REPRODUCTION
REVIEW

Early sex-dependent differences in response to environmental stress

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Abstract

Developmental plasticity enables the appearance of long-term effects in offspring caused by exposure to environmental stressors during embryonic and foetal life. These long-term effects can be traced to pre- and post-implantation development, and in both cases, the effects are usually sex specific. During preimplantation development, male and female embryos exhibit an extensive transcriptional dimorphism mainly driven by incomplete X chromosome inactivation. These early developmental stages are crucial for the establishment of epigenetic marks that will be conserved throughout development, making it a particularly susceptible period for the appearance of long-term epigenetic-based phenotypes. Later in development, gonadal formation generates hormonal differences between the sexes, and male and female placentae exhibit different responses to environmental stressors. The maternal environment, including hormones and environmental insults during pregnancy, contributes to sex-specific placental development that controls genetic and epigenetic programming during foetal development, regulating sex-specific differences, including sex-specific epigenetic responses to environmental hazards, leading to long-term effects. This review summarizes several human and animal studies examining sex-specific responses to environmental stressors during both the periconception period (caused by differences in sex chromosome dosage) and placental development (caused by both sex chromosomes and hormones). The identification of relevant sex-dependent trajectories caused by sex chromosomes and/or sex hormones is essential to define diagnostic markers and prevention/intervention protocols.

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Introduction

Thefoetal origins of adult disease hypothesis (so-called 'Barker hypothesis') (Barker & Osmond 1986) proposes that undernutrition during gestation is, in part, responsible for adult cardiac and metabolic disorders due to foetal programming *in utero* that permanently shapes the body's structure, function and metabolism and contributes to susceptibility to adult diseases. This hypothesis has led to a wider theory, known as the 'Developmental Origins of Health and Disease (DOHaD)', which is based on developmental plasticity (the ability of the genotype to produce different phenotypes in response to different environments) and the concept of evolutionary mismatch (evolved traits that were once advantageous but became maladaptive due to changes in the environment). There are several critical stages when development is malleable (periconception, pregnancy and early postnatal life) and exhibits an enhanced plasticity that enables the organism to fine-tune epigenetic control of gene expression in accordance with environmental cues. During these periods, the embryo can adapt to novel conditions and diverse environments, reprograming developmental trajectories in order to confer the best chance of survival and reproductive success. However, these adaptive changes can conflict with the postnatal environment and impair adult heath. The time of maximal plasticity appears during periconception, as plasticity is reduced progressively as foetal development proceeds (Fig. 1). There are three key periods (embryonic, foetal, lactation) during which environmental stress exerts a greater effect. Sexual dimorphism in the DOHaD context of health and disease is a phenomenon among a variety of species, but it generally occurs in most common noncommunicable diseases (NCDs), including cardiovascular disease, metabolic diseases, hypertension, neurological disorders and cancer (Junien *et al*. 2012, Kalisch-Smith *et al*. 2017). NCDs continue to be the leading cause of deaths worldwide and were responsible for 38 million of the world's 56 million deaths in 2012, and WHO predicts a 17% increase in NCDs during the next decade (Mendis *et al*. 2015). The concept of DOHaD represents an important approach to the understanding and prevention of the alarming and increasing incidence of NCD, as recent studies of variable regions of neonatal methylome

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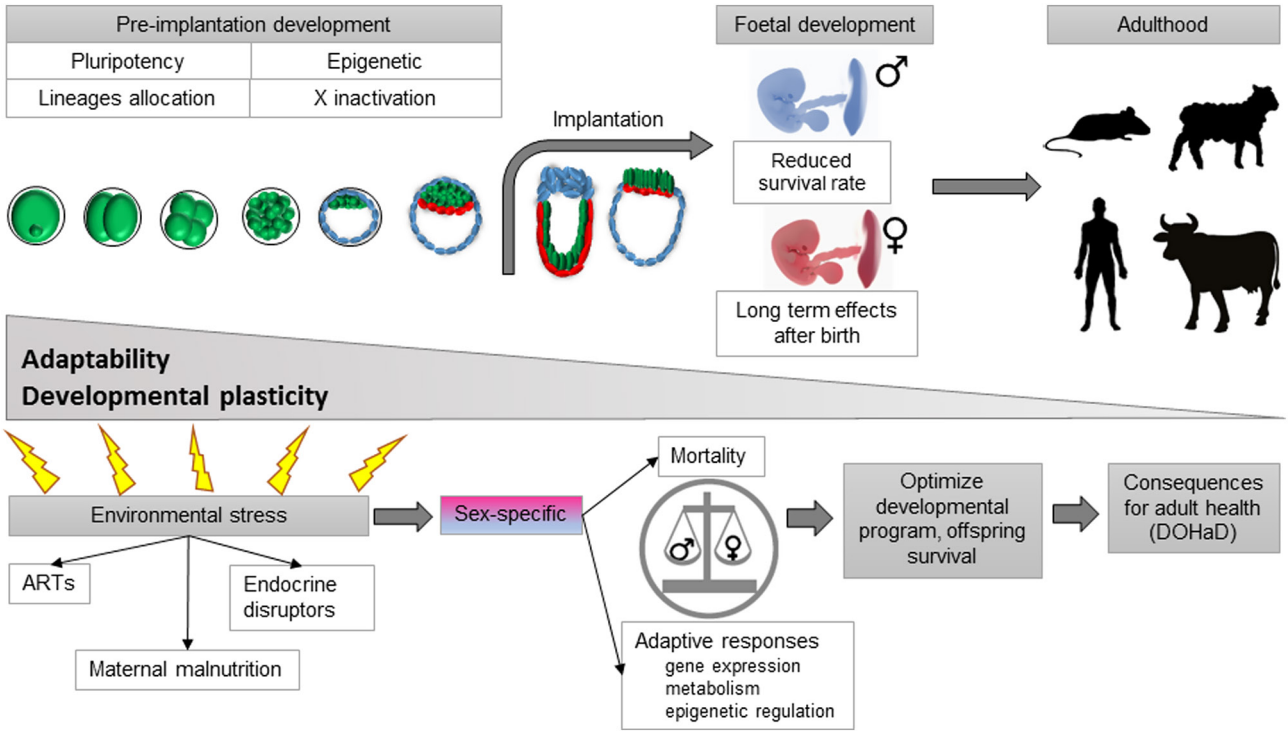

Figure 1 Pre- and peri-implantation embryo adaptations to environmental stress. Comparative pre- and peri-implantation embryo development in mammals. Critical events taking place during this developmental window (pluripotency emergence, epigenetic reprogramming, lineage allocation and X chromosome inactivation) render the embryo especially sensitive to environmental stress. Such embryos and the resulting foetus respond to stressors by sex-specific mortality or by adaptive responses in order to optimize their developmental program and offspring survival. The adaptability due to developmental plasticity (the ability of the genotype to produce different phenotypes in response to different environments) decrease throughout embryo and foetal development until early postnatal life. Later in adult life, this adaptability disappears and only the brain maintains a certain degree of plasticity. The female placenta (in pink), due to its higher adaptability, buffers more efficiently the impact of endogenous and exogenous stressors on the foetus and it is less compromised than male foetus (in blue) under similar stress conditions. However, compensatory mechanisms can compromise adult health according to the developmental origins of adult health and disease (DOHaD).

demonstrate that the interaction of genotype with the uterine environment accounts for 75% of unexplained variability (Teh *et al*. 2014).

In the context of DOHaD, the epigenetic marks that respond to the environment register the effects during the development of a specific sex (Heijmans *et al*. 2009). Environmental epigenetics investigates environmental factors that impact on the organism to modulate the expression of genes across the life course. The two most prominent stages are the perimplantation period and the period of foetal development, where epigenetic alterations may affect not only birth outcomes but also lifelong health. Some early environmental events such as stress, nutrition, behavior and environmental pollutants may disturb the precisely timed processes that sculpt the embryo and foetus in a sex-dependent manner and thus influence its health in later life (Attig *et al*. 2010). We have reported that sex differences in embryonic metabolism, gene expression and epigenetics start from the blastocyst stage (Gutierrez-Adan *et al*. 2000, 2006, Bermejo-Alvarez *et al*. 2008), and these differences are caused by extra gonadal and dosage effects of genes encoded on sex chromosomes. Sex has been proposed as a major factor determining the type and severity of the long-term effects originating during the preimplantation period (Bermejo-Alvarez *et al*. 2011*a*). Preimplantation developmental plasticity has evolved in order to offer the best chances of survival under changing environments. Conversely, environmental conditions experienced in early life can dramatically influence neonatal and adult biology, which may result in detrimental long-term effects (Laguna-Barraza *et al*. 2012). There are many human and animal studies demonstrating sexual differences in response to various developmental insults during periconception (Fleming *et al*. 2015*a*) and pregnancy (Sundrani *et al*. 2017) and demonstrating sexual differences in response to various developmental insults during pregnancy (Sundrani *et al*. 2017). However, there is a gap in knowledge of the mechanism(s) mediating environmental and maternal programing of preimplantation development. For example, how can environmental conditions during the preimplantation period have a long-term effect? How does the early embryo sense its environment and how

are morphogenesis, metabolism, genetic and epigenetic alterations induced? What are the sex-specific differences and how are they maintained, erased or gave rise to new ones?

Preimplantation embryo development, a crucial period for the appearance of sex-specific long-term effects

Sex differences in gene expression emerge with embryonic genome activation, and they are dynamic throughout the lifespan. Although some authors have suggested that transcriptional sex differences are higher in adult tissues compared with preimplantation embryos at the 8-cell stage (Lowe *et al*. 2015), no transcriptional analysis in adult tissues has reported the massive transcriptional sex differences observed in both mouse (Kobayashi *et al*. 2006) and bovine (Bermejo-Alvarez *et al*. 2010) blastocysts. Preimplantation embryo development constitutes a critical period for the establishment of epigenetic marks, being one of the two unique moments during the lifespan where genome-wide demethylation occurs (Seisenberger *et al*. 2012). During these early developmental stages, key epigenetic processes, such as X chromosome inactivation (XCI) or initial telomere lengthening, take place (de Frutos *et al*. 2016).

Table 1 Different studies showing sex-specific effects of environmental alterations during preimplantation development, either *in vivo* through maternal stress or *in vitro* by assisted reproductive technologies (ARTs).

	Treatment	Effect			Reference
		Both sexes	Females only	Males only	
Maternal stress					
Mouse	Heat stress-induced oxidative damage in pregnant mice and *in vitro* cultured embryos			Increased embryo loss	Perez-Crespo *et al*. (2005)
Sheep	Restriction of B vitamins and methionine in maternal diet	Obesity, insulin resistance, elevated blood pressure, DNA methylation alterations		More severe phenotype	Sinclair *et al*. (2007)
Mouse, rat	Low-protein maternal diet	Cardiovascular, metabolic and behavioural alterations	More severe phenotype		Fleming *et al*. (2015*b*)
Assisted reproductive technologies					
Bovine	High glucose environment *in vitro*		Increased embryo loss		Gutierrez-Adan *et al*. (2001)
Mouse, bovine	High glucose environment *in vitro*	Increased apoptosis	Increased survival and implantation		Jimenez *et al*. (2003)
Mouse	*In vitro* culture with serum	Altered behavior, imprinted genes expression	Increased body weight, liver steatosis	Hyperactivity, anxiety	Fernandez-Gonzalez *et al*. (2004)
Mouse	*In vitro* culture without CSH2	Restricted foetal growth, increased body weight and adiposity, placenta alterations		Increased body weight and adiposity, decreased brain size	Sjoblom *et al*. (2005)
Mouse	ICSI with DNA-fragmented sperm	Reduced embryo development and offspring, altered gene transcription and methylation, increased mortality, premature aging	Behavioural alterations, higher body weight and organ size, increased tumor incidence	Delayed active demethylation of male pronucleus	Fernandez-Gonzalez *et al*. (2008)
Mouse	IVF, ISCI, SCNT	Increased body weight	Altered glucose clearance, higher body weight and adiposity		Scott *et al*. (2010)
Mouse	IVF			Increased body weight and heart size, glucose intolerance	Donjacour *et al*. (2014)
Mouse	IVF	Sex-specific differences in sterol metabolism, redox state, mobilization and oxidation of fatty acids	Increased fat accumulation, altered fat metabolite composition	Altered liver metabolite composition	Feuer *et al*. (2014)
Mouse	IVF		Increased apoptosis and pregnancy loss	Placental overgrowth, postnatal overgrowth	Tan *et al*. (2016)

These dynamic changes of the epigenetic landscape exert a double impact on sex-specific long-term effects. On the one hand, incomplete XCI results in sex-unbalanced expression of X-linked genes, leading not only to an upregulation of these genes in females, but also to a genome-wide sex-specific transcriptional regulation in autosomal genes. On the other hand, the dynamic changes in the epigenome make this period particularly vulnerable to the appearance of aberrant epigenetic marks. In this perspective, the combination of the greatest transcriptional sexual dimorphism with the most susceptible period for the appearance of stable epigenetic alterations results in the most relevant period for the generation of sex-specific responses to stressors, leading to sex-specific phenotypic consequences for the offspring (Table 1).

Sex chromosomes drive sex differences in the absence of gonads

In the absence of gonads, and therefore without any sex-specific hormonal bias, sexual dimorphism during the preimplantation period relies solely on the differences in sex chromosome dosage. Thus, Y-linked genes will only be expressed in males, whereas X-linked genes will be upregulated in females if XCI is not accomplished, which is actually the case for most preimplantation embryos (Bermejo-Alvarez *et al*. 2012*a*). The dynamics and mechanism leading to XCI during preimplantation development have been the subject of debate over the last 10 years. Initial experiments conducted in the mouse observed that XCI was effectively accomplished in an imprinted manner by the blastocyst stage, experiencing a reactivation exclusively in the inner cell mass (Kay *et al*. 1994). This situation was thought to be comparable to other mammalian species, but transcriptional studies in bovine blastocysts refuted this notion, as most X-linked genes were upregulated in female blastocysts compared to their male counterparts (Bermejo-Alvarez *et al*. 2010). This situation was also observed in rabbit and human blastocysts by conducting *in situ* hybridization (ISH) studies (Okamoto *et al*. 2011), and in pigs, by transcriptional (Park *et al*. 2012) and methylation analyses (Hwang *et al*. 2015), so the mouse model was deemed to be more an exception than a rule for XCI dynamics (Bermejo-Alvarez *et al*. 2012*a*). Indeed, bovine (Bermejo-Alvarez *et al*. 2010) and rabbit (Okamoto *et al*. 2011) embryos achieve extensive XCI later, around the time of gastrulation, which opens a large window when the upregulation of X-linked genes in female embryos occurs and provides a molecular basis for the appearance of sex-specific phenotypes. In the case of human embryos, a recent study using single-cell RNA-Seq has observed that before XCI is accomplished, biallelic expression of *XIST* in female embryos causes progressive dampening of X chromosome expression (Petropoulos *et al*. 2016), an unexpected phenomenon that urges the revisiting of XCI dynamics in human stem cells (Lamas-Toranzo *et al*. 2017). This progressive downregulation, occurring in both X-chromosomes, gradually compensates for the differences in the expression of X-linked genes between the sexes, but it still leaves a large period of significant transcriptional sexual dimorphism – almost all preimplantation development, in fact – which may result in sex-specific long-term effects.

Sex chromosomes regulate autosomes, amplifying transcriptional sex differences

Sex-biased expression of Y- and X-linked genes is not the only transcriptional difference between male and female preimplantation embryos. Extensive transcriptional sex differences, affecting not only genes located on sex chromosomes but also many autosomal genes has been uncovered in preimplantation embryos by genome-wide transcriptional analyses in mice (Kobayashi *et al*. 2006) and, especially, cattle blastocysts, where almost one-third of the genes actively expressed showed transcriptional sex differences (Bermejo-Alvarez *et al*. 2010). The wider extent of transcriptional sex differences in bovine blastocysts compared with murine can be explained as a consequence of the incomplete XCI status in cattle compared to mice. In agreement with this notion, transcriptional sex differences are dramatically reduced in more advanced stages of bovine embryogenesis, such as in Day 14 (Bermejo-Alvarez *et al*. 2011*b*) or Day 19 (Forde *et al*. 2016) elongated conceptuses, once XCI has been mostly accomplished. Besides, gene co-expression networks analysis between X-linked genes upregulated in bovine female blastocysts and autosomal genes showing transcriptional sex-dependent differences has identified a major module of autosomal genes for which sex differences are likely driven by the X genes (Itoh & Arnold 2014). Effects of X chromosome on autosomal transcriptional regulation are also evident in sex-reversed mouse models: over 1000 autosomal genes were found differentially expressed between XY and XX males or XX and XY females (Wijchers *et al*. 2010).

The mechanisms by which upregulated X-linked genes affect the expression of autosomal genes remain to be determined; however, the presence of different genes encoding for chromatin modifiers on the X chromosome provides a plausible explanation. Both *KDM5C* (H3K4 demethylase, also known as *JARID1C*) and *UTX* (H3K27 demethylase, also known as *KDM6A*) are X-linked genes, and the chromatin marks they regulate are particularly relevant for preimplantation development. H3K4me3 is involved in transcriptional activation, whereas H3K27me3 is associated with transcriptional repression. Both opposing marks are

present in early blastomeres on the promoters for genes involved in early cell differentiation. Bivalent domains keep these genes, including *Sox2*, *Lifr*, *Nanog*, *Cdx2*, *Eomes* and *Tbpa*, poised for activation, occurring once they lose the repressive H3K27me3 mark (Dahl *et al*. 2010). Given the sex differences in the transcription of key regulators of these bivalent domains, the effect of different stressors on the differentiation processes occurring during preimplantation development may also be sex specific. This effect may depend on the stressor, as although high glucose exposure does not have a sex-dependent effect on inner cell mass and trophectoderm cell counts in mouse blastocysts (Bermejo-Alvarez *et al*. 2012*b*), human male blastocysts have been reported to exhibit a better quality trophectoderm (TE) than female when cultured *in vitro* (Ebner *et al*. 2016), which may have short-term consequences on implantation or long-term consequences on placental development.

Developmental consequences of preimplantation transcriptional sex differences

Genes which transcription is sex dependent exert different functions which may determine a sex-specific response to a given stressor (Fig. 1). Metabolic differences between male and female embryos could determine a differential response to nutritional stress that may result in sex-specific early embryonic death or long-term effects. Glucose metabolism was initially reported to be different between sexes based on the upregulation of glucose-6-phosphate dehydrogenase (*G6PD*) in bovine (Gutierrez-Adan *et al*. 2000, Wrenzycki *et al*. 2002) and human (Taylor *et al*. 2001) female blastocysts, and on the overexpression of the autosomal *SLC2A3* in bovine male blastocysts (Morton *et al*. 2007). However, gene ontology analysis of global transcriptional differences between male and female bovine blastocysts did not highlight glucose metabolism as a sexually dimorphic pathway (Bermejo-Alvarez *et al*. 2010). The specific transcriptional analysis of genes involved in either anaerobic glycolysis or the pentose phosphate pathway do not show a clear sex bias (Bermejo-Alvarez *et al*. 2011*a*), and the transcriptional response of bovine blastocysts to the presence of glucose was found to be unrelated to sex (Cagnone *et al*. 2011), all in agreement with the overall inconsistent reports regarding glucose-mediated sex biases (reviewed in Bermejo-Alvarez *et al*. 2012*b*). On the other hand, gene ontology analysis of bovine blastocysts highlighted mitochondria and protein translation, proteolysis and protein transport as sex-dependent pathways (Bermejo-Alvarez *et al*. 2010), which correlates with the differences between sexes in mtDNA content (Bermejo-Alvarez *et al*. 2008) and amino acid metabolism (Sturmey *et al*. 2010) as previously reported. Sex-specific differences in these and other pathways provide a molecular basis for the appearance of a sex-specific response to nutritional stressors and may also be used for non-invasive embryo sexing methods (Sturmey *et al*. 2010, Gomez *et al*. 2016).

Other pathways reported to exhibit sex differences include those related to apoptosis and embryo–maternal communication. The X-linked inhibitor of apoptosis *XIAP* is, as are most X-linked genes, upregulated in female embryos (Gutierrez-Adan *et al*. 2001), which is consistent with the notion that male embryos are more sensitive to oxidative-induced heat stress (Perez-Crespo *et al*. 2005). Nevertheless, under normal *in vitro* culture conditions, female bovine blastocysts seem to be more prone to apoptosis (Ghys *et al*. 2016). The differential apoptotic rates between sexes under different stressors may result in one sex being more susceptible than the other to blastomere loss, which may have consequences for subsequent development. Furthermore, sex differences may also affect embryo–maternal signaling, which is essential for proper late embryonic development and implantation. Interferon tau, *IFNT*, a major embryo-derived signaling molecule for pregnancy recognition in ruminants, and *PGRMC1*, a progesterone receptor, are upregulated in female bovine embryos (Larson *et al*. 2001, Arias-Alvarez *et al*. 2011, Bermejo-Alvarez *et al*. 2011*b*), and the embryokine CSF2 exerts a sex-specific response (Dobbs *et al*. 2014).

Finally, transcriptional differences between sexes may directly affect epigenetics in a sexually dimorphic manner, thereby exerting a direct impact on the appearance of sex-specific long-term developmental consequences. Apart from the X-linked genes already discussed, autosomal genes including both *de novo* DNA methyltransferases (*DNMT3A* and *DNMT3B*) and two genes related with histone methylation (*HMT1* and *ILF3*) are upregulated in male bovine blastocysts compared to their female counterparts (Bermejo-Alvarez *et al*. 2008). These transcriptional differences are reflected in the methylation levels of specific sequences such as the repetitive sequence *VNTR* (Bermejo-Alvarez *et al*. 2008) and one differentially methylated region (DMR) in the imprinted gene *IGF2* (Gebert *et al*. 2009), both being hypomethylated in female bovine blastocysts compared to males. Similar methylation differences are observed in closely related biological systems such as murine embryonic stem cells (ES), where XX cell lines are hypomethylated compared to XY lines (Zvetkova *et al*. 2005). Sexual dimorphism in histone modification may also arise, as differences in histone modifications methyltransferases of H3K4me3, H3K27me3 and H3K9me3, as well as DNA methyltransferases, have been observed in male and female E10 pig embryos (Gao *et al*. 2011).

Sexual dimorphism at the epigenetic level is directly bound to the appearance of sex-specific epigenetic-based phenotypes. An example of sex-biased epigenetic alteration is Beckwith–Wiedemann syndrome, a human epigenetic disorder to which the so-called 'Large Offspring Syndrome' in animals is considered equivalent

(Farin *et al*. 2004). This syndrome is caused by altered methylation patterns at imprinting domains; it occurs at a relatively high frequency in monozygotic twins, and in almost all cases, the affected twins are female (Lubinsky & Hall 1991, Weksberg *et al*. 2002). External stressors acting during the periconceptional period have also been reported to exert a sex-specific effect. In sheep, a methyl-deficient maternal diet during the periconceptional period leads to epigenetic alterations, which are more pronounced in males than in females (Sinclair *et al*. 2007). In rats, a restriction of dietary protein supplementation during the periconceptional period results in sex-specific cardiovascular and behavioral diseases in the offspring (Kwong *et al*. 2000, Watkins *et al*. 2008*b*), with females being more susceptible (Fleming *et al*. 2015*b*). The effect of this low-protein diet was mediated through changes in branched-chain amino acids and insulin levels in the uterine fluid, which were detected by embryos via the mTOR signaling pathway. These embryos were able to activate compensatory mechanisms in order to enhance maternal nutrient retrieval, by stimulating trophectoderm and primitive endoderm proliferation, endocytosis and cellular motility (Eckert *et al*. 2012, Sun *et al*. 2014, 2015). Although these responses protected foetal growth, at the same time, they led to abnormal growth and increased adult adiposity, resulting in adverse long-term effects, with female offspring more severely affected. Moreover, these effects were observed even when the embryos were transferred to mothers on a normal nutritional regime (Watkins *et al*. 2008*a*, 2010, 2011). Consistent with these studies, other authors have reported a greater response to stress by the female placenta compared to the male placenta in terms of gene expression (Clifton 2010, Osei-Kumah *et al*. 2011).

Sex-specific long-term effects mediated by assisted reproductive technologies

Assisted reproductive technologies may constitute an environmental stressor to the preimplantation embryo. Several studies reporting long-term consequences of *in vitro* culture or other ART identified a sex bias in the frequency and nature of the long-term effect (Fernandez-Gonzalez *et al*. 2004, Sjoblom *et al*. 2005, Feuer & Rinaudo 2012, Tarin *et al*. 2014). These deleterious effects have pointed to epigenetic alterations produced by ARTs. A number of reports have demonstrated that *in vitro* culture conditions induce alterations and errors in the epigenetic reprogramming of bovine embryos, leading to alterations in their DNA methylation pattern (Fernandez-Gonzalez *et al*. 2004, Niemann *et al*. 2010, Salilew-Wondim *et al*. 2015). Studies in mice have demonstrated that genomic imprinting in preimplantation embryos can be disturbed by specific culture conditions (Doherty *et al*. 2000, Khosla *et al*. 2001). Furthermore, mouse embryos cultured to the blastocyst stage using commercially available sequential media have been shown to suffer a shift in the expression of some non-imprinted genes (Morgan *et al*. 2008). Recently, we have shown that the presence of oviductal fluid within the culture medium during *in vitro* culture of bovine embryos provoked alterations of the methylation level of regions CpG in the developmental genes *MTERF2*, *ABCA7* and *OLFM1* and in the retrotransposon *LINE-1* at the blastocyst stage when compared to control conditions using conventional culture medium supplemented with BSA (Barrera *et al*. 2017). Others have also shown that the methylation levels of the embryonic epigenome are affected by culture conditions in a developmental stage-dependent manner (Salilew-Wondim *et al*. 2015). These epigenetic alterations, especially those provoked under suboptimal *in vitro* culture conditions, have been blamed as the cause of diverse disorders in the offspring, such as the 'Large Offspring Syndrome' in cattle and sheep (Chen *et al*. 2013). This condition is characterized by a disproportionate growth and reduced viability of the foetus. Similar effects are observed when mouse embryos are cultured *in vitro* with serum, but only in females, together with sex-dependent behavioral abnormalities (Fernandez-Gonzalez *et al*. 2004). However, in a similar model in mice, increased body weight and decreased brain size were only observed in males when they were cultured in the presence or absence of a specific growth factor, granulocyte-macrophage colony-stimulating factor, also known as colony-stimulating factor 2 (CSF2) (Sjoblom *et al*. 2005). CSF2 is a cytokine produced in the oviduct and endometrium that has been implicated in developmental programming (Giacomini *et al*. 1995, de Moraes *et al*. 1999, O'Leary *et al*. 2004, Nahar & Kadokawa 2016) and its expression is modified by environmental factors. For example, seminal plasma triggers CSF2 expression (Tremellen *et al*. 1998, O'Leary *et al*. 2004, Bromfield *et al*. 2014), while maternal obesity can suppress it (Nahar & Kadokawa 2016). In cattle, treatment with CSF2 from Day 5 to Day 7 after fertilization improved blastocyst development for female but not for male embryos. Furthermore, this treatment decreased embryo elongation and intrauterine accumulation of IFNT in females and affected the transcriptome and methylome in a different way for males and females (Siqueira & Hansen 2016).

Metabolic alterations in the offspring associated with ARTs are also dependent on sex according to different studies. In mice, long-term effects associated with *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT) were found to be sex specific, with females showing altered glucose clearance, higher body weight and adiposity (Scott *et al*. 2010*a*). In agreement, another study reported sex-specific differences in sterol metabolism, redox state, mobilization and oxidation of fatty acids after IVF in murine offspring, with females being more predisposed to increased fat accumulation (Feuer *et al*. 2014).

However, in another study in mice produced from IVF, altered postnatal growth and glucose intolerance were only observed in males (Donjacour *et al*. 2014). Additional sexual dimorphic patterns induced by IVF were reported by Tan and coworkers (Tan *et al*. 2016). In this study, female embryos were more susceptible to apoptosis during the preimplantation and mid-gestation stages. Later in gestation, both males and females showed reduced placental angiogenesis, but compensatory placental overgrowth was more evident in males. Finally, overgrowth was only observed in males after birth (Tan *et al*. 2016). Our group has also reported a large number of sex-dependent alterations after ICSI using fresh or frozen sperm, including behavioral alterations, higher body weight and organ size and increased tumor incidence in females (Fernandez-Gonzalez *et al*. 2008, Ramos-Ibeas *et al*. 2014).

Sex differences after implantation: another source for sex-specific long-term effects

Once the embryo has been implanted and XCI has been accomplished, transcriptional sex differences driven by sex chromosome dosage are reduced, but not abolished (Wijchers *et al*. 2010), compared to preimplantation development. However, once the gonads develop, a new factor for sexual dimorphism arises: sex hormones. From an evolutionary point of view, sex differences in foetal survival is seen as a way to adjust the energy invested by parents in male vs female offspring to the postnatal environment faced by the offspring (Trivers & Willard 1973, Koskela *et al*. 2009). According to the Trivers–Willard hypothesis, mothers with plentiful resources invest in the sex with a reproductive disadvantage while those mothers facing adverse environment preferentially produce offspring of the sex with a greater chance of reproductive success (Trivers & Willard 1973). The dominant concept is that female and male foetuses have different growth strategies, leading to differential survival and pregnancy outcomes. Thus, by their greater sensitivity to the maternal environment, females would follow modest growth changes and therefore better adaptation to deleterious signals such as maternal asthma and a restricted or high-fat diet. In contrast, male foetuses show a more divergent growth curve and as consequence poor adaptation to adverse environment (Clifton 2010, Eriksson *et al*. 2010, Cox *et al*. 2013). Although multiple observations support this hypothesis, there is a lack of solid experimental data from animal models in the literature unequivocally proving that male growth and survival in the womb is more affected than that of females when the environment is perturbed. It has been reported that a high-fat diet provokes under-methylation only of female placentas in mice, which is indicative of an active response to this stressor (Gallou-Kabani *et al*. 2010); this was confirmed by another study showing that female placenta was more responsive to nutritional perturbations by more significant changes in the placental transcriptome (Mao *et al*. 2010). In rats, this higher adaptation was also observed for the female placenta in contrast to its male counterpart in response to ethanol by decreasing the 11β-hydroxysteroid dehydrogenase-2 activity (Wilcoxon *et al*. 2003). In addition, in humans, the female placenta responds to antenatal steroid administration by upregulating 11β-hydroxysteroid dehydrogenase-2 (Stark *et al*. 2009). These observations suggest that male foetuses are less buffered than females against certain environmental insults. Thus, under various stressors, male foetuses exhibit higher late foetal mortality due to a greater *in utero* vulnerability (Eriksson *et al*. 2010), and their foetal growth is more affected than that of females (Sundrani *et al*. 2017) (Fig. 1).

Placental response to environmental stress

The placenta is a gestational interface between mother and foetus that controls foetal development and growth through the exchange of gases, nutrients and waste products and also in the production of pregnancy-induced hormones, growth factors and immune response. Thus, the adaptive properties of this organ are essential for foetal survival during specific stresses and seem to be the main reason for the sex differences associated with stress observed during early and later foetal stages, affecting adult life as well (Gabory *et al*. 2013). The reported higher protection of female offspring *in utero* (Wilcoxon *et al*. 2003, Vickers *et al*. 2011) could be due to inherent sex differences in the placenta making the female placenta more adaptive and plastic. The sex differences of the human placenta have been revealed at the transcriptomic level. Global transcriptomic analyses revealed that females possess more upregulated autosomal genes, including immune-regulating genes, than males (Sood *et al*. 2006). This may indicate that human female placentae respond better to potential infections. In contrast, analyses of isolated cells derived from human placentae revealed sex-dependent differences in four placental cell types: cytotrophoblasts, synctiotrophoblasts and arterial and venous endothelial cells. In these analyses, male placentae showed enrichment of signaling pathways reported to mediate graft vs host disease and other transcripts involved in immune function and inflammation (Cvitic *et al*. 2013). The authors suggested that male placentae may be forced to upregulate immune-associated transcripts in an attempt to counteract the response of the maternal immune system due to a reduced maternal–foetal compatibility compared to female placentae. It has also been suggested that the structural and functional differences may contribute to enhance male susceptibility to *in utero* environmental perturbations (Kalisch-Smith *et al*. 2016). The placenta of human males invades more deeply into the spiral arteries than

female placenta. In contrast, the latter exhibits more effective placental surface differentiation (Alwasel *et al*. 2014). In the spiny mouse (*Acomys cahirinus*), the female placenta contains a larger labyrinth but smaller spongy region than that of the male, and genes related to glucose transport were found to be differentially expressed among sexes in the two placental regions (O'Connell *et al*. 2013*a*). Based on rodent literature, it has been suggested that perturbation during early placental development may have a greater impact on viability and growth of the female foetus while those occurring later in gestation may preferentially affect the male foetus (Kalisch-Smith *et al*. 2016).

Placental response to maternal diet

All these inherent differences between male and female placenta are likely behind the differential response to the maternal environment. The available literature demonstrates that environmental exposures can disrupt placental morphology, epigenetic regulation and gene expression in a sex-dependent manner (Tarrade *et al*. 2015). The placenta adapts to the maternal diet and metabolic status altering foetal nutrient supply. This is done in a different way in male and female placentae showing different profiles of energy metabolism including glycogen storage and metabolism throughout gestation (O'Connell *et al*. 2013*b*). Animal studies have observed transcriptional sex differences in placentae under different maternal dietary regimes. In mice, it has been found that a low-fat vs high-fat diet deregulates significantly more genes in female than in male placentae (Mao *et al*. 2010) mainly affecting cell signaling of immune cells and uptake and metabolism of amino acids. In contrast, the genes affected in male placenta were related to development and function of the vascular system as well as uptake and metabolism of glucose and fatty acids (Gabory *et al*. 2012). These differences in gene expression can be explained by changes in epigenetic regulation. Thus, female placentae were found to be hypomethylated in response to a high-fat diet in mice (Gallou-Kabani *et al*. 2010, Gabory *et al*. 2012). In rats, high-fat and high-salt diets lowered placental size of males, increasing the expression of genes associated with metabolism and pro-inflammatory mediators, which has been related to future cardio-metabolic disturbances (Reynolds *et al*. 2015). In rabbits, high-fat and high-cholesterol diets provoke higher accumulation of triacylglycerol in males while they upregulate genes of the lipid pathway in females (Tarrade *et al*. 2013). In non-human primates, nutritional restriction has been reported to suppress genes related to programmed cell death and enhance genes associated with cell proliferation in female placentae. In contrast, male placentae showed less responsiveness in terms of the number of affected genes and pertinent pathways (Cox *et al*. 2013). In humans, it has been reported that obese women show thicker and less efficient placentae showing significant alterations meant for adaptation only in the female placenta (Mandò *et al*. 2016).

Placental response to maternal psychological stress

In utero exposure to maternal psychological distress affects the function of the foetal hypothalamus–adrenal–pituitary (HPA) axis, which has implications for the development of diverse foetal tissues (Cottrell & Seckl 2009, Harris & Seckl 2011). Although this response to stress could be physiological, high stress and prolonged levels of prenatal stress and cortisol exposure can produce negative effects on foetal development (Laplante *et al*. 2004, Davis & Sandman 2010). In response to this signaling, differential sex-dependent responses have been reported in both human and animal studies at both normal and pathological distress levels (Sandman *et al*. 2013). In order to buffer the impact of maternal psychological stress, placentae express 11β-HSD2 that degrades cortisol to cortisone. Interestingly, the female placenta is more reactive than the male, and higher expression of 11β-HSD2 has been shown in female placenta exposed to glucocorticoid treatment than that of males (Stark *et al*. 2009). Treatments with glucocorticoids have been shown to induce other alterations such as increased placental oxidative stress, expression of pro-inflammatory cytokine and 5 alpha-reductase (Scott *et al*. 2009, Vu *et al*. 2009, Stark *et al*. 2011), showing a more pro-oxidative state in male placentae.

According to the hypothesis of DOHaD (Barker 2007), the glucocorticoids transmitted from mother to foetus during gestation could be exploited by the foetus to optimize development in preparation for survival and success after birth (Gluckman *et al*. 2009, Ellis & Del Giudice 2014). However, it has been argued that while sex-specific foetal growth strategies result in greater adaptive flexibility for females in the short term, especially under maternal distress, it increases the long-term risk of developmental psychopathology. Thus, anxiety and depression are two examples of stress-related psychopathology for which there are clear sex differences in presentation and prevalence (Altemus *et al*. 2014).

Response to sex hormones and endocrine disruptors

From the early stages of development, the foetus is very susceptible to steroid hormone exposure, which plays important roles in tissue and organ differentiation (Sundrani *et al*. 2017). Thus, androgens have a role not only in the maturation of the male foetus but also in the development of mammary gland and folliculogenesis in the female foetus. Changes in these hormones due to maternal distress or endocrine disruptors differentially affect male and female foetuses. Low levels of androgen

affect the development of testes in male foetuses and ovaries and adrenal cortex in female foetuses (Abbott *et al*. 2006) and an excess provokes alteration in testicular development (Rojas-García *et al*. 2013) and induces masculine characteristics in female foetuses (Wolf *et al*. 2002), foetal growth retardation (Manikkam *et al*. 2004) and polycystic ovarian syndrome in adult life (Hogg *et al*. 2011). At the same level as the sex hormones, also affecting the overall development of the foetus, endocrine-disrupting chemicals (EDC) have been highlighted as the main effectors altering placental and foetal performance in a deleterious way. EDC has been shown to alter gene transcription, signaling pathways (Tan *et al*. 2013, Xu *et al*. 2015), DNA methylation patterns (Nahar *et al*. 2015), miRNAs (Avissar-Whiting *et al*. 2010) and placental structure (Tachibana *et al*. 2007). However, a sex-dependent placental response is still not strongly supported by the literature. Thus, the only evidence to date is the differential DNA methylation of AluYb8 in response to the xenoestrogen burden in human placentas as higher methylation in males and no response in females (Vilahur *et al*. 2014). Other studies have also suggested that EDC can affect imprinted genes in the placenta (Susiarjo *et al*. 2013, Shin *et al*. 2014), but sex differences were not explored. Further studies are thus essential in addressing this critical gap in our understanding of how environmental chemicals interact with sex to affect placental outcomes.

Conclusions

Exposure to environmental stressors during preimplantation and peri-implantation development occurring either *in vivo*, such as during maternal diet imbalance, or *in vitro*, due to ARTs, activates compensatory responses in the embryo. These responses are often sex specific, due to the marked sex differences in transcriptional profiles at these stages, and may lead to long-term consequences, given the relevant epigenetic changes occurring during these early stages of development. Later, during foetal development, sex-specific foetal and placental responses may determine sex-specific long-term consequences to the exposure of environmental stressors such as maternal diet, stress or the exposure to endocrine disruptors. The epigenetic marks induced by specific environmental factors need to be identified in order to improve our understanding of the ontology of chronic diseases in response to these environmental factors by taking into account that male and female embryos and fetuses respond differently to the same environmental insult. Advances in transcriptomic and epigenomic tools make it possible to uncover the epigenetic roots of chronic diseases and their link to environmental insults and sex.

Declaration of interest

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

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References

Abbott DH, Padmanabhan V & Dumesic DA 2006 Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. *Reproductive Biology and Endocrinology* **4** 17. (https://doi.org/10.1186/1477-7827-4-17)

Altemus M, Sarvaiya N & Neill Epperson C 2014 Sex differences in anxiety and depression clinical perspectives. *Frontiers in Neuroendocrinology* **35** 320–330. (https://doi.org/10.1016/j.yfrne.2014.05.004)

Alwasel SH, Harrath AH, Aldahmash WM, Abotalib Z, Nyengaard JR, Osmond C, Dilworth MR, Al Omar SY, Jerah AA & Barker DJP 2014 Sex differences in regional specialisation across the placental surface. *Placenta* **35** 365–369. (https://doi.org/10.1016/j.placenta.2014.03.003)

Arias-Alvarez M, Bermejo-Alvarez P, Gutierrez-Adan A, Rizos D, Lorenzo PL & Lonergan P 2011 Effect of leptin supplementation during in vitro oocyte maturation and embryo culture on bovine embryo development and gene expression patterns. *Theriogenology* **75** 887–896. (https://doi.org/10.1016/j.theriogenology.2010.10.031)

Attig L, Gabory A & Junien C 2010 Early nutrition and epigenetic programming: chasing shadows. *Current Opinion in Clinical Nutrition and Metabolic Care* **13** 284–293. (https://doi.org/10.1097/MCO.0b013e328338aa61)

Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, Moen EL & Marsit CJ 2010 Bisphenol A exposure leads to specific microRNA alterations in placental cells. *Reproductive Toxicology* **29** 401–406. (https://doi.org/10.1016/j.reprotox.2010.04.004)

Barker DJ 2007 The origins of the developmental origins theory. *Journal of Internal Medicine* **261** 412–417. (https://doi.org/10.1111/j.1365-2796.2007.01809.x)

Barker DJ & Osmond C 1986 Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* **1** 1077–1081. (https://doi.org/10.1016/S0140-6736(86)91340-1)

Barrera AD, Garcia EV, Hamdi M, Sanchez-Calabuig MJ, Lopez-Cardona AP, Balvis NF, Rizos D & Gutierrez-Adan A 2017 Embryo culture in presence of oviductal fluid induces DNA methylation changes in bovine blastocysts. *Reproduction* **154** 1–12. (https://doi.org/10.1530/REP-16-0651)

Bermejo-Alvarez P, Rizos D, Rath D, Lonergan P & Gutierrez-Adan A 2008 Epigenetic differences between male and female bovine blastocysts produced in vitro. *Physiological Genomics* **32** 264–272. (https://doi.org/10.1152/physiolgenomics.00234.2007)

Bermejo-Alvarez P, Rizos D, Rath D, Lonergan P & Gutierrez-Adan A 2010 Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. *PNAS* **107** 3394–3399. (https://doi.org/10.1073/pnas.0913843107)

Bermejo-Alvarez P, Rizos D, Lonergan P & Gutierrez-Adan A 2011*a* Transcriptional sexual dimorphism during preimplantation embryo development and its consequences for developmental competence and adult health and disease. *Reproduction* **141** 563–570. (https://doi.org/10.1530/REP-10-0482)

Bermejo-Alvarez P, Rizos D, Lonergan P & Gutierrez-Adan A 2011*b* Transcriptional sexual dimorphism in elongating bovine embryos: implications for XCI and sex determination genes. *Reproduction* **141** 801–808. (https://doi.org/10.1530/REP-11-0006)

Bermejo-Alvarez P, Ramos-Ibeas P & Gutierrez-Adan A 2012*a* Solving the 'X' in embryos and stem cells. *Stem Cells and Development* **21** 1215–1224. (https://doi.org/10.1089/scd.2011.0685)

Bermejo-Alvarez P, Roberts RM & Rosenfeld CS 2012*b* Effect of glucose concentration during in vitro culture of mouse embryos on development to blastocyst, success of embryo transfer, and litter sex ratio. *Molecular Reproduction and Development* **79** 329–336. (https://doi.org/10.1002/mrd.22028)

Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ & Robertson SA 2014 Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *PNAS* **111** 2200–2205. (https://doi.org/10.1073/pnas.1305609111)

Cagnone GL, Dufort I, Vigneault C & Sirard MA 2011 Differential gene expression profile in bovine blastocysts resulting from hyperglycemia exposure during early cleavage stages. *Biology of Reproduction* **86** 50. (https://doi.org/10.1095/biolreprod.111.094391)

Clifton VL 2010 Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta* **31** (Supplement) S33–S39. (https://doi.org/10.1016/j.placenta.2009.11.010)

Cottrell EC & Seckl JR 2009 Prenatal stress, glucocorticoids and the programming of adult disease. *Frontiers in Behavioral Neuroscience* **3** 19. (https://doi.org/10.3389/neuro.08.019.2009)

Cox LA, Li C, Glenn JP, Lange K, Spradling KD, Nathanielsz PW & Jansson T 2013 Expression of the placental transcriptome in maternal nutrient reduction in baboons is dependent on fetal sex. *Journal of Nutrition* **143** 1698–1708. (https://doi.org/10.3945/jn.112.172148)

Cvitic S, Longtine MS, Hackl H, Wagner K, Nelson MD, Desoye G & Hiden U 2013 The human placental sexome differs between trophoblast epithelium and villous vessel endothelium. *PLoS ONE* **8** e79233. (https://doi.org/10.1371/journal.pone.0079233)

Chen Z, Robbins KM, Wells KD & Rivera RM 2013 Large offspring syndrome: a bovine model for the human loss-of-imprinting overgrowth syndrome Beckwith-Wiedemann. *Epigenetics* **8** 591–601. (https://doi.org/10.4161/epi.24655)

Dahl JA, Reiner AH, Klungland A, Wakayama T & Collas P 2010 Histone H3 lysine 27 methylation asymmetry on developmentally-regulated promoters distinguish the first two lineages in mouse preimplantation embryos. *PLoS ONE* **5** e9150. (https://doi.org/10.1371/journal.pone.0009150)

Davis EP & Sandman CA 2010 The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Development* **81** 131–148. (https://doi.org/10.1111/j.1467-8624.2009.01385.x)

de Frutos C, Lopez-Cardona AP, Fonseca Balvis N, Laguna-Barraza R, Rizos D, Gutierrez-Adan A & Bermejo-Alvarez P 2016 Spermatozoa telomeres determine telomere length in early embryos and offspring. *Reproduction* **151** 1–7. (https://doi.org/10.1530/REP-15-0375)

de Moraes AA, Paula-Lopes FF, Chegini N & Hansen PJ 1999 Localization of granulocyte-macrophage colony-stimulating factor in the bovine reproductive tract. *Journal of Reproductive Immunology* **42** 135–145. (https://doi.org/10.1016/s0165-0378(98)00075-8)

Dobbs KB, Gagne D, Fournier E, Dufort I, Robert C, Block J, Sirard MA, Bonilla L, Ealy AD, Loureiro B *et al*. 2014 Sexual dimorphism in developmental programming of the bovine preimplantation embryo caused by colony-stimulating factor 2. *Biology of Reproduction* **91** 80. (https://doi.org/10.1095/biolreprod.114.121087)

Doherty AS, Mann MR, Tremblay KD, Bartolomei MS & Schultz RM 2000 Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biology of Reproduction* **62** 1526–1535. (https://doi.org/10.1095/biolreprod62.6.1526)

Donjacour A, Liu X, Lin W, Simbulan R & Rinaudo PF 2014 In vitro fertilization affects growth and glucose metabolism in a sex-specific manner in an outbred mouse model. *Biology of Reproduction* **90** 80. (https://doi.org/10.1095/biolreprod.113.113134)

Ebner T, Tritscher K, Mayer RB, Oppelt P, Duba HC, Maurer M, Schappacher-Tilp G, Petek E & Shebl O 2016 Quantitative and qualitative trophectoderm grading allows for prediction of live birth and gender. *Journal of Assisted Reproduction and Genetics* **33** 49–57. (https://doi.org/10.1007/s10815-015-0609-9)

Eckert JJ, Porter R, Watkins AJ, Burt E, Brooks S, Leese HJ, Humpherson PG, Cameron IT & Fleming TP 2012 Metabolic induction and early responses of mouse blastocyst developmental programming following maternal low protein diet affecting life-long health. *PLoS ONE* **7** e52791. (https://doi.org/10.1371/journal.pone.0052791)

Ellis BJ & Del Giudice M 2014 Beyond allostatic load: rethinking the role of stress in regulating human development. *Development and Psychopathology* **26** 1–20. (https://doi.org/10.1017/S0954579413000849)

Eriksson JG, Kajantie E, Osmond C, Thornburg K & Barker DJP 2010 Boys live dangerously in the womb. *American Journal of Human Biology* **22** 330–335. (https://doi.org/10.1002/ajhb.20995)

Farin CE, Farin PW & Piedrahita JA 2004 Development of fetuses from in vitro-produced and cloned bovine embryos. *Journal of Animal Science* **82** (E-Supplement) E53–E62. (https://doi.org/10.2527/2004.8213_supplE53x)

Fernandez-Gonzalez R, Moreira P, Bilbao A, Jimenez A, Perez-Crespo M, Ramirez MA, Rodriguez De Fonseca F, Pintado B & Gutierrez-Adan A 2004 Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *PNAS 101* 5880–5885. (https://doi.org/10.1073/pnas.0308560101)

Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M, Sanchez-Martin M, Ramirez MA, Pericuesta E, Bilbao A, Bermejo-Alvarez P, de Dios Hourcade J, de Fonseca FR *et al*. 2008 Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. *Biology of Reproduction* **78** 761–772. (https://doi.org/10.1095/biolreprod.107.065623)

Feuer S & Rinaudo P 2012 Preimplantation stress and development. *Birth Defects Research Part C: Embryo Today* **96** 299–314. (https://doi.org/10.1002/bdrc.21022)

Feuer SK, Donjacour A, Simbulan RK, Lin W, Liu X, Maltepe E & Rinaudo PF 2014 Sexually dimorphic effect of in vitro fertilization (IVF) on adult mouse fat and liver metabolomes. *Endocrinology* **155** 4554–4567. (https://doi.org/10.1210/en.2014-1465)

Fleming TP, Velazquez MA & Eckert JJ 2015*a* Embryos, DOHaD and David Barker. *Journal of Developmental Origins of Health and Disease* **6** 377–383. (https://doi.org/10.1017/S2040174415001105)

Fleming TP, Watkins AJ, Sun C, Velazquez MA, Smyth NR & Eckert JJ 2015*b* Do little embryos make big decisions? How maternal dietary protein restriction can permanently change an embryo's potential, affecting adult health. *Reproduction, Fertility, and Development* **27** 684–692. (https://doi.org/10.1071/RD14455)

Forde N, Maillo V, O'Gaora P, Simintiras CA, Sturmey RG, Ealy AD, Spencer TE, Gutierrez-Adan A, Rizos D & Lonergan P 2016 Sexually dimorphic gene expression in bovine conceptuses at the initiation of implantation. *Biology of Reproduction* **95** 92. (https://doi.org/10.1095/biolreprod.116.139857)

Gabory A, Ferry L, Fajardy I, Jouneau L, Gothié JD, Vigé A, Fleur C, Mayeur S, C Gallou-Kabani, Gross MS *et al*. 2012 Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS ONE* **7** e47986. (https://doi.org/10.1371/journal.pone.0047986)

Gabory A, Roseboom TJ, Moore T, Moore LG & Junien C 2013 Placental contribution to the origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics. *Biology of Sex Differences* **4** 5. (https://doi.org/10.1186/2042-6410-4-5)

Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A *et al*. 2010 Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. *PLoS ONE* **5** e14398. (https://doi,org/10.1371/journal.pone.0014398)

Gao Y, Hyttel P & Hall VJ 2011 Dynamic changes in epigenetic marks and gene expression during porcine epiblast specification. *Cell Reprogram* **13** 345–360. (https://doi.org/10.1089/cell.2010.0110)

Gebert C, Wrenzycki C, Herrmann D, Groger D, Thiel J, Reinhardt R, Lehrach H, Hajkova P, Lucas-Hahn A, Carnwath JW *et al*. 2009 DNA methylation in the IGF2 intragenic DMR is re-established in a sex-specific manner in bovine blastocysts after somatic cloning. *Genomics* **94** 63–69. (https://doi.org/10.1016/j.ygeno.2009.03.004)

Ghys E, Dallemagne M, De Troy D, Sauvegarde C, Errachid A & Donnay I 2016 Female bovine blastocysts are more prone to apoptosis than male ones. *Theriogenology* **85** 591–600. (https://doi.org/10.1016/j.theriogenology.2015.09.050)

Giacomini G, Tabibzadeh SS, Satyaswaroop PG, Bonsi L, Vitale L, Bagnara GP, Strippoli P & Jasonni VM 1995 Epithelial cells are the

major source of biologically active granulocyte macrophage colony-stimulating factor in human endometrium. *Human Reproduction* **10** 3259–3263. (https://doi.org/10.1093/oxfordjournals.humrep.a135899)

Gluckman PD, Hanson MA, Bateson P, Beedle AS, Law CM, Bhutta ZA, Anokhin KV, Bougnères P, Chandak GR, Dasgupta P *et al*. 2009 Towards a new developmental synthesis: adaptive developmental plasticity and human disease. *Lancet* **373** 1654–1657. (https://doi.org/10.1016/S0140-6736(09)60234-8)

Gomez E, Munoz M, Simo C, Ibanez C, Carrocera S, Martin-Gonzalez D & Cifuentes A 2016 Non-invasive metabolomics for improved determination of embryonic sex markers in chemically defined culture medium. *Journal of Chromatography A* **1474** 138–144. (https://doi.org/10.1016/j.chroma.2016.10.080)

Gutierrez-Adan A, Oter M, Martinez-Madrid B, Pintado B & DeLa Fuente J 2000 Differential expression of two genes located on the X chromosome between male and female in vitro-produced bovine embryos at the blastocyst stage. *Molecular Reproduction and Development* **55** 146–151. (https://doi.org/10.1002/(SICI)1098-2795(200002)55:2<146::AID-MRD3>3.0.CO;2-F)

Gutierrez-Adan A, Granados J, Pintado B & De La Fuente J 2001 Influence of glucose on the sex ratio of bovine IVM/IVF embryos cultured in vitro. *Reproduction, Fertility, and Development* **13** 361–365. (https://doi.org/10.1071/RD00039)

Gutierrez-Adan A, Perez-Crespo M, Fernandez-Gonzalez R, Ramirez MA, Moreira P, Pintado B, Lonergan P & Rizos D 2006 Developmental consequences of sexual dimorphism during pre-implantation embryonic development. *Reproduction in Domestic Animals* **41** (Supplement 2) 54–62. (https://doi.org/10.1111/j.1439-0531.2006.00769.x)

Harris A & Seckl J 2011 Glucocorticoids, prenatal stress and the programming of disease. *Hormones and Behavior* **59** 279–289. (https://doi.org/10.1016/j.yhbeh.2010.06.007)

Heijmans BT, Tobi EW, Lumey LH & Slagboom PE 2009 The epigenome: archive of the prenatal environment. *Epigenetics* **4** 526–531. (https://doi.org/10.4161/epi.4.8.10265)

Hogg K, McNeilly AS & Duncan WC 2011 Prenatal androgen exposure leads to alterations in gene and protein expression in the ovine fetal ovary. *Endocrinology* **152** 2048–2059. (https://doi.org/10.1210/en.2010-1219)

Hwang JY, Oh JN, Park CH, Lee DK & Lee CK 2015 Dosage compensation of X-chromosome inactivation center-linked genes in porcine preimplantation embryos: non-chromosome-wide initiation of X-chromosome inactivation in blastocysts. *Mechanisms of Development* **138** 246–255. (https://doi.org/10.1016/j.mod.2015.10.005)

Itoh Y & Arnold AP 2014 X chromosome regulation of autosomal gene expression in bovine blastocysts. *Chromosoma* **123** 481–489. (https://doi.org/10.1007/s00412-014-0461-1)

Jimenez A, Madrid-Bury N, Fernandez R, Perez-Garnelo S, Moreira P, Pintado B, de la Fuente J & Gutierrez-Adan A 2003 Hyperglycemia-induced apoptosis affects sex ratio of bovine and murine preimplantation embryos. *Molecular Reproduction and Development* **65** 180–187. (https://doi.org/10.1002/mrd.10286)

Junien C, Gabory A & Attig L 2012 Sexual dimorphism in the XXI(st) century. *Medical Sciences* **28** 185–192. (https://doi.org/10.1051/medsci/2012282017)

Kalisch-Smith JI, Simmons DG, Dickinson H & Moritz KM 2016 Review: Sexual dimorphism in the formation, function and adaptation of the placenta. *Placenta* **54** 10–16. (https://doi.org/10.1016/j.placenta.2016.12.008)

Kalisch-Smith JI, Simmons DG, Pantaleon M & Moritz KM 2017 Sex differences in rat placental development: from pre-implantation to late gestation. *Biology of Sex Differences* **8** 17. (https://doi.org/10.1186/s13293-017-0138-6)

Kay GF, Barton SC, Surani MA & Rastan S 1994 Imprinting and X chromosome counting mechanisms determine Xist expression in early mouse development. *Cell* **77** 639–650. (https://doi.org/10.1016/0092-8674(94)90049-3)

Khosla S, Dean W, Brown D, Reik W & Feil R 2001 Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biology of Reproduction* **64** 918–926. (https://doi.org/10.1095/biolreprod64.3.918)

Kobayashi S, Isotani A, Mise N, Yamamoto M, Fujihara Y, Kaseda K, Nakanishi T, Ikawa M, Hamada H, Abe K *et al*. 2006 Comparison of gene expression in male and female mouse blastocysts revealed imprinting of the X-linked gene, Rhox5/Pem, at preimplantation stages. *Current Biology* **16** 166–172. (https://doi.org/10.1016/j.cub.2005.11.071)

Koskela E, Mappes T, Niskanen T & Rutkowska J 2009 Maternal investment in relation to sex ratio and offspring number in a small mammal – a case for Trivers and Willard theory? *Journal of Animal Ecology* **78** 1007–1014. (https://doi.org/10.1111/j.1365-2656.2009.01574.x)

Kwong WY, Wild AE, Roberts P, Willis AC & Fleming TP 2000 Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* **127** 4195–4202.

Laguna-Barraza R, Bermejo-Alvarez P, Ramos-Ibeas P, de Frutos C, Lopez-Cardona AP, Calle A, Fernandez-Gonzalez R, Pericuesta E, Ramirez MA & Gutierrez-Adan A 2012 Sex-specific embryonic origin of postnatal phenotypic variability. *Reproduction, Fertility, and Development* **25** 38–47. (https://doi.org/10.1071/RD12262)

Lamas-Toranzo I, Pericuesta E & Bermejo-Alvarez P 2017 Memories of an X-chromosome. *Stem Cell Investigation* **4** 27. (https://doi.org/10.21037/sci.2017.03.01)

Laplante DP, Barr RG, Brunet A, Du Fort GG, Meaney ML, Saucier J-Fo, Zelazo PR & King S 2004 Stress during pregnancy affects general intellectual and language functioning in human toddlers. *Pediatric Research* **56** 400–410. (https://doi.org/10.1203/01.PDR.0000136281.34035.44)

Larson MA, Kimura K, Kubisch HM & Roberts RM 2001 Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocyst and in the expression of the signaling molecule IFN-tau. *PNAS* **98** 9677–9682. (https://doi.org/10.1073/pnas.171305398)

Lowe R, Gemma C, Rakyan VK & Holland ML 2015 Sexually dimorphic gene expression emerges with embryonic genome activation and is dynamic throughout development. *BMC Genomics* **16** 295. (https://doi.org/10.1186/s12864-015-1506-4)

Lubinsky MS & Hall JG 1991 Genomic imprinting, monozygous twinning, and X inactivation. *Lancet* **337** 1288. (https://doi.org/10.1016/0140-6736(91)92956-3)

Mandò C, Calabrese S, Mazzocco MI, Novielli C, Anelli GM, Antonazzo P & Cetin I 2016 Sex specific adaptations in placental biometry of overweight and obese women. *Placenta* **38** 1–7. (https://doi.org/10.1016/j.placenta.2015.12.008)

Manikkam M, Crespi EJ, Doop DD, Herkimer C, Lee JS, Yu S, Brown MB, Foster DL & Padmanabhan V 2004 Fetal programming: prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. *Endocrinology* **145** 790–798. (https://doi.org/10.1210/en.2003-0478)

Mao J, Zhang X, Sieli PT, Falduto MT, Torres KE & Rosenfeld CS 2010 Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *PNAS* **107** 5557–5562. (https://doi.org/10.1073/pnas.1000440107)

Mendis S, Davis S & Norrving B 2015 Organizational update: the world health organization global status report on noncommunicable diseases 2014; one more landmark step in the combat against stroke and vascular disease. *Stroke* **46** e121–e122. (https://doi.org/10.1161/STROKEAHA.115.008097)

Morgan HD, Jin XL, Li A, Whitelaw E & O'Neill C 2008 The culture of zygotes to the blastocyst stage changes the postnatal expression of an epigentically labile allele, agouti viable yellow, in mice. *Biology of Reproduction* **79** 618–623. (https://doi.org/10.1095/biolreprod.108.068213)

Morton KM, Herrmann D, Sieg B, Struckmann C, Maxwell WM, Rath D, Evans G, Lucas-Hahn A, Niemann H & Wrenzycki C 2007 Altered mRNA expression patterns in bovine blastocysts after fertilisation in vitro using flow-cytometrically sex-sorted sperm. *Molecular Reproduction and Development* **74** 931–940. (https://doi.org/10.1002/mrd.20573)

Nahar A & Kadokawa H 2016 Suppressed expression of macrophage migration inhibitory factor in the oviducts of lean and obese cows. *Reproduction, Fertility, and Development* **28** 655–662. (https://doi.org/10.1071/RD14164)

Nahar MS, Liao C, Kannan K, Harris C & Dolinoy DC 2015 In utero bisphenol A concentration, metabolism, and global DNA methylation across matched placenta, kidney, and liver in the human fetus. *Chemosphere* **124** 54–60. (https://doi.org/10.1016/j.chemosphere.2014.10.071)

Niemann H, Carnwath JW, Herrmann D, Wieczorek G, Lemme E, A Lucas-Hahn & Olek S 2010 DNA methylation patterns reflect epigenetic reprogramming in bovine embryos. *Cell Reprogram* **12** 33–42. (https://doi.org/10.1089/cell.2009.0063)

O'Connell BA, Moritz KM, Walker DW & Dickinson H 2013*a* Sexually dimorphic placental development throughout gestation in the spiny mouse (Acomys cahirinus). *Placenta* **34** 119–126. (https://doi.org/10.1016/j.placenta.2012.11.009)

O'Connell BA, Moritz KM, Walker DW & Dickinson H 2013*b* Treatment of pregnant spiny mice at mid gestation with a synthetic glucocorticoid has sex-dependent effects on placental glycogen stores. *Placenta* **34** 932–940. (https://doi.org/10.1016/j.placenta.2013.06.310)

O'Leary S, Jasper MJ, Warnes GM, Armstrong DT & Robertson SA 2004 Seminal plasma regulates endometrial cytokine expression, leukocyte recruitment and embryo development in the pig. *Reproduction* **128** 237–247. (https://doi.org/10.1530/rep.1.00160)

Okamoto I, Patrat C, Thepot D, Peynot N, Fauque P, Daniel N, Diabangouaya P, Wolf JP, Renard JP, Duranthon V *et al*. 2011 Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. *Nature* **472** 370–374. (https://doi.org/10.1038/nature09872)

Osei-Kumah A, Smith R, Jurisica I, Caniggia I & Clifton VL 2011 Sex-specific differences in placental global gene expression in pregnancies complicated by asthma. *Placenta* **32** 570–578. (https://doi.org/10.1016/j.placenta.2011.05.005)

Park CH, Jeong YH, Jeong YI, Lee SY, Jeong YW, Shin T, Kim NH, Jeung EB, Hyun SH, Lee CK *et al*. 2012 X-linked gene transcription patterns in female and male in vivo, in vitro and cloned porcine individual blastocysts. *PLoS ONE* **7** e51398. (https://doi.org/10.1371/journal.pone.0051398)

Perez-Crespo M, Ramirez MA, Fernandez-Gonzalez R, Rizos D, Lonergan P, Pintado B & Gutierrez-Adan A 2005 Differential sensitivity of male and female mouse embryos to oxidative induced heat-stress is mediated by glucose-6-phosphate dehydrogenase gene expression. *Molecular Reproduction and Development* **72** 502–510. (https://doi.org/10.1002/mrd.20366)

Petropoulos S, Edsgard D, Reinius B, Deng Q, Panula SP, Codeluppi S, Plaza Reyes A, Linnarsson S, Sandberg R & Lanner F 2016 Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell* **165** 1012–1026. (https://doi.org/10.1016/j.cell.2016.03.023)

Ramos-Ibeas P, Calle A, Fernandez-Gonzalez R, Laguna-Barraza R, Pericuesta E, Calero A, Ramirez MA & Gutierrez-Adan A 2014 Intracytoplasmic sperm injection using DNA-fragmented sperm in mice negatively affects embryo-derived embryonic stem cells, reduces the fertility of male offspring and induces heritable changes in epialleles. *PLoS ONE* **9** e95625. (https://doi.org/10.1371/journal.pone.0095625)

Reynolds CM, Vickers MH, Harrison CJ, Segovia SA & Gray C 2015 Maternal high fat and/or salt consumption induces sex-specific inflammatory and nutrient transport in the rat placenta. *Physiological Reports* **3** e12399–e12399. (https://doi.org/10.14814/phy2.12399)

Rojas-García PP, Recabarren MP, Sir-Petermann T, Rey R, Palma S, Carrasco A, Perez-Marin CC, Padmanabhan V & Recabarren SE 2013 Altered testicular development as a consequence of increase number of sertoli cell in male lambs exposed prenatally to excess testosterone. *Endocrine* **43** 705–713. (https://doi.org/10.1007/s12020-012-9818-5)

Salilew-Wondim D, Fournier E, Hoelker M, Saeed-Zidane M, Tholen E, Looft C, Neuhoff C, Besenfelder U, Havlicek V, Rings F *et al*. 2015 Genome-wide DNA methylation patterns of bovine blastocysts developed in vivo from embryos completed different stages of development in vitro. *PLoS ONE* **10** e0140467. (https://doi.org/10.1371/journal.pone.0140467)

Sandman CA, Glynn LM & Davis EP 2013 Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research* **75** 327–335. (https://doi.org/10.1016/j.jpsychores.2013.07.009)

Scott NM, Hodyl NA, Murphy VE, Osei-Kumah A, Wyper H, Hodgson DM, Smith R & Clifton VL 2009 Placental cytokine expression covaries with maternal asthma severity and fetal sex. *Journal of Immunology* **182** 1411–1420. (https://doi.org/10.4049/jimmunol.182.3.1411)

Scott KA, Yamazaki Y, Yamamoto M, Lin Y, Melhorn SJ, Krause EG, Woods SC, Yanagimachi R, Sakai RR & Tamashiro KL 2010 Glucose parameters are altered in mouse offspring produced by assisted reproductive technologies and somatic cell nuclear transfer. *Biology of Reproduction* **83** 220–227. (https://doi.org/10.1095/biolreprod.109.082826)

Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W & Reik W 2012 The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Molecular Cell* **48** 849–862. (https://doi.org/10.1016/j.molcel.2012.11.001)

Shin H-S, Seo J-H, Jeong S-H, Park S-W, Park Y, Son S-W, Kim JS & Kang H-G 2014 Exposure of pregnant mice to chlorpyrifos-methyl alters embryonic H19 gene methylation patterns. *Environmental Toxicology* **29** 926–935. (https://doi.org/10.1002/tox.21820)

Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA *et al*. 2007 DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *PNAS* **104** 19351–19356. (https://doi.org/10.1073/pnas.0707258104)

Siqueira LG & Hansen PJ 2016 Sex differences in response of the bovine embryo to colony-stimulating factor 2. *Reproduction* **152** 645–654. (https://doi.org/10.1530/REP-16-0336)

Sjoblom C, Roberts CT, Wikland M & Robertson SA 2005 Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. *Endocrinology* **146** 2142–2153. (https://doi.org/10.1210/en.2004-1260)

Sood R, Zehnder JL, Druzin ML & Brown PO 2006 Gene expression patterns in human placenta. *PNAS* **103** 5478–5483. (https://doi.org/10.1073/pnas.0508035103)

Stark MJ, Wright IMR & Clifton VL 2009 Sex-specific alterations in placental 11beta-hydroxysteroid dehydrogenase 2 activity and early postnatal clinical course following antenatal betamethasone. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **297** R510–R514. (https://doi.org/10.1152/ajpregu.00175.2009)

Stark MJ, Hodyl NA, Wright IMR & Clifton VL 2011 Influence of sex and glucocorticoid exposure on preterm placental pro-oxidant-antioxidant balance. *Placenta* **32** 865–870. (https://doi.org/10.1016/j.placenta.2011.08.010)

Sturmey RG, Bermejo-Alvarez P, Gutierrez-Adan A, Rizos D, Leese HJ & Lonergan P 2010 Amino acid metabolism of bovine blastocysts: a biomarker of sex and viability. *Molecular Reproduction and Development* **77** 285–296. (https://doi.org/10.1002/mrd.21145)

Sun C, Velazquez MA, Marfy-Smith S, Sheth B, Cox A, Johnston DA, Smyth N & Fleming TP 2014 Mouse early extra-embryonic lineages activate compensatory endocytosis in response to poor maternal nutrition. *Development* **141** 1140–1150. (https://doi.org/10.1242/dev.103952)

Sun C, Denisenko O, Sheth B, Cox A, Lucas ES, Smyth NR & Fleming TP 2015 Epigenetic regulation of histone modifications and Gata6 gene expression induced by maternal diet in mouse embryoid bodies in a model of developmental programming. *BMC Developmental Biology* **15** 3. (https://doi.org/10.1186/s12861-015-0053-1)

Sundrani DP, Roy SS, Jadhav AT & Joshi SR 2017 Sex-specific differences and developmental programming for diseases in later life. *Reproduction, Fertility and Development*.

Susiarjo M, Sasson I, Mesaros C & Bartolomei MS 2013 Bisphenol a exposure disrupts genomic imprinting in the mouse. *PLoS Genetics* **9** e1003401. (https://doi.org/10.1371/journal.pgen.1003401)

Tachibana T, Wakimoto Y, Nakamuta N, Phichitraslip T, Wakitani S, Kusakabe K, Hondo E & Kiso Y 2007 Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. *Journal of Reproduction and Development* **53** 509–514. (https://doi.org/10.1262/jrd.18171)

Tan W, Huang H, Wang Y, Wong TY, Wang CC & Leung LK 2013 Bisphenol A differentially activates protein kinase C isoforms in murine placental tissue. *Toxicology and Applied Pharmacology* **269** 163–168. (https://doi.org/10.1016/j.taap.2013.03.016)

Tan K, Wang Z, Zhang Z, An L & Tian J 2016 IVF affects embryonic development in a sex-biased manner in mice. *Reproduction* **151** 443–453. (https://doi.org/10.1530/REP-15-0588)

Tarin JJ, Garcia-Perez MA, Hermenegildo C & Cano A 2014 Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment

cycles. *Reproductive Biology and Endocrinology* **12** 56. (https://doi.org/10.1186/1477-7827-12-56)

Tarrade A, Rousseau-Ralliard D, Aubrière M-C, Peynot N, Dahirel M, Bertrand-Michel J, Aguirre-Lavin T, Morel O, Beaujean N, Duranthon V *et al*. 2013 Sexual dimorphism of the feto-placental phenotype in response to a high fat and control maternal diets in a rabbit model. *PLoS ONE* **8** e83458. (https://doi.org/10.1371/journal.pone.0083458)

Tarrade A, Panchenko P, Junien C & Gabory A 2015 Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. *Journal of Experimental Biology* **218** 50–58. (https://doi.org/10.1242/jeb.110320)

Taylor DM, Handyside AH, Ray PF, Dibb NJ, Winston RM & Ao A 2001 Quantitative measurement of transcript levels throughout human preimplantation development: analysis of hypoxanthine phosphoribosyl transferase. *Molecular Human Reproduction* **7** 147–154. (https://doi.org/10.1093/molehr/7.2.147)

Teh AL, Pan H, Chen L, Ong ML, Dogra S, Wong J, Mac IsaacJL, Mah SM, McEwen LM, Saw SM *et al*. 2014 The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. *Genome Research* **24** 1064–1074. (https://doi.org/10.1101/gr.171439.113)

Tremellen KP, Seamark RF & Robertson SA 1998 Seminal transforming growth factor beta1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus. *Biology of Reproduction* **58** 1217–1225. (https://doi.org/10.1095/biolreprod58.5.1217)

Trivers RL & Willard DE 1973 Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179** 90–92. (https://doi.org/10.1126/science.179.4068.90)

Vickers MH, Clayton ZE, Yap C & Sloboda DM 2011 Maternal fructose intake during pregnancy and lactation alters placental growth and leads to sex-specific changes in fetal and neonatal endocrine function. *Endocrinology* **152** 1378–1387. (https://doi.org/10.1210/en.2010-1093)

Vilahur N, Bustamante M, Byun HM, Fernandez MF, Santa Marina L, Basterrechea M, Ballester F, Murcia M, Tardon A, Fernandez-Somoano A *et al*. 2014 Prenatal exposure to mixtures of xenoestrogens and repetitive element DNA methylation changes in human placenta. *Environment International* **71** 81–87. (https://doi.org/10.1016/j.envint.2014.06.006)

Vu TT, Hirst JJ, Stark M, Wright IMR, Palliser HK, Hodyl N & Clifton VL 2009 Changes in human placental 5alpha-reductase isoenzyme expression with advancing gestation: effects of fetal sex and glucocorticoid exposure. *Reproduction, Fertility, and Development* **21** 599–607. (https://doi.org/10.1071/RD08224)

Watkins AJ, Ursell E, Panton R, Papenbrock T, Hollis L, Cunningham C, Wilkins A, Perry VH, Sheth B, Kwong WY *et al*. 2008*a* Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biology of Reproduction* **78** 299–306. (https://doi.org/10.1095/biolreprod.107.064220)

Watkins AJ, Wilkins A, Cunningham C, Perry VH, Seet MJ, Osmond C, Eckert JJ, Torrens C, Cagampang FR, Cleal J *et al*. 2008*b* Low protein diet fed exclusively during mouse oocyte maturation leads to behavioural and cardiovascular abnormalities in offspring. *Journal of Physiology* **586** 2231–2244. (https://doi.org/10.1113/jphysiol.2007.149229)

Watkins AJ, Lucas ES, Torrens C, Cleal JK, Green L, Osmond C, Eckert JJ, Gray WP, Hanson MA & Fleming TP 2010 Maternal low-protein diet during mouse pre-implantation development induces vascular dysfunction and altered renin-angiotensin-system homeostasis in the offspring. *British Journal of Nutrition* **103** 1762–1770. (https://doi.org/10.1017/S0007114509993783)

Watkins AJ, Lucas ES, Wilkins A, Cagampang FR & Fleming TP 2011 Maternal periconceptional and gestational low protein diet affects mouse offspring growth, cardiovascular and adipose phenotype at 1 year of age. *PLoS ONE* **6** e28745. (https://doi.org/10.1371/journal.pone.0028745)

Weksberg R, Shuman C, Caluseriu O, Smith AC, Fei YL, Nishikawa J, Stockley TL, Best L, Chitayat D, Olney A *et al*. 2002 Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Human Molecular Genetics* **11** 1317–1325. (https://doi.org/10.1093/hmg/11.11.1317)

Wijchers PJ, Yandim C, Panousopoulou E, Ahmad M, Harker N, Saveliev A, Burgoyne PS & Festenstein R 2010 Sexual dimorphism in mammalian autosomal gene regulation is determined not only by Sry but by sex chromosome complement as well. *Developmental Cell* **19** 477–484. (https://doi.org/10.1016/j.devcel.2010.08.005)

Wilcoxon JS, Schwartz J, Aird F & Redei EE 2003 Sexually dimorphic effects of maternal alcohol intake and adrenalectomy on left ventricular hypertrophy in rat offspring. *American Journal of Physiology: Endocrinology and Metabolism* **285** E31–E39. (https://doi.org/10.1152/ajpendo.00552.2002)

Wolf CJ, Hotchkiss A, Ostby JS, LeBlanc GA & Gray LE 2002 Effects of prenatal testosterone propionate on the sexual development of male and female rats: a dose-response study. *Toxicological Sciences* **65** 71–86. (https://doi.org/10.1093/toxsci/65.1.71)

Wrenzycki C, Lucas-Hahn A, Herrmann D, Lemme E, Korsawe K & Niemann H 2002 In vitro production and nuclear transfer affect dosage compensation of the X-linked gene transcripts G6PD, PGK, and Xist in preimplantation bovine embryos. *Biology of Reproduction* **66** 127–134. (https://doi.org/10.1095/biolreprod66.1.127)

Xu X, Chiung YM, Lu F, Qiu S, Ji M & Huo X 2015 Associations of cadmium, bisphenol A and polychlorinated biphenyl co-exposure in utero with placental gene expression and neonatal outcomes. *Reproductive Toxicology* **52** 62–70. (https://doi.org/10.1016/j.reprotox.2015.02.004)

Zvetkova I, Apedaile A, Ramsahoye B, Mermoud JE, Crompton LA, John R, Feil R & Brockdorff N 2005 Global hypomethylation of the genome in XX embryonic stem cells. *Nature Genetics* **37** 1274–1279. (https://doi.org/10.1038/ng1663)

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