposes sexual identity soon after fertilization. *BioEssays* **40**, e1800073 (2018).

78. Burgoyne, P. S. & Arnold, A. P. A primer on the use of mouse models for identifying direct sex chromosome effects that cause sex differences in non-gonadal tissues. *Biol. Sex Differ* **7**, 68 (2016).
79. Wijchers, P. J. *et al.* Developmental Cell Sexual Dimorphism in Mammalian Autosomal Gene Regulation Is Determined Not Only by Sry but by Sex Chromosome Complement As Well. *Dev. Cell* **19**, 477–484 (2010).
80. Bhan, A. *et al.* Histone Methyltransferase EZH2 Is Transcriptionally Induced by Estradiol as Well as Estrogenic Endocrine Disruptors Bisphenol-A and Diethylstilbestrol. *J. Mol. Biol.* **426**, 3426–3441 (2014).
81. Yamane, K. *et al.* JHDM2A, a JmjC-Containing H3K9 Demethylase, Facilitates Transcription Activation by Androgen Receptor. *Cell* **125**, 483–495 (2006).
82. Tang, W. W., Kobayashi, T., Irie, N., Dietmann, S. & Surani, M. A. Specification and epigenetic programming of the human germ line. *Nat. Rev. Genet.* **17**, 585–600 (2016).
83. Seisenberger, S. *et al.* The Dynamics of Genome-wide DNA Methylation Reprogramming in Mouse Primordial Germ Cells. *Mol. Cell* **48**, 849–862 (2012).
84. Tang, W. W. C. *et al.* A unique gene regulatory network resets the human germline epigenome for development. *Cell* **161**, 1453–1467 (2015).
85. Huang, T.C. *et al.* Sex-specific chromatin remodelling safeguards transcription in germ cells. *Nature* **600**, 737–742 (2021). **This study uncovered sex-specific differences in the dynamics of repressive histone modifications remodelling during germline development, leading to different epigenomic landscapes in males and females.**
86. Sasaki, H. & Matsui, Y. Epigenetic events in mammalian germ-cell development:

941 reprogramming and beyond. *Nat. Rev. Genet.* **9**, 129–140 (2008).
942 87. Xu, Q. *et al.* SETD2 regulates the maternal epigenome, genomic imprinting and
943 embryonic development. *Nat. Genet.* **51**, 844–856 (2019).
944 88. Shirane, K., Miura, F., Ito, T. & Lorincz, M. C. NSD1-deposited H3K36me2 directs de novo
945 methylation in the mouse male germline and counteracts Polycomb-associated silencing.
946 *Nat. Genet.* **52**, 1088–1098 (2020).
947 89. Jaenisch, R. & Bird, A. Epigenetic regulation of gene expression: how the genome
948 integrates intrinsic and environmental signals. *Nat. Genet* **33**, 245–254 (2003).
949 90. Han, L. *et al.* Embryonic defects induced by maternal obesity in mice derive from Stella
950 insufficiency in oocytes. *Nat. Genet.* **50**, 432–442 (2018).
951 91. Radford, E.J. *et al.* In utero effects. In utero undernourishment perturbs the adult sperm
952 methylome and intergenerational metabolism. *Science* **345**, 1255903 (2014).
953 92. Ding, T., Mokshagundam, S., Rinaudo, P.F., Osteen, K.G. & Bruner-Tran, K.L. Paternal
954 developmental toxicant exposure is associated with epigenetic modulation of sperm and
955 placental Pgr and Igf2 in a mouse model. *Biol. Reprod.* **99**, 864–876 (2018).
956 93. Pepin, A.S., Lafleur, C., Lambrot, R., Dumeaux, V. & Kimmins, S. Sperm Histone H3 Lysine
957 4 tri-methylation serves as a metabolic sensor of paternal obesity and is associated with
958 the inheritance of metabolic dysfunction. *Mol. Metab.* Feb 17:101463 doi:
959 10.1016/j.molmet.2022.101463. Online ahead of print. (2022).
960 94. Stanford, K.I. *et al.* Paternal Exercise Improves Glucose Metabolism in Adult Offspring.
961 *Diabetes* **67**, 2530–2540 (2018).
962 95. Sharma, U. *et al.* Biogenesis and function of tRNA fragments during sperm maturation
963 and fertilization in mammals. *Science* **351**, 391–396 (2016).
964 96. Khulan, B. *et al.* Periconceptional maternal micronutrient supplementation is associated

with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia. *Hum. Mol. Genet.* **21**, 2086–2101 (2012). **This pilot interventional study performed in rural Gambia uncovered sex dimorphic DNA methylation changes induced by periconceptional maternal micronutrient supplementation.**

97. Milagre, I. *et al.* Gender Differences in Global but Not Targeted Demethylation in iPSC Reprogramming. *Cell Rep.* **18**, 1079–1089 (2017).

98. Pasque, V. *et al.* X Chromosome Dosage Influences DNA Methylation Dynamics during Reprogramming to Mouse iPSCs. *Stem Cell Reports* **10**, 1537–1550 (2018).

99. Rossmann, M. P., Dubois, S. M., Agarwal, S. & Zon, L. I. Mitochondrial function in development and disease. *Dis. Model. Mech.* **14**, 48912 (2021).

100. Medini, H., Cohen, T. & Mishmar, D. Mitochondria Are Fundamental for the Emergence of Metazoans: On Metabolism, Genomic Regulation, and the Birth of Complex Organisms. *Annu. Rev. Genet.* **54**, 151–166 (2020).

101. Ramalho-Santos, J. & Amaral, S. Mitochondria and mammalian reproduction. *Mol. Cell. Endocrinol.* **379**, 74–84 (2013).

102. Sutovsky, P. *et al.* Ubiquitin tag for sperm mitochondria. *Nature* **402**, 371–372 (1999).

103. Mishra, P. & Chan, D. C. Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat. Rev. Mol. Cell. Biol.* **15**, 634–646 (2014).

104. May-Panloup, P. *et al.* Low oocyte mitochondrial DNA content in ovarian insufficiency. *Hum. Reprod.* **20**, 593–597 (2005).

105. Jenuth, J. P., Peterson, A. C., Fu, K. & Shoubridge, E. A. Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nat. Genet.* **14**, 146–151 (1996).

106. Gemmell, N.J., Metcalf, V.J. & Allendorf, F.W. Mother's curse: the effect of mtDNA on

individual fitness and population viability. *Trends Ecol. Evol.* **19**, 238–244 (2004).

107. Dowling, D.K. & Adrian, R.E. Challenges and Prospects for Testing the Mother's Curse Hypothesis. *Integr. Comp. Biol.* **59**, 875–889 (2019).

108. Nakada, K. *et al.* Mitochondria-related male infertility. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15148–15153 (2006).

109. Inoue, K. *et al.* Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. *Nat. Genet.* **26**, 176–181 (2000).

110. Martikainen, M.H. *et al.* Decreased male reproductive success in association with mitochondrial dysfunction. *Eur. J. Hum. Genet.* **25**, 1162–1164 (2017).

111. Gorman, G.S., Grady, J.P. & Turnbull, D.M. Mitochondrial donation--how many women could benefit? *N. Engl. J. Med.* **372**, 885–887 (2015).

112. Ande, S. R. *et al.* Prohibitin overexpression in adipocytes induces mitochondrial biogenesis, leads to obesity development, and affects glucose homeostasis in a sex-specific manner. *Diabetes* **63**, 3734–3741 (2014).

113. Miotto, P. M., McGlory, C., Holloway, T. M., Phillips, S. M. & Holloway, G. P. Sex differences in mitochondrial respiratory function in human skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **314**, R909–R915 (2018).

114. Woodman, A. G. *et al.* Prenatal iron deficiency causes sex-dependent mitochondrial dysfunction and oxidative stress in fetal rat kidneys and liver. *FASEB J.* **32**, 3254–3263 (2018).

115. Nicholas, L. M. *et al.* Exposure to maternal obesity programs sex differences in pancreatic islets of the offspring in mice. *Diabetologia* **63**, 324–337 (2020). **This study demonstrated that maternal obesity programs offspring islets in a sex-specific manner, with islets of female offspring being better at coping with a nutritionally-rich postnatal**

environment.

116. Chella Krishnan, K. *et al.* Sex-specific genetic regulation of adipose mitochondria and metabolic syndrome by Ndufv2. *Nat. Metab.* **3**, 1552–1568 (2021). **This study identified a genetic variant at the *Ndufv2* locus that regulates its activity, as well as that of at least 89 mitochondrial genes in the adipose tissue, in a sex-dependant manner.**

117. Mitchell, S.J. *et al.* Effects of Sex, Strain, and Energy Intake on Hallmarks of Aging in Mice. *Cell Metab.* **23**, 1093–1112 (2016).

118. Napso, T. *et al.* Diet-induced maternal obesity impacts feto-placental growth and induces sex-specific alterations in placental morphology, mitochondrial bioenergetics, dynamics, lipid metabolism and oxidative stress in mice. *Acta Physiol. (Oxf).* **3**, e13795 (2022).

119. Theys, N., Bouckenooghe, T., Ahn, M.T., Remacle, C. & Reusens, B. Maternal low-protein diet alters pancreatic islet mitochondrial function in a sex-specific manner in the adult rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R1516–R1525 (2009).

120. Shelley, P. *et al.* Altered skeletal muscle insulin signaling and mitochondrial complex II-III linked activity in adult offspring of obese mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R675–R681 (2009).

121. Christians, J. K. The Placenta's Role in Sexually Dimorphic Fetal Growth Strategies. *Reprod. Sci.* (2021) doi:10.1007/S43032-021-00780-3.

122. Kalisch-Smith, J. I., Simmons, D. G., Pantaleon, M. & Moritz, K. M. Sex differences in rat placental development: from pre-implantation to late gestation. *Biol. Sex Differ.* **8**, 17 (2017).

123. O'Connell, B. A., Moritz, K. M., Roberts, C. T., Walker, D. W. & Dickinson, H. The placental response to excess maternal glucocorticoid exposure differs between the male

and female conceptus in spiny mice. *Biol. Reprod.* **85**, 1040–1047 (2011).

124. Wang, Y., Bucher, M. & Myatt, L. Use of Glucose, Glutamine and Fatty Acids for Trophoblast Respiration in Lean, Obese and Gestational Diabetic Women. *J. Clin. Endocrinol. Metab.* **104**, 4178–4187 (2019).

125. Gong, S. *et al.* Placental polyamine metabolism differs by fetal sex, fetal growth restriction, and preeclampsia. *JCI Insight* **3**, e120723 (2018). **This study identified for the first time a link between placental polyamine metabolism and sex-related differences in placenta-related complications of human pregnancy.**

126. Wieczorek, A. *et al.* Sex-specific regulation of stress-induced fetal glucocorticoid surge by the mouse placenta. *Am. J. Physiol. Endocrinol. Metab.* **317**, E109–E120 (2019).

127. Gonzalez, T. L. *et al.* Sex differences in the late first trimester human placenta transcriptome. *Biol. Sex Differ.* **9**, 4 (2018).

128. Sun, T. *et al.* Sexually Dimorphic Crosstalk at the Maternal-Fetal Interface. *J. Clin. Endocrinol. Metab.* **105**, e4831–e4847 (2020).

129. Gong, S. *et al.* Genome-wide oxidative bisulfite sequencing identifies sex-specific methylation differences in the human placenta. *Epigenetics* **13**, 228–239 (2018).

130. Braun, A. E. *et al.* Examining Sex Differences in the Human Placental Transcriptome During the First Fetal Androgen Peak. *Reprod. Sci.* **28**, 801–818 (2021).

131. Hamada, H. *et al.* Allele-Specific Methylome and Transcriptome Analysis Reveals Widespread Imprinting in the Human Placenta. *Am. J. Hum. Genet.* **99**, 1045–1058 (2016).

132. Nugent, B. M., O'Donnell, C. M., Epperson, C. N. & Bale, T. L. Placental H3K27me3 establishes female resilience to prenatal insults. *Nat. Commun.* **9**, 2555 (2018). **This study found sex-differences in placental expression of X-linked *OGT* gene (O-linked N-**

acetylglucosamine transferase), leading to higher levels of the repressive histone mark H3K27me3 in female placentae.

133. Hardivillé, S. & Hart, G. W. Nutrient regulation of signaling, transcription, and cell physiology by O-GlcNAcylation. *Cell Metab.* **20**, 208–213 (2014).

134. Gabory, A. et al. Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS One* **7**, e47986 (2012).

135. Chen, P.Y. *et al.* Intrauterine calorie restriction affects placental DNA methylation and gene expression. *Physiol. Genomics* **45**, 565–576 (2013).

136. Kim, D.W., Young, S.L., Grattan, D.R. & Jasoni, C.L. Obesity during pregnancy disrupts placental morphology, cell proliferation, and inflammation in a sex-specific manner across gestation in the mouse. *Biol. Reprod.* **90**, 130 (2014).

137. Claycombe-Larson, K.G., Bundy, A.N. & Roemmich, J.N. Paternal high-fat diet and exercise regulate sperm miRNA and histone methylation to modify placental inflammation, nutrient transporter mRNA expression and fetal weight in a sex-dependent manner. *J. Nutr. Biochem.* **81**, 108373 (2020).

138. Madeja, Z. *et al.* Paternal MHC expression on mouse trophoblast affects uterine vascularization and fetal growth. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 4012–4017 (2011).

139. Napso, T., Yong, H. E. J., Lopez-Tello, J. & Sferruzzi-Perri, A. N. The Role of Placental Hormones in Mediating Maternal Adaptations to Support Pregnancy and Lactation. *Front. Physiol.* **9**, 1091 (2018).

140. Napso, T. *et al.* Placental secretome characterization identifies candidates for pregnancy complications. *Commun. Biol.* **4**, 701 (2021).

141. Cleaton, M. A. *et al.* Fetus-derived DLK1 is required for maternal metabolic adaptations to pregnancy and is associated with fetal growth restriction. *Nat. Genet.* **48**,

1085 1473–1480 (2016).
1086 142. Douglas, C. *et al.* CRISPR-Cas9 effectors facilitate generation of single-sex litters and
1087 sex-specific phenotypes. *Nat. Commun.* **12**, 6926 (2021).
1088 143. Winkler, T. W. *et al.* The Influence of Age and Sex on Genetic Associations with Adult
1089 Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *PLoS Genet.* **11**,
1090 e1005378 (2015).
1091 144. Savva, C. *et al.* Obese mother offspring have hepatic lipidic modulation that
1092 contributes to sex-dependent metabolic adaptation later in life. *Commun. Biol.* **4**, 14
1093 (2021).
1094 145. Litzenburger, T. *et al.* Maternal high-fat diet induces long-term obesity with sex-
1095 dependent metabolic programming of adipocyte differentiation, hypertrophy and
1096 dysfunction in the offspring. *Clin. Sci. (London)* **134**, 921–939 (2020).
1097 146. Fullston, T. *et al.* Paternal obesity initiates metabolic disturbances in two generations
1098 of mice with incomplete penetrance to the F 2 generation and alters the transcriptional
1099 profile of testis and sperm microRNA content. *FASEB J.* **27**, 4226–4243 (2013).
1100 147. Pramfalk, C. *et al.* Sex-specific differences in hepatic fat oxidation and synthesis may
1101 explain the higher propensity for NAFLD in men. *J. Clin. Endocrinol. Metab.* **100**, 4425–
1102 4433 (2015).
1103 148. Samuelsson, A.M., Matthews, P. A., Jansen, E., Taylor, P. D. & Poston, L. Sucrose
1104 feeding in mouse pregnancy leads to hypertension, and sex-linked obesity and insulin
1105 resistance in female offspring. *Front. Physiol.* **4**, 14 (2013).
1106 149. Dearden, L. & Balthasar, N. Sexual Dimorphism in Offspring Glucose-Sensitive
1107 Hypothalamic Gene Expression and Physiological Responses to Maternal High-Fat Diet
1108 Feeding. *Endocrinology* **155**, 2144–2154 (2014).

150. Almeida, M. M. *et al.* Perinatal maternal high-fat diet induces early obesity and sex-specific alterations of the endocannabinoid system in white and brown adipose tissue of weanling rat offspring. *Br. J. Nutr.* **118**, 788–803 (2017).

151. Hufnagel, A. *et al.* Maternal but not fetoplacental health can be improved by metformin in a murine diet-induced model of maternal obesity and glucose intolerance. *J. Physiol.* (2021) doi: 10.1113/JP281902.

152. Schoonejans, J. M. *et al.* Maternal Metformin Intervention during Obese Glucose-Intolerant Pregnancy Affects Adiposity in Young Adult Mouse Offspring in a Sex-Specific Manner. *Int. J. Mol. Sci.* **22**, 8104 (2021).

153. Petry, C. J., Dorling, M. W., Pawlak, D. B., Ozanne, S. E. & Hales, C. N. Diabetes in Old Male Offspring of Rat Dams Fed a Reduced Protein Diet. *Int. J. Exp. Diabetes Res.* **2**, 139–143 (2001).

154. Fernandez-Twinn, D. S. *et al.* Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R368–R373 (2005).

155. Samuelsson, A. M. *et al.* Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: A novel murine model of developmental programming. *Hypertension* **51**, 383–392 (2008).

156. Lomas-Soria, C. *et al.* Maternal obesity has sex-dependent effects on insulin, glucose and lipid metabolism and the liver transcriptome in young adult rat offspring. *J. Physiol.* **596**, 4611– 4628 (2018).

157. Della Torre, S. Non-alcoholic Fatty Liver Disease as a Canonical Example of Metabolic Inflammatory-Based Liver Disease Showing a Sex-Specific Prevalence: Relevance of Estrogen Signaling. *Front. Endocrinol. (Lausanne)* **11**, 572490 (2020).

158. Villa, A. *et al.* Tetradian oscillation of estrogen receptor α is necessary to prevent liver lipid deposition. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11806–11811 (2012).

159. Kozlov, A. V. *et al.* Effect of estrogen on mitochondrial function and intracellular stress markers in rat liver and kidney following trauma-hemorrhagic shock and prolonged hypotension. *Mol. Med.* **16**, 254–261 (2010).

160. Yasuda, M., Shimizu, I., Shiba, M. & Ito, S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology* **29**, 719–727 (1999).

161. Sampathkumar, N. K. *et al.* Widespread sex-dimorphism in aging and age-related diseases. *Hum. Genet.* **139**, 333–356 (2020).

162. Blackburn, E. H., Epel, E. S. & Lin, J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science* **350**, 1193–1198 (2015).

163. Gardner, M. *et al.* Gender and telomere length: Systematic review and meta-analysis. *Exp. Gerontol.* **51**, 15–27 (2014).

164. Horvath, S. *et al.* An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. *Genome Biol.* **17**, 171 (2016). **This study found that epigenetic aging rates are significantly associated with sex, which may contribute to the lower mortality rates in women.**

165. Sugrue, V. J. *et al.* Castration delays epigenetic aging and feminizes DNA methylation at androgen-regulated loci. *Elife* **10**, e64932 (2021).

166. Zambrano, E., Lomas-Soria, C. & Nathanielsz, P. W. Rodent studies of developmental programming and ageing mechanisms: Special issue: In utero and early life programming of ageing and disease. *Eur. J. Clin. Invest.* **51**, e13631 (2021).

167. Parkinson, J. R. C. *et al.* Clinical and molecular evidence of accelerated ageing following very preterm birth. *Pediatr. Res.* **87**, 1005–1010 (2019).

168. Girchenko, P. *et al.* Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin. Epigenetics* **9**, 49 (2017).

169. Shrestha, D., Workalemahu, T. & Tekola-Ayele, F. Maternal dyslipidemia during early pregnancy and epigenetic ageing of the placenta. *Epigenetics* **14**, 1030–1039 (2019).

170. Accounting for sex in the genome. *Nat. Med.* **23**, 1243 (2017).

171. Mank, J. E. & Rideout, E. J. Developmental mechanisms of sex differences: from cells to organisms. *Development* **148**, 199750 (2021).

172. Christians, J. K., Shergill, H. K. & Albert, A. Y. K. Sex-dependent effects of prenatal food and protein restriction on offspring physiology in rats and mice: systematic review and meta-analyses. *Biol. Sex Differ.* **12**, 21 (2021).

173. Chin, E. H. & Christians, J. K. When are sex-specific effects really sex-specific? *J. Dev. Orig. Health Dis.* **6**, 438–442 (2015).

174. Pietzner, M. *et al.* Mapping the proteo-genomic convergence of human diseases. *Science* **374**, 1541 (2021).

175. Karp, N. A. *et al.* Prevalence of sexual dimorphism in mammalian phenotypic traits. *Nat. Commun.* **8**, 15475 (2017). **This large high-throuput study analysed 234 traits in thousands of mice and found that a large proportion of mammalian traits in both wild-type and mutants are influenced by sex.**

176. van der Bijl, W. & Mank, J. E. Widespread cryptic variation in genetic architecture between the sexes. *Evol. Lett.* **5**, 359–369 (2021).

177. Juliusdottir, T. *et al.* Distinction between the effects of parental and fetal genomes on fetal growth. *Nat. Genet. 2021 538* **53**, 1135–1142 (2021).

178. Keleher, M. R. *et al.* Maternal high-fat diet associated with altered gene expression,

DNA methylation, and obesity risk in mouse offspring. *PLoS One* **13**, e0192606 (2018).

179. Chang, E. *et al.* Programming effects of maternal and gestational obesity on offspring metabolism and metabolic inflammation. *Sci. Rep.* **9**, 16027 (2019).

180. Lecoutre, S. *et al.* Depot- and sex-specific effects of maternal obesity in offspring's adipose tissue. *J. Endocrinol.* **230**, 39–53 (2016).

181. Khamoui, A. V., Desai, M., Ross, M. G. & Rossiter, H. B. Sex-Specific Effects of Maternal and Postweaning High-Fat Diet on Skeletal Muscle Mitochondrial Respiration. *J. Dev. Orig. Health Dis.* **9**, 670–677 (2018).

182. Marchese, E. *et al.* Enumerating b-Cells in whole human islets: Sex differences and associations with clinical outcomes after islet transplantation. *Diabetes Care* **38**, e176–e177 (2015).

183. Argente-Arizón, P. *et al.* The Hypothalamic Inflammatory/Gliosis Response to Neonatal Overnutrition Is Sex and Age Dependent. *Endocrinology* **159**, 368–387 (2018).

184. Óvilo, C. *et al.* Prenatal programming in an obese swine model: sex-related effects of maternal energy restriction on morphology, metabolism and hypothalamic gene expression. *Br. J. Nutr.* **111**, 735–746 (2014).

185. Bohacek, J. & Mansuy, I. M. Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. *Nat. Rev. Genet.* **16**, 641–652 (2015).

186. Jawaid, A., Jehle, K.-L. & Mansuy, I. M. Impact of Parental Exposure on Offspring Health in Humans. *Trends Genet.* **37**, 373–388 (2021).

187. Heard, E. & Martienssen, R. A. Transgenerational Epigenetic Inheritance: myths and mechanisms. *Cell* **157**, 95–109 (2014).

188. Lawson, H. A., Cheverud, J. M. & Wolf, J. B. Genomic imprinting and parent-of-origin effects on complex traits. *Nat. Rev. Genet.* **14**, 609–617 (2013).

189. Surani, M.A., Barton, S.C. & Norris, M.L. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature* **308,** 548–550 (1984).

190. McGrath, J. & Solter, D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* **37**, 179–183 (1984).

191. Angiolini, E. *et al.* Regulation of placental efficiency for nutrient transport by imprinted genes. *Placenta* **27 Suppl A**, S98–S102 (2006).

192. Hanna, C.W. Placental imprinting: Emerging mechanisms and functions. *PLoS Genet.* **16**, e1008709 (2020).

193. Rahimi, S. *et al.* Moderate maternal folic acid supplementation ameliorates adverse embryonic and epigenetic outcomes associated with assisted reproduction in a mouse model. *Hum. Reprod.* **34**, 851–862 (2019).

194. Thomas, K.N. *et al.* Maternal background alters the penetrance of growth phenotypes and sex-specific placental adaptation of offspring sired by alcohol-exposed males. *FASEB J.* **35**, e22035 (2021).

195. Aykroyd, B.R.L., Tunster, S.J. & Sferruzzi-Perri, A.N. Igf2 deletion alters mouse placenta endocrine capacity in a sexually dimorphic manner. *J. Endocrinol.* **246**, 93–108 (2020).

196. Aykroyd, B.R.L., Tunster, S.J. & Sferruzzi-Perri, A.N. Loss of imprinting of the Igf2-H19 ICR1 enhances placental endocrine capacity via sex-specific alterations in signalling pathways in the mouse. *Development* **149**, dev199811 (2022).

197. Sandovici, I., Hoelle, K., Angiolini, E. & Constância, M. Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. *Reprod. Biomed. Online* **25**, 68–89 (2012).

198. Small, K.S. *et al.* Regulatory variants at KLF14 influence type 2 diabetes risk via a

female-specific effect on adipocyte size and body composition. *Nat. Genet.* **50**, 572–580 (2018). **This human study demonstrated that the metabolic risk associated with a genetic variation at the imprinted *KLF14* locus locus depends on the sex both of the subject and of the parent from whom the risk allele derives.**

199. Yang, Q. *et al.* Adipocyte-Specific Modulation of KLF14 Expression in Mice Leads to Sex-Dependent Impacts on Adiposity and Lipid Metabolism. *Diabetes* Jan 26:db210674. doi: 10.2337/db21-0674 (2022).

200. Messerschmidt, D.M. *et al.* Trim28 is required for epigenetic stability during mouse oocyte to embryo transition. *Science* **335**, 1499–1502 (2012).

201. Bojkowska, K. *et al.* Liver-specific ablation of Krüppel-associated box-associated protein 1 in mice leads to male-predominant hepatosteatosis and development of liver adenoma. *Hepatology* **56**, 1279–1290 (2012).

202. Bond, S.T. *et al.* Deletion of Trim28 in committed adipocytes promotes obesity but preserves glucose tolerance. *Nat. Commun.* **12**, 74 (2021).

203. Tanaka K, *et al.* Paternally expressed gene 3 (Pw1/Peg3) promotes sexual dimorphism in metabolism and behavior. *PLoS Genet.* **18**, e1010003 (2022). **This study identified the paternally expressed imprinted *Pw1*/*Peg3* gene as an important regulator of male-specific metabolic characteristics, acting through sex steroid pathways.**

Acknowledgements

This work was supported by funding from the Medical Research Council (MRC_MC_UU_00014/4 to M.C. and S.E.O.) and the British Heart Foundation (RG/17/12/33167 to S.E.O.). A.H. was supported by a Wellcome Trust studentship (108926/B/15/Z).

Contributions

S.E.O. and D.F.-T. conceived the outline and wrote the synopsis of the review. S.E.O. and M.C. elaborated the concepts, wrote the introductory and concluding sections, gave feedback on all other sections, and had oversight of this review. A.H. wrote the section on sexual dimorphism vulnerabilities during pre-conception and intrauterine development, including Table 1, and drew Fig. 1. I.S. wrote the section on biological mechanisms leading to sex differences in intergenerational programming of metabolic traits and drew Figs. 2–6. D.F.-T. wrote the section on consequences for long-term and intergenerational inheritance of metabolic traits. All authors read and gave feedback on all sections, and approved the final version of the paper.

Competing interests

All authors declare no competing interests.

Figure legends

Fig. 1: Factors that influence the intergenerational inheritance of metabolic traits at each stage of organismal development. Parental germline exposures prior to pregnancy affect sperm and oocytes. *In utero* exposures experienced by the developing embryo during pregnancy due to adverse maternal environments may programme sex-related dimorphic effects on the short- and long-term health of the offspring.

Fig. 2: Sexual dimorphic effects related to sex chromosomes and sex hormones. a, X and Y-chromosome bearing spermatozoa may have differences in their proteome content and some of these proteins are related to metabolic processes. **b**, X-linked genes that escape XCI (X-chromosome inactivation) result in gene dosage differences between males and females, e.g. *OGT*, encoding O-linked N-acetylglucosamine transferase or *Kdm5c*, encoding a histone demethylase (Xa – active X chromosome; Xi – inactive X chromosome). **c**, Gonadal steroid hormones have been suggested as the

underlying mechanism responsible for the sexual dimorphism observed in metabolic diseases. **d**, Upper panel: binding of testosterone (T) to the promoter regions of genes containing androgen-response elements leads to their transcriptional up-regulation (thicker blue arrow), which in turn is responsible for sex-related differential expression (sex-DE). Lower panel: interaction between estrogen (E) and the splicing machinery induces retention of exon 2 into the mature mRNA of a hypothetical gene and leads to expression of a female-specific alternative transcript. **e**, Sex hormones influence the composition of the gut microbiota, with lower glutamine/glutamate ratios (Gln/Glu) being described in male mice.

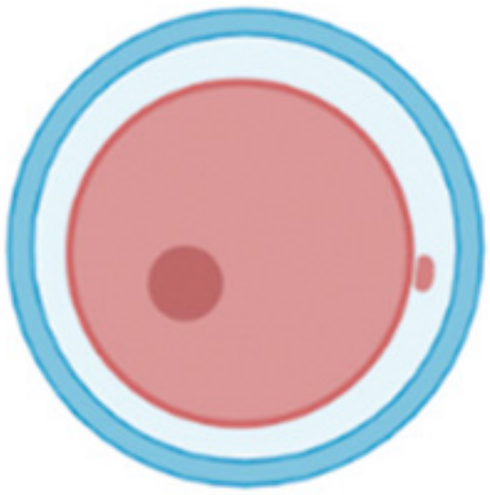
Fig. 3. Developmental epigenetic reprogramming leading to sex-related effects on gene expression patterns. Key sex-related differences identified in mouse studies are colour-coded (red – female-specific, blue – male-specific). Mature oocytes have large chromatin blocks enriched in H3K4me3, associated with low DNA methylation levels and contribute with histone variants, such as histone H1foo, acting as maternal factors in the zygote. Actively transcribed gene bodies exhibit high DNA methylation levels. In sperm, most histones are replaced by protamines and DNA is overall highly methylated. Males also contribute to the zygote and early embryogenesis by small non-coding RNAs, such as transfer RNA fragments (tRFs). At fertilization, the paternal pro-nucleus (PN) is subject to active DNA demethylation. Oocyte-derived Stella protects maternal PN against active demethylation, and methylation of maternal DNA is gradually diluted through DNA replication during subsequent cell divisions, in the absence of nuclear Dnmt1. H3K4me3 inherited from the mother controls the maternal-to-zygotic transition (MZT). Imprinted DMRs (differentially methylated regions) are protected against DNA demethylation by binding of Zfp57 and Zfp445 in both sexes. Prior to implantation, female embryos initiate XCI. Binding of HP1 to the inactive X chromosome depletes its levels on autosomes, inducing sex-related differences in gene expression for hundreds of genes. A few genomic loci are transiently imprinted because of the inheritance of maternal H3K27me3. The embryo reaches its lowest DNA methylation levels at the blastocyst stage; the first cell differentiation

1305 event corresponds to the formation of the inner cell mass (ICM, the future embryo proper) and
1306 trophectoderm (TE, the future placenta). After implantation, lineage establishment is accompanied
1307 by cell-type-specific *de novo* DNA methylation. After the formation of the gonads, sex hormones
1308 recruit specific epigenetic modifiers (e.g. Ezh2, which contains an estrogen-response element, and
1309 Jhmd2a, which interacts with the androgen-receptor). In the developing embryo, primordial germ
1310 cells (PGCs) are first de-methylated (with the exception of DNA demethylation resistant sequences
1311 such as IAPs – intracisternal A particles), followed by gain of DNA methylation in a sex-specific
1312 manner. *De novo* DNA methylation is mediated by Nsd1 prior to birth in sperm, and guided by Setd2
1313 in oocytes, in the post-natal life.
1314
1315 **Fig. 4: Sexual dimorphic effects related to mitochondria. a**, Mitochondria are implicated in the
1316 conversion of cholesterol (Ch) into pregnenolone (Pg), which is then converted into sex hormones in
1317 the endoplasmic reticulum, in a sex-dependent manner. Mitochondrial metabolism (through the
1318 Krebs cycle) is also the source of substrates and co-factors used for chromatin remodelling. **b**, During
1319 female gametogenesis, differentiation of PGCs into mature oocytes is accompanied by an increase in
1320 the total number of mitochondria. In addition, oocyte maturity is associated with a selective
1321 amplification of a fraction of mitochondria present in PGCs, which leads to a more homoplasmic
1322 mature oocyte in comparison with the heteroplasmic progenitor (depicted here by a reduction in the
1323 number of colours painting mitochondria in the oocyte), a process known as mitochondrial
1324 bottleneck. **c**, In the white adipocytes of female mice, a locus on chromosome 17 containing *Ndufv2*,
1325 controls *in trans* the expression of at least 89 genes implicated in mitochondrial biogenesis and
1326 oxidative phosphorylation.
1327
1328 **Fig. 5: Sex differences in placenta and maternal adaptations to pregnancy.** Sex-related differences
1329 in feto-placental unit development and function favour growth in males and survival in females. The
1330 fetus and the placenta are also influencing maternal adaptations to pregnancy in a sex-dependent

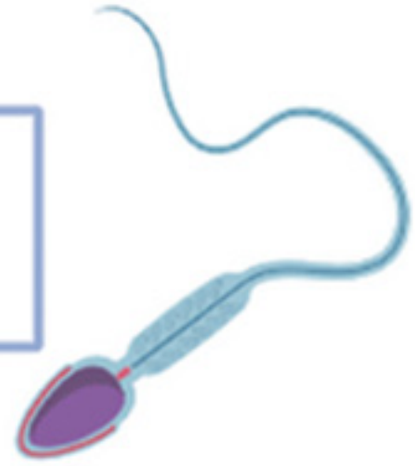
manner, leading to increased risk for pregnancy-related diseases in male-bearing pregnant women and intergenerational effects. Characteristics and parameters enhanced in a sex-specific manner are shown in blue (males) and red (females), respectively, while those that have not been explored so far for sex-related effects are shown in black (11βHSD2 – 11β-hydroxysteroid dehydrogenase type 2, DiAcSpm – N1,N12-diacetylspermine).

Fig. 6. Pathways leading to sex differences in the intergenerational inheritance of metabolic disease. The diagram summarizes the sex-related molecular changes identified through studies of developmental programming performed in animal models and humans, as discussed in this review. Suboptimal exposures during peri-conceptional or intrauterine development can lead to sexually dimorphic molecular changes that contribute to sex-related differences in the frequency, age-of-onset and severity of metabolic disease in adult life. The sub-optimal metabolic milieu can exert detrimental effects on the germline, thus contributing to the intergenerational inheritance of metabolic diseases.

Stress
Nutrition
Diabetes
Smoking
Age
Chemicals
ART



Peri-conceptional exposures



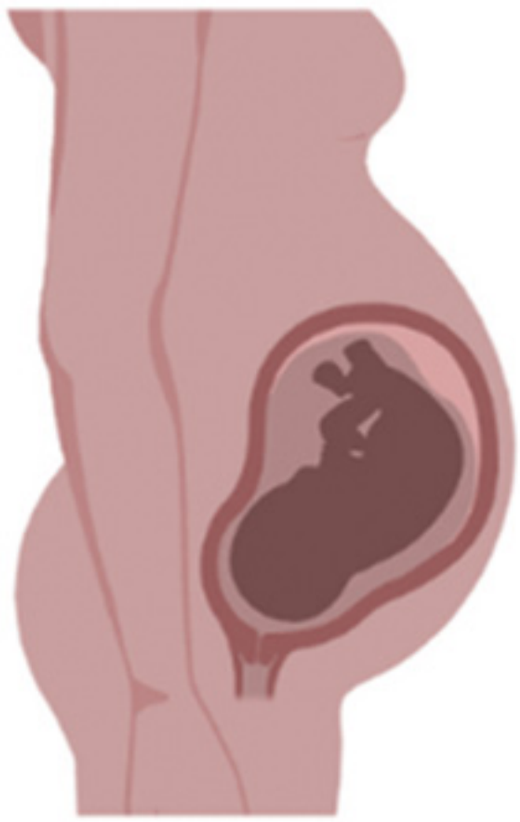
Stress
Nutrition
Diabetes
Smoking
Age
Chemicals
ART

In utero exposures

Stress

Gestational diabetes

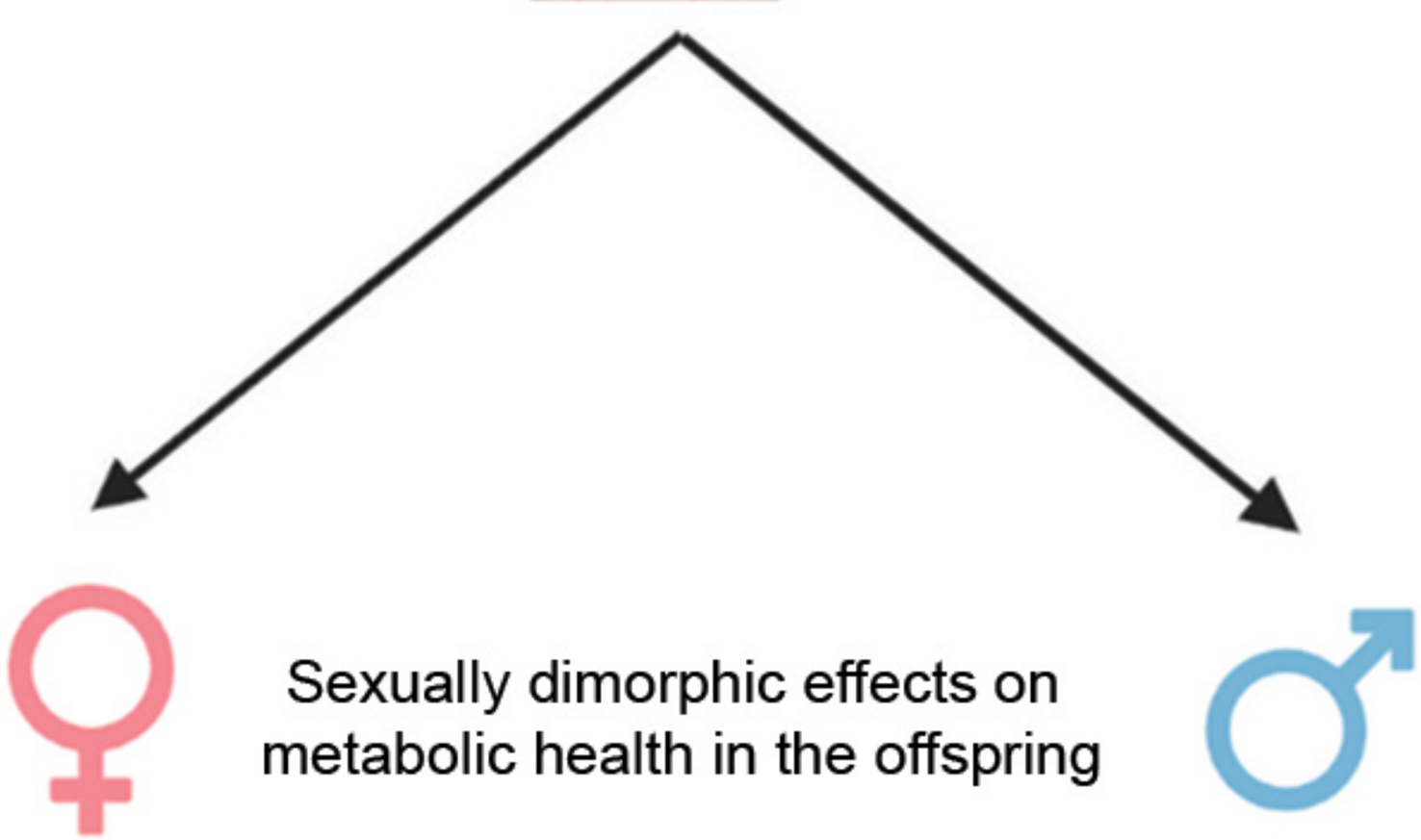
Impaired pregnancy adaptations



Maternal nutrition

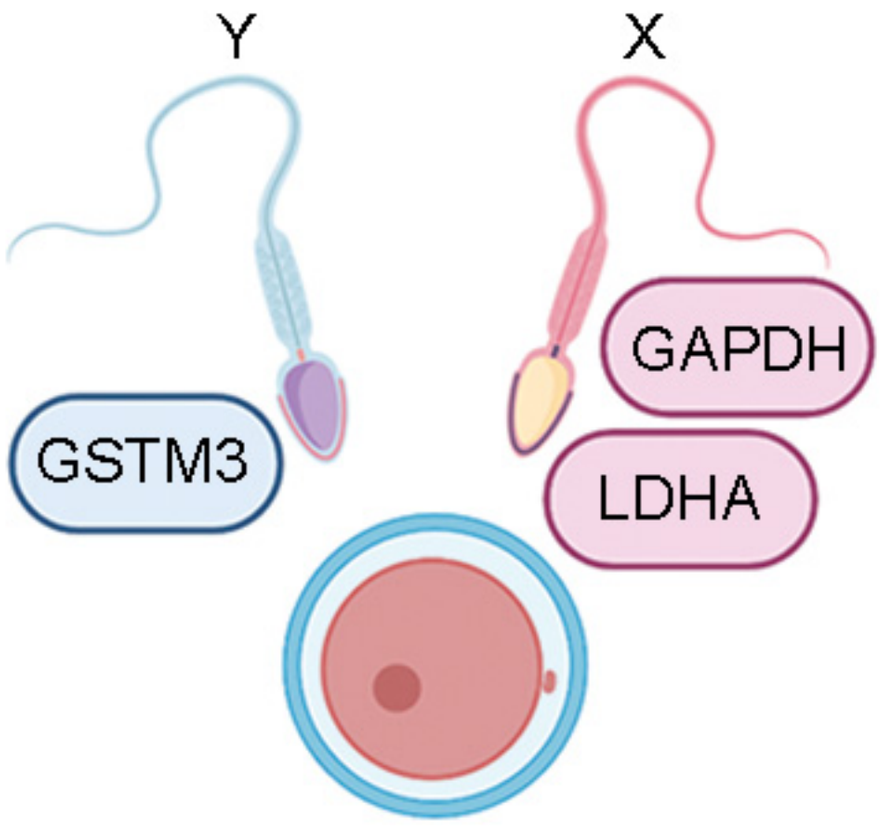
Smoking

Preeclampsia

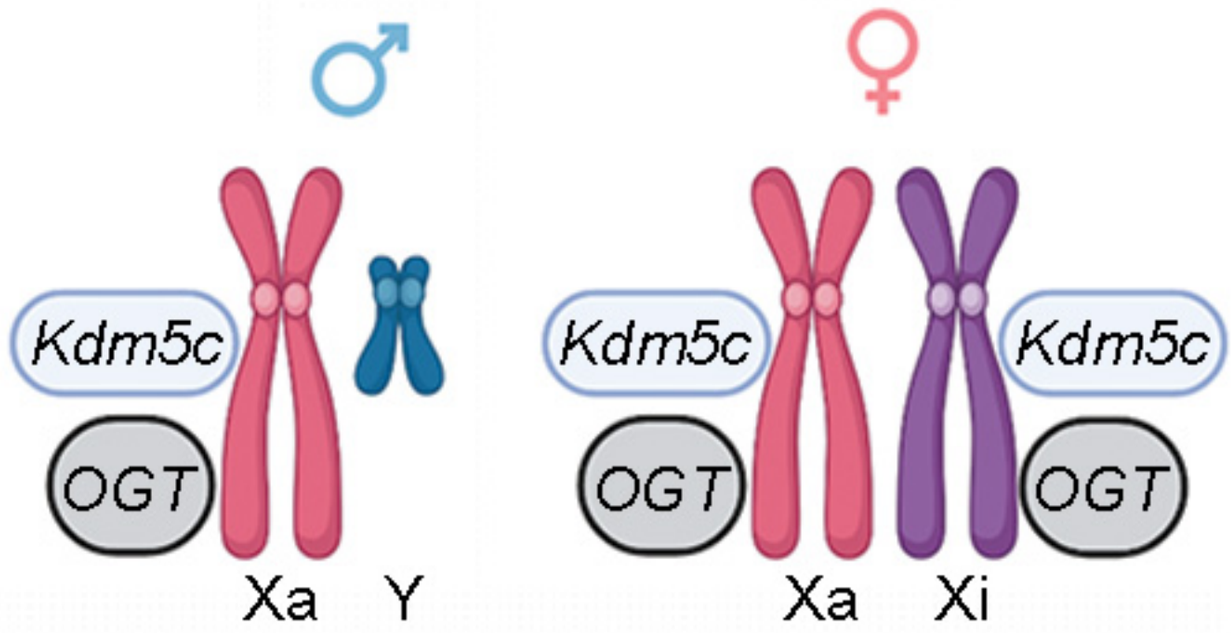


Sexually dimorphic effects on metabolic health in the offspring

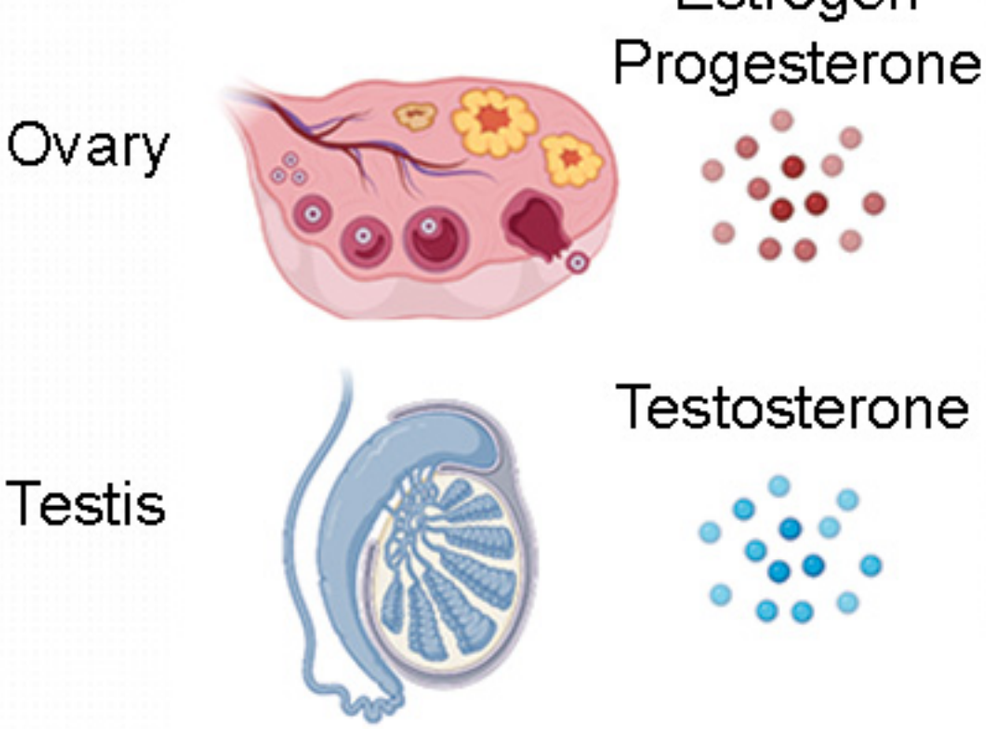
a


Y
X
GAPDH
GSTM3
LDHA

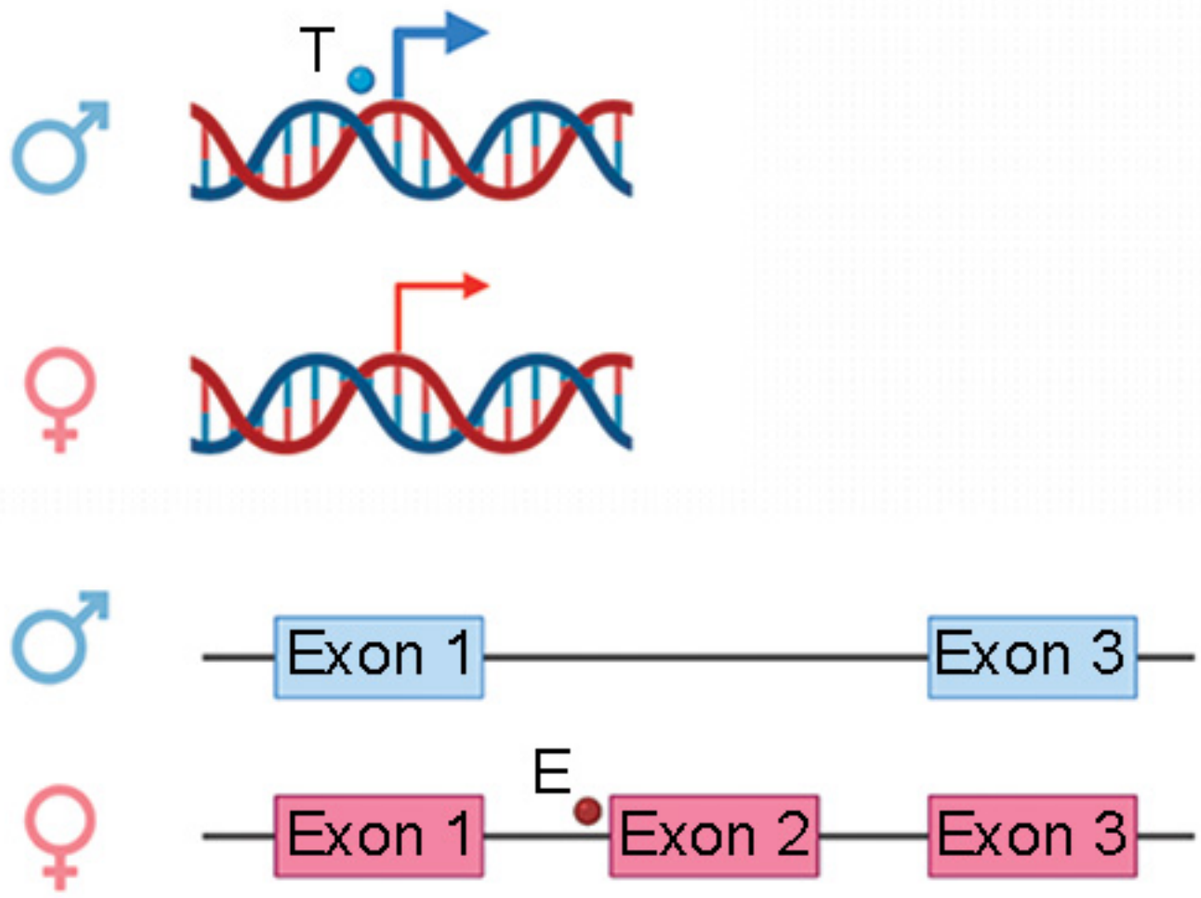
b


Kdm5c
OGT
Xa
Y
Kdm5c
Kdm5c
OGT
OGT
Xa
Xi

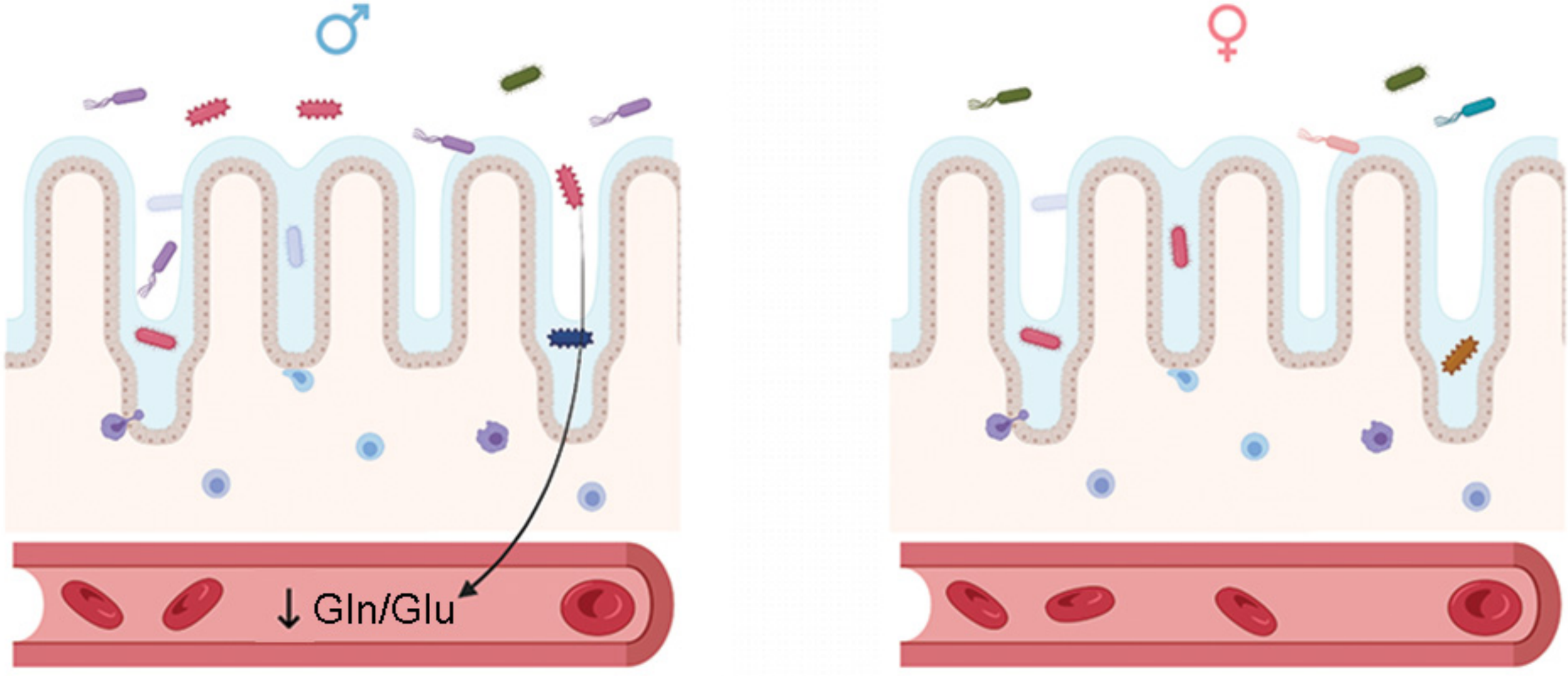
c

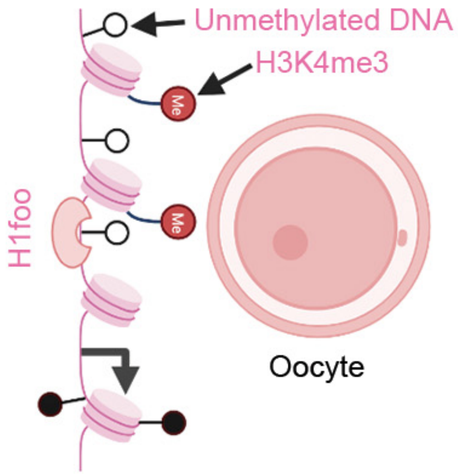

Estrogen
Progesterone
Ovary
Testosterone
Testis

d

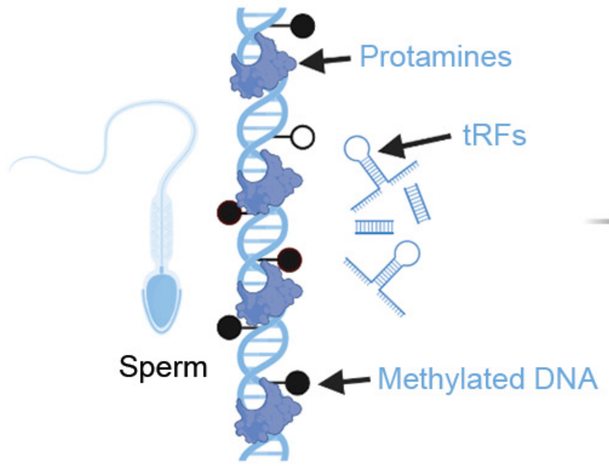

T
Exon 1
Exon 3
E
Exon 1
Exon 2
Exon 3

e


↓ Gln/Glu



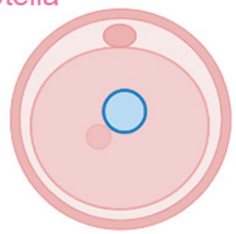
Oocyte



Sperm

Maternal PN:
- DNA methylation protected by Stella
- H3K4me3 controls MZT

Paternal PN:
- Active DNA demethylation
- tRFs control early gene expression



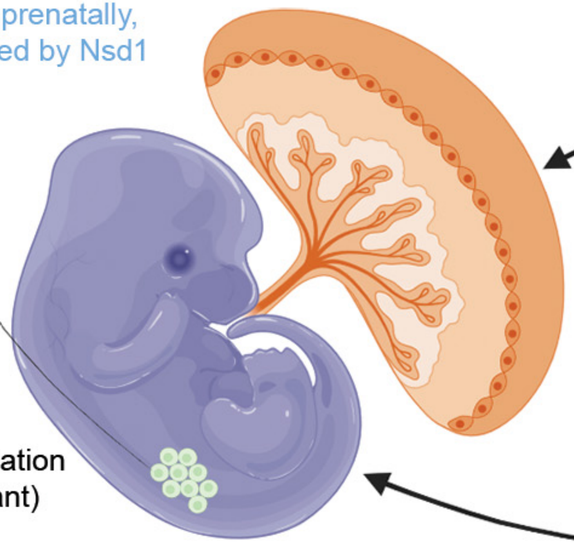
Zygote

- Passive DNA demethylation
- XCI
- HP1 depleted from autosomes
- H3K27me3-dependent imprinting

- Zfp57 and Zfp445 protect imprinted DMRs

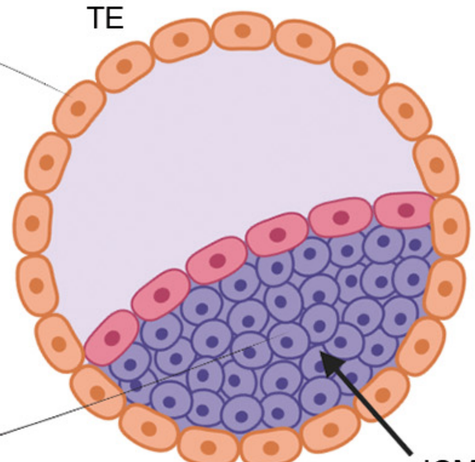
DNA methylation
postnatally,
guided by Setd2

DNA methylation
prenatally,
led by Nsd1



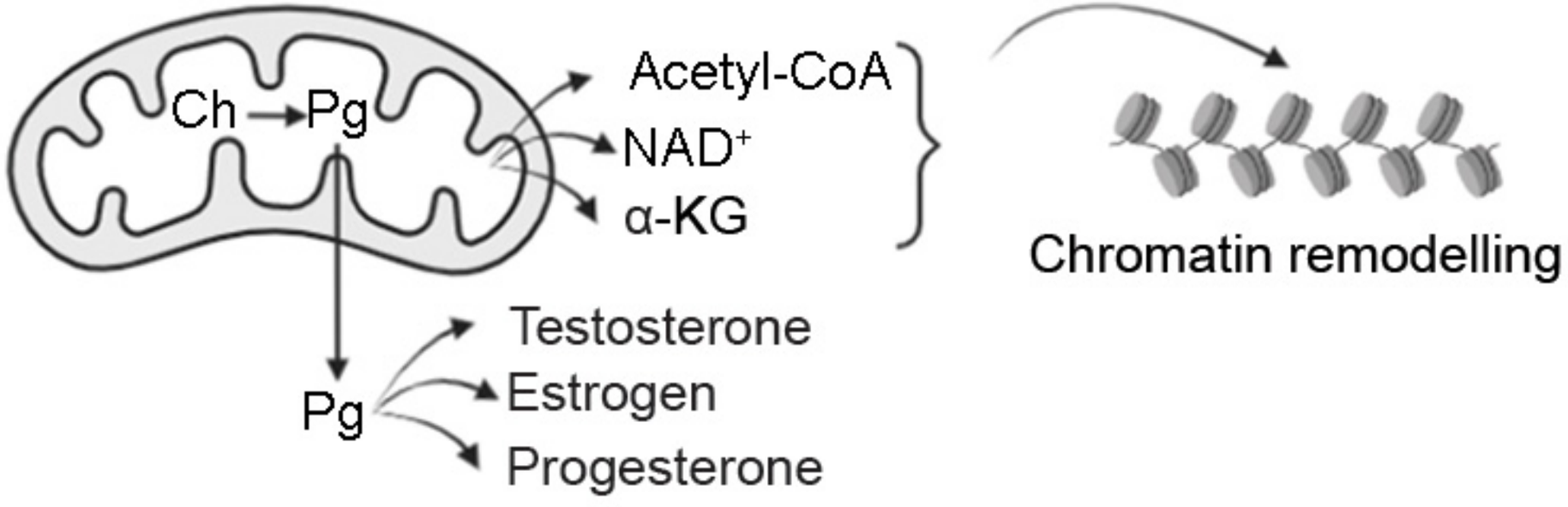
DNA demethylation
(IAPs resistant)

PGCs

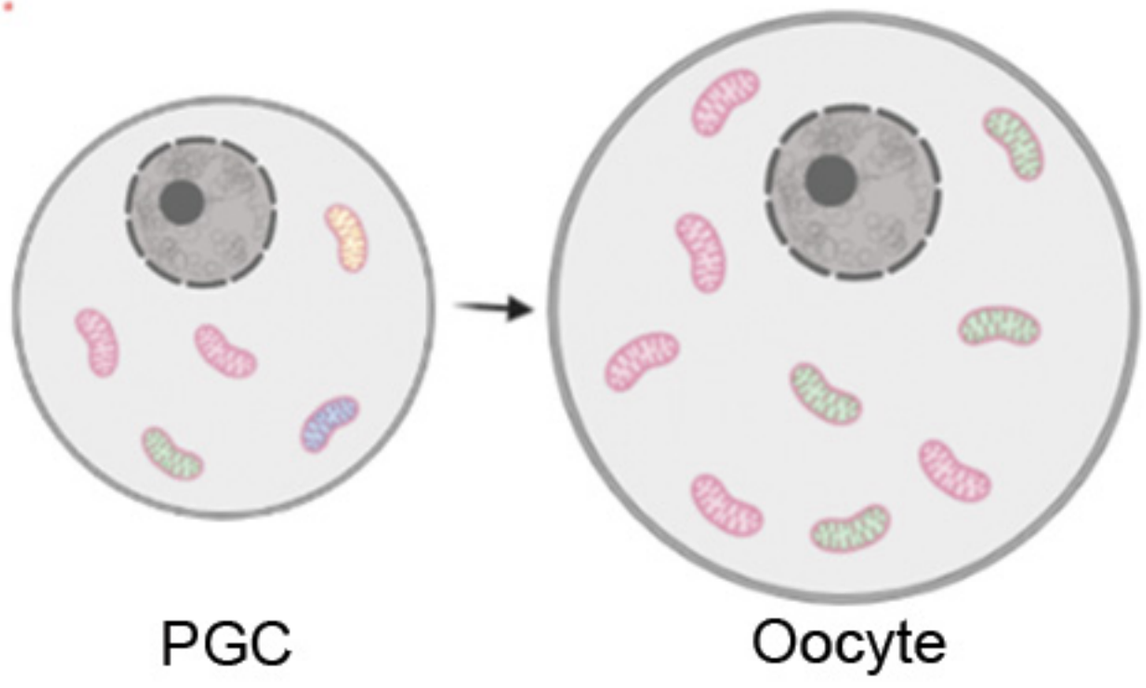


- De novo DNA methylation
-Ezh2 recuited by estrogen
- Jhmd2a recruited by testosterone

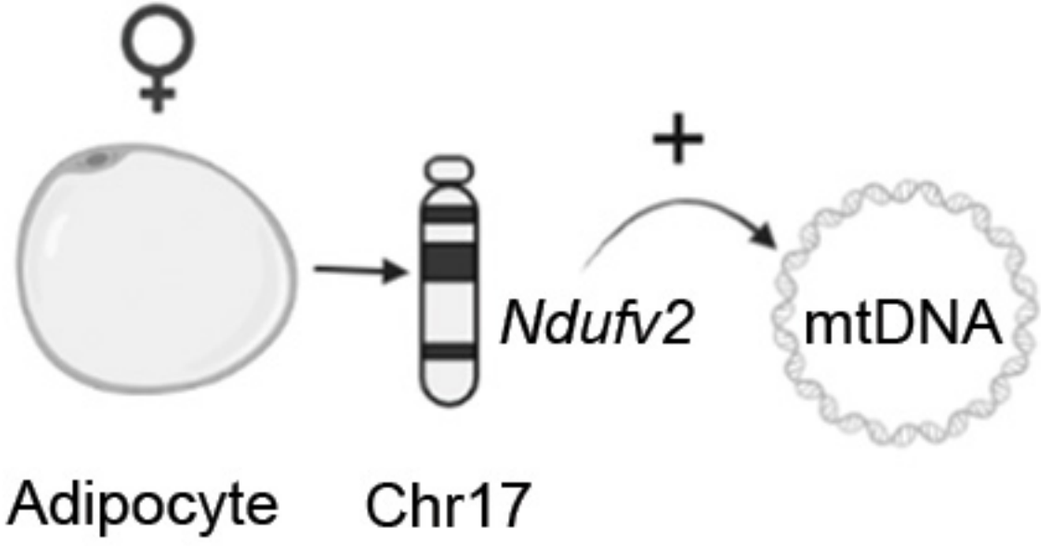
a


Ch
Pg
Acetyl-CoA
NAD+
α-KG
Chromatin remodelling
Pg
Testosterone
Estrogen
Progesterone

b

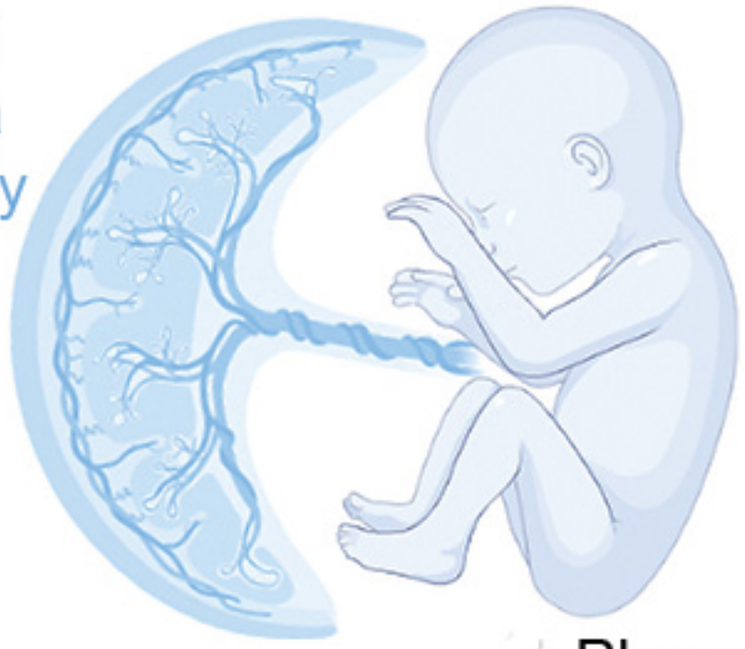

PGC
Oocyte

c


+
Ndufv2
mtDNA
Adipocyte
Chr17

Size
Glutamine use
in mitochondria
11βHSD2 activity

♂ Size



Survival ♀

Efficiency
Functional reserve
Polyamine metabolism
H3K27me3 levels

Placental hormones
Placental metabolites
(DiAcSpm)
Fetal hormones
(Testosterone)

Pre-eclampsia
Gestational diabetes

Suboptimal pre-conceptional exposures

Suboptimal intrauterine exposures

Metabolic disease (type 2 diabetes, obesity, metabolic syndrome)

Maternal germline

Ovary

Oocyte

Sex modifiable responses:
- Histone marks
- Histone variants
- Imprinted DMRs
- ncRNAs (tRFs - sperm)
- Mitochondria

Sperm

Testis

Paternal germline

Fertilization

Maternal effects

Altered epigenetic reprogramming:
- DNA methylation (LTRs, ERVs)
- Histone marks

Altered epigenetic programming:
- Cell differentiation
- XCI
- DNA methylation
- Histone marks
- ncRNAs
- Gene expression

Placental hormones

Age effects

Estrogen decline

Sexually dimorphic responses:
- Epigenome
- Transcriptome
- Microbiota
- Mitochondria
- Cellular metabolism
- Hormones

Age effects

Testosterone decline
Accelerated ageing