Human Reproduction Update, Vol.25, No.6, pp. 777–801, 2019
Advance Access Publication on October 21, 2019 doi:10.1093/humupd/dmz025

human reproduction update

REVIEW

In-utero stress and mode of conception: impact on regulation of imprinted genes, fetal development and future health

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Submitted on March 6, 2018; resubmitted on July 4, 2019; editorial decision on July 12, 2019

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BACKGROUND: Genomic imprinting is an epigenetic gene regulatory mechanism; disruption of this process during early embryonic development can have major consequences on both fetal and placental development. The periconceptional period and intrauterine life are crucial for determining long-term susceptibility to diseases. Treatments and procedures in assisted reproductive technologies (ART) and adverse *in-utero* environments may modify the methylation levels of genomic imprinting regions, including insulin-like growth factor 2 (*IGF2*)/*H19*, mesoderm-specific transcript (*MEST*), and paternally expressed gene 10 (*PEG10*), affecting the development of the fetus. ART, maternal psychological stress, and gestational exposures to chemicals are common stressors suspected to alter global epigenetic patterns including imprinted genes.

OBJECTIVE AND RATIONALE: Our objective is to highlight the effect of conception mode and maternal psychological stress on fetal development. Specifically, we monitor fetal programming, regulation of imprinted genes, fetal growth, and long-term disease risk, using the imprinted genes *IGF2/H19*, *MEST*, and *PEG10* as examples. The possible role of environmental chemicals in genomic imprinting is also discussed.

SEARCH METHODS: A PubMed search of articles published mostly from 2005 to 2019 was conducted using search terms *IGF2/H19, MEST, PEG10,* imprinted genes, DNA methylation, gene expression, and imprinting disorders (IDs). Studies focusing on maternal prenatal stress, psychological well-being, environmental chemicals, ART, and placental/fetal development were evaluated and included in this review.

OUTCOMES: *IGF2/H19, MEST*, and *PEG10* imprinted genes have a broad developmental effect on fetal growth and birth weight variation. Their disruption is linked to pregnancy complications, metabolic disorders, cognitive impairment, and cancer. Adverse early environment has a major impact on the developing fetus, affecting mostly growth, the structure, and subsequent function of the hypothalamic–pituitary–adrenal axis and neurodevelopment. Extensive evidence suggests that the gestational environment has an impact on epigenetic patterns including imprinting, which can lead to adverse long-term outcomes in the offspring. Environmental stressors such as maternal prenatal psychological stress have been found to associate with altered DNA methylation patterns in placenta and to affect fetal development. Studies conducted during the past decades have suggested that ART pregnancies are at a higher risk for a number of complications such as birth defects and IDs. ART procedures involve multiple steps that are conducted during critical windows for imprinting establishment and maintenance, necessitating long-term evaluation of children conceived through ART. Exposure to environmental chemicals can affect placental imprinting and fetal growth both in humans and in experimental animals. Therefore, their role in imprinting should be better elucidated, considering the ubiquitous exposure to these chemicals.

WIDER IMPLICATIONS: Dysregulation of imprinted genes is a plausible mechanism linking stressors such as maternal psychological stress, conception using ART, and chemical exposures with fetal growth. It is expected that a greater understanding of the role of imprinted genes and their regulation in fetal development will provide insights for clinical prevention and management of growth and IDs. In a broader context, evidence connecting impaired imprinted gene function to common diseases such as cancer is increasing. This implies early regulation of imprinting may enable control of long-term human health, reducing the burden of disease in the population in years to come.

Key words: imprinting / ART / *IGF2/H19* / *MEST* / *PEG10* / fetal development / imprinting disorders / chemical exposures / maternal psychological stress

Introduction

Genomic imprinting is a well-conserved mammalian gene regulatory mechanism, estimated to affect ∼1% (∼200 genes) of the human protein-coding genome. There are >100 known imprinted genes at present (http://www.geneimprint.com/site/genes-by-species), but this number is likely to increase in the near future, as methods to validate them become more sensitive (Cordeiro *et al.*, 2014; Kappil *et al.*, 2015a; Monk, 2015; Moore *et al.*, 2015). Imprinted genes display mono-allelic expression in a parent-of-origin-specific fashion and play a key role in the regulation of fetal growth and placental development (Lim and Ferguson-Smith, 2010). Silencing of one of the alleles in a parent-specific manner is achieved by epigenetic modifications, including differential methylation of DNA, histone modifications (acetylation/methylation), and long noncoding RNAs (Huang and Kim, 2009; Lambertini *et al.*, 2012; Patten *et al.*, 2016). In general, these epigenetic regulatory mechanisms modify the chromatin structure allowing control of its transcriptional activity. Differential DNA methylation is directly applied to the DNA strand and is a widely recognized epigenetic modification associated with imprinting and its maintenance. When located within a gene promoter, DNA methylation typically acts to repress gene transcription, and this is essential for normal development.

Epigenetic modulation of gene activity is involved in a number of processes including genomic imprinting, aging, X-chromosome inactivation, and carcinogenesis. In genomic imprinting, the active and silent alleles are determined by their methylation status, and loss of DNA methylation leads to loss of imprinting (Koukoura *et al.*, 2011; Patten *et al.*, 2016). Most imprinted genes are only expressed in certain tissues. In humans, imprinted genes are mostly expressed in the placenta (∼80 of the ∼100 known imprinted genes), an organ that facilitates interactions between the mother and the fetus. Imprinted genes are also expressed in umbilical cord blood (Monk, 2015). DNA methylation patterns that control the expression of imprinted genes are established early in development. Alterations in the environmental milieu during these early stages may result in imprinting aberrations with the potential for adverse health effects later in life. For example, conception through assisted reproductive technologies (ART) may be associated with imprinting disorders (IDs) (Huntriss and Picton, 2008). Furthermore, maternal psychological stress during pregnancy can result in differential DNA methylation levels on imprinted genes (Soubry *et al.*, 2011; Liu *et al.*, 2012; Vidal *et al.*, 2014b). In summary, DNA methylation and genomic imprinting take place during early critical periods of development and can be affected by stressors with possible long-term health effects. These environmental factors may also affect broader imprinting mechanisms, such as histone modifications and recruitment of DNA methyltransferases (DNMTs).

Here we use the word ‘stressor’ to describe common external and internal factors that affect women of reproductive age and may be connected to fetal growth and development via epigenetic mechanisms including imprinting. We focus on maternal psychological stress during

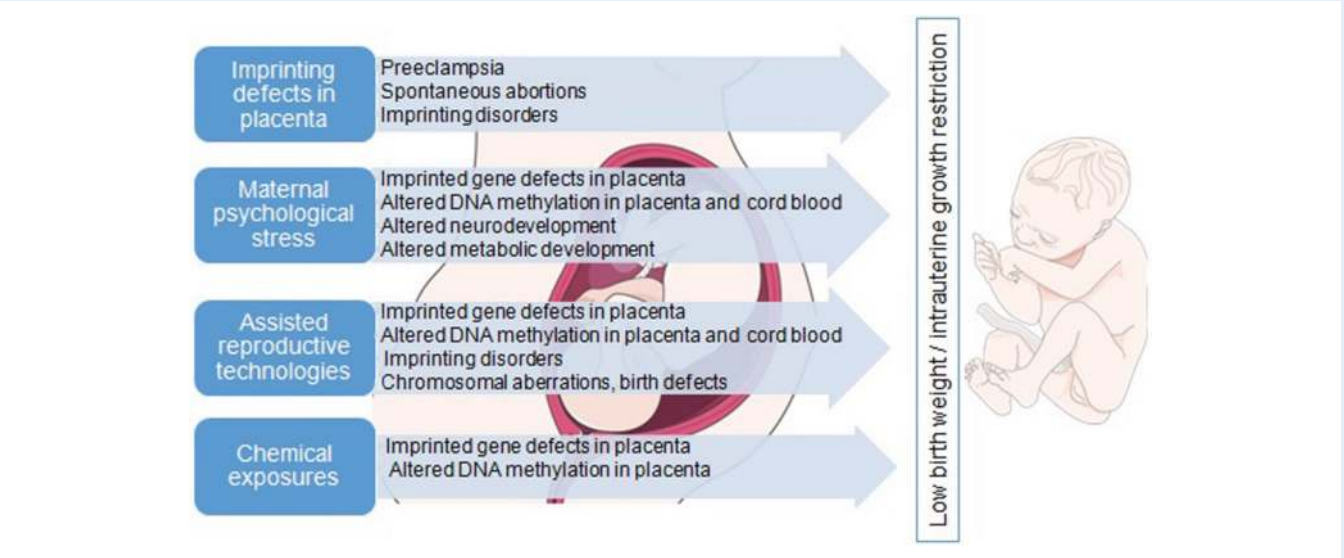

Figure 1 Effects of imprinting and stressors on fetal development. Disruption of imprinting in the placenta is associated with preeclampsia, spontaneous abortions, and IDs and can be observed in LBW pregnancies. Stressors such as maternal psychological stress, conception using ART, and exposure to chemicals have all been found to associate with imprinting defects in placenta and altered DNA methylation patterns in placenta and/or cord blood. In all scenarios, fetal development is affected with various consequences varying from LBW (common to all stressors) to neurodevelopmental delays and birth defects.

pregnancy, mode of conception, and environmental chemicals. The imprinted genes insulin-like growth factor 2 (*IGF2*), *H19*, mesoderm-specific transcript (*MEST*), and paternally expressed gene 10 (*PEG10*) are used as examples as precise regulation of these genes is critical for normal fetal development. The chosen stressors represent commonly occurring factors that can coincide in the same woman and affect offspring health. Many women suffer from psychological stress in their everyday life, even during pregnancy. Exposure to chemicals is inescapable. Infertility rates are constantly increasing, with many couples seeking help through ART, which is both a maternal and fetal stress. Although maternal nutritional status is another well-known stressor affecting imprinting and fetal development, we will not focus on it in this review as it has been recently extensively reviewed elsewhere (Lillycrop and Burdge, 2011). Diverse cellular responses are triggered in response to stress (Kültz, 2005), and the exact mechanism depends on the type of stressor. For example, maternal psychological stress has effects on the hypothalamic–pituitary–adrenal (HPA) axis, and chemicals may trigger xenobiotic metabolism or function as endocrine disruptors. A common mechanism for the three stressors in our review is modulation of epigenetic patterns including imprinting (Fig. 1).

IGF2, *H19*, *MEST*, and *PEG10* have a broad developmental effect on fetal growth, and their disruption is linked to low birth weight (LBW), metabolic disorders, cognitive impairment, and certain types of cancer. More specifically, *IGF2* acts as a mitogenic growth factor that promotes differentiation and metabolism, and together with *H19* they are linked to nervous and connective tissue development. *MEST* is involved in the control of embryonic and placental growth, and *PEG10* displays a functional role in growth-promoting activities and human placenta formation at a later stage of the first trimester. These imprinted genes are responsive to different *in-utero* environments and thus may serve as mediators of environmental signals to fetal development during pregnancy.

Molecular mechanisms of imprinting

Organization and regulation of imprinted genes

Imprinted genes often aggregate in well-conserved imprinted domains of 3–12 genes spread over a few mega bases of DNA, although single imprinted genes can be found too (Lewis and Reik, 2006; Lambertini *et al.*, 2012). Each domain contains cytosine-guanine (CpG)-rich differentially methylated regions (DMRs). Some of these DMRs, referred to as imprinting control regions (ICRs), can have a regulatory role and govern the expression of the genes in the domain. There are typically one ICR and possibly several DMRs per imprinted domain. There are two types of DMRs commonly found within imprinted loci: germline DMRs (gDMRs) that are applied in the germline and their imprints are maintained throughout development even in somatic cells, while some loci also have somatic DMRs (sDMRs) gained after fertilization. Some imprinted domains have secondary gDMRs or sDMRs that appear after fertilization. There are also some imprinted domains with no identified gDMRs/ICR, and these are possibly controlled by histone methylation.

Accurate timing and positioning of imprinting demand a highly specialized set of molecular mechanisms. Epigenetic mechanisms control DMRs and ICRs and regulate the expression of the associated genes including silencing of one of the alleles (Huang and Kim, 2009; Lambertini *et al.*, 2012; Patten *et al.*, 2016). Allele-specific DNA methy-

lation of ICRs has been considered as the key molecular mechanism in establishment and maintenance of imprinting (Feng et al., 2011). In humans, DNA methylation is regulated by DNMTs (DNMT1, DNMT3a, and DNMT3b), which add a methyl group at the C(5) position of cytosine in CpG dinucleotides and have distinct roles (Carless et al., 2013). Maintenance of the inherited DNA methylation patterns is performed by DNMT1, which preferably methylates hemimethylated CpGs. DNMT3a and DNMT3b are essential for *de-novo* DNA methylation that occurs after embryo implantation (Moss and Wallrath, 2007). DNMTs receive methyl groups from the cyclical cellular process called 'one-carbon metabolism', which converts universal methyl donor S-adenosyl-methionine to S-adenosyl-homocysteine (Jiang et al., 2012).

The DNA methylation pathway is highly dependent on nutritional status, and many dietary micronutrients are essential for its regulation (McKee and Reyes, 2018). These nutrients are known as methyl donors, and they serve as cofactors for enzymes involved in one-carbon metabolism. Imbalances in methyl donors can negatively affect DNMTs function, S-adenosyl-methionine regeneration, and DNA methylation (McKee and Reyes, 2018). Methyl-group donors derived from food (such as choline, betaine, folate, vitamin B6, vitamin B12, and methionine) and supplements (like folic acid) are necessary cofactors for donating a methyl-group in the DNA methyl-activation cycle. They are also crucial for the normal development of the central nervous system (McKee and Reyes, 2018). Prenatal malnutrition in humans can alter the supply of methyl donors and the activities of DNMTs and subsequently disrupt the correct establishment of DNA and histone methylation marks (Lillycrop and Burdge, 2011; Jiang et al., 2012; Pauwels et al., 2017).

Erasure, establishment, and maintenance of imprinting

During human gametogenesis and early embryogenesis, two major genome-wide epigenetic reprogramming events take place to erase methylation marks (Reik et al., 2001). The first wave of DNA demethylation occurs between fertilization and implantation when many DNA methylation marks are erased (Fig. 2). A large decrease in DNA methylation is seen between gametes and the zygote, with a further reduction at the two-cell stage, providing support for active DNA demethylation. Genome-wide DNA methylation studies reveal decreasing DNA methylation levels from cleavage-stage to blastocyst-stage embryos in the inner cell mass (Guo et al., 2014; Smith et al., 2014). This DNA demethylation likely occurs through passive demethylation mechanisms. Following implantation these methylation marks are re-established, seen as a sharp increase in methylation post-implantation. This first reprogramming event does not include imprinted genes, which retain their methylation marks despite otherwise global demethylation (Guo et al., 2014; Okae et al., 2014). Parental imprinting marks are protected from this event and eventually reconfigure to the specific imprinting profile of each somatic tissue as the embryo develops (Guo et al., 2014, 2015). The theoretical level of methylation at each imprinted DMR is 50%, since one of the alleles is methylated and the other one is not (Reik et al., 2001; Liu et al., 2012; McCullough et al., 2015). However, imprinting methylation marks are sensitive to adverse environmental exposures during pregnancy that can result in hypo- or hyper-methylation of the DMRs.

A second global epigenetic reprogramming event takes place during embryonic germ cell development and involves also erasure of methylation marks on the imprinted genes. Male primordial germ cells (PGCs) exhibit low methylation levels at weeks 7 and 8, decreasing to the lowest levels at weeks 9–13, and these low levels are maintained until week 19 (Fig. 2). In females, PGCs exhibit low methylation levels at week 5.5, and by week 7 DNA methylation decreases to its lowest levels, where it is maintained through to week 11. In weeks 16 and 17 DNA methylation levels remain low (Guo et al., 2015; Tang et al., 2015). There is, however, data that report some differences in the methylation levels of male and female PGCs (Gkountela et al., 2015) compared to the previously mentioned studies. The erasure and re-establishment of the methylation marks are achieved by a not yet completely known mechanism (Guo et al., 2014, 2015; White et al., 2016). Human PGCs seem to be strongly enriched with genes involved in the base excision repair (BER) pathway (Gkountela et al., 2013, 2015; Guo et al., 2015). This finding is compatible with the possibility that the BER pathway is involved in the global DNA demethylation process in human PGCs, especially in active demethylation. The established marks are then maintained through cell divisions by DNMT1 that faithfully copies the methylation pattern from the template DNA strand to the nascent new strand (Moss and Wallrath, 2007).

As far as the imprinted gene demethylation is concerned, the parental-specific imprints are erased in PGCs. These imprints are re-established during gametogenesis in accordance with the sex of the fetus then maintained after fertilization and throughout subsequent development (King et al., 2015). This methylation loss may also occur by active DNA demethylation. Timing of methylation erasure at imprinted domains in PGCs remains controversial. Current data indicate that imprinted DNA methylation erasure follows a similar pattern to global DNA demethylation (Fig. 2). In female week 5.5 PGCs, a subset of imprinted gDMRs contain 20–40% methylation, indicating that some imprinted gDMRs have begun methylation erasure (Guo et al., 2015; Tang et al., 2015). Subsequent DNA demethylation continues, with imprinted gDMRs having 10–20% methylation in week 7 and 9 female and male PGCs, respectively. In week 10 PGCs through to week 16 female and week 19 male germ cells, imprinted gDMRs have low methylation levels. In contrast to these studies, another group found delayed DNA methylation erasure at imprinted gDMRs in PGCs, occurring weeks after global demethylation (Gkountela et al., 2013, 2015). To explain the possible methodology or sample differences between these human genome-wide studies, more extensive confirmatory studies are required.

Following erasure of methylation marks, the genome-wide epigenetic programming involves DNA methylation acquisition. In humans, acquisition of DNA methylation presents differences between spermatogenesis and oogenesis. In the male germline, the exact timing of DNA methylation acquisition is yet unknown (White et al., 2016). In mature sperm, methylation levels are reported to be ~54% (Guo et al., 2014), but some studies indicate higher methylation levels (Smallwood et al., 2011; Okae et al., 2014). In the female germline, acquisition of methylation occurs during folliculogenesis, when primordial follicles grow and mature to reach the ovulatory stage. Germinal vesicles (GVs) metaphase I and metaphase II oocytes have ~50% of global DNA methylation and are less methylated than sperm (White et al.,

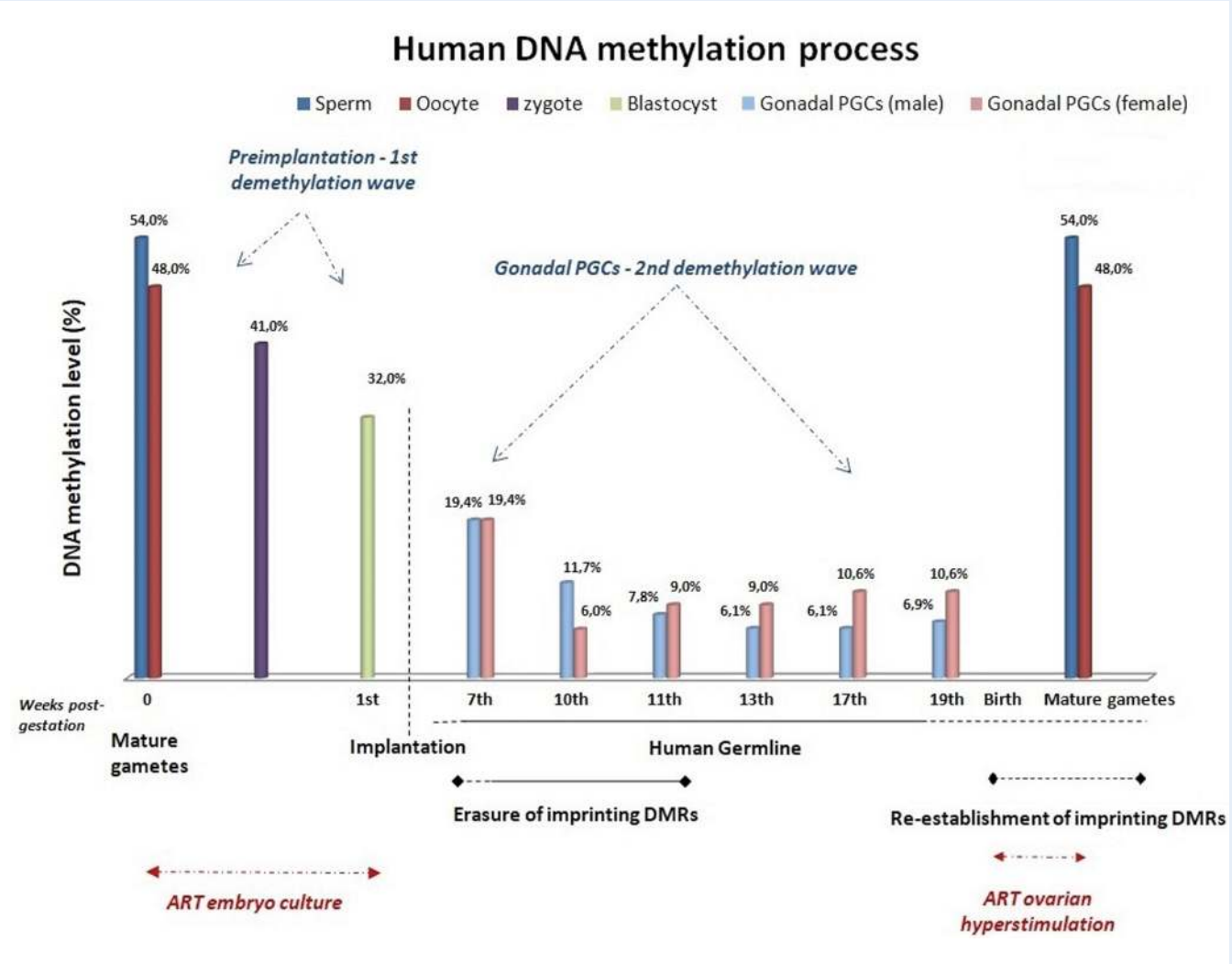

Figure 2 DNA methylation of human sperm, oocyte, blastocyst, post-implantation embryos, and PGCs. A genome-wide DNA demethylation takes place after fertilization. The DNA methylation level is reduced from 54% and 48% (median level) in sperm and metaphase II oocytes, respectively, to 41% in the zygotes and further to 32% in the inner cell mass of blastocyst-stage embryos, but the imprinted genes escape this first wave of DNA demethylation. Afterwards, there is a global re-methylation process shortly after implantation and the DNA methylation level reaches 92% (gonadal somatic cells). During PGC development, there is a global DNA de-methylation process that also involves the imprinted genes. The DNA methylation level is reduced from 92% in post-implantation embryos to 7.8% in the 11th week of male PGCs and 6.0% in the 10th week of female PGCs during this developmental period. DNA methylation levels remain low until week 19. In subsequent weeks and after birth, DNA methylation levels are gradually increased until they reach the level of mature germ cells. The imprints are re-established during germ cell maturation. Figure adapted from Guo *et al.* (2014).

2016). Studies. concerning the acquisition of imprinted methylation during spermatogenesis have shown that certain imprinted genes have acquired methylation at the stage of adult spermatogonia (Kerjean *et al.*, 2000; Marques *et al.*, 2011). In human oogenesis, acquisition of imprinted DNA methylation occurs from primary to preantral/antral follicle stages and is completed in the GV, metaphase I, and metaphase II stages (White *et al.*, 2016). This evidence, however, concerns only certain imprinted genes, while earlier developmental stages have not been studied yet. Regulation of human demethylation and remethylation processes are difficult to untangle, and further studies are needed to decipher the complex epigenetic reprogramming of the human germline.

In summary, imprinted genes are regulated through epigenetic mechanisms, mostly through DNA methylation. Proper function of DNMTs and supply of methyl donors is essential for correct DNA methylation establishment. Imprinting demethylation and remethylation occur in DMRs during different stages of germ cell development. Precise regulation of DMR methylation is important for establishing and maintaining the right imprints, affecting the expression levels of the associated genes. Many imprinted genes occur in clusters that are enriched for growth regulators that interact to coordinate early growth. Thus, methylation alterations at a single DMR may lead to changes in the regulation of multiple genes with subsequent effects on growth (Liu *et al.*, 2012).

Genomic imprinting, fetal growth, and development

Imprinting in human placenta and fetal development

The human placenta starts forming when the embryo implants in the maternal endometrium. It is composed of a fetal and a maternal component, and it grows throughout pregnancy, assisting in the development of the fetus. The fetal component of the placenta originates from the outer layer of the blastocyst, the trophectoderm, and gives rise to placental disc, amniotic and chorionic membranes, chorionic villi, and the umbilical cord (Caruso *et al.*, 2012). The maternal component of the placenta is termed the decidua; it develops from the maternal endometrium, and its role is to support the structure and function of the placenta as a whole (Caruso *et al.* 2012). Normal placental function is indispensable for intrauterine fetal viability and growth. As the placenta forms the interface between maternal and embryonic environments, it may be particularly susceptible to signals from both the mother and the fetus (Robins *et al.*, 2011; Non *et al.*, 2012. Human placenta produces pregnancy-related hormones and supports fetal development through numerous functions. Examples of these functions are transport of nutrients and waste between maternal and fetal circulations, regulation of fetal growth, and protection of the fetus from the maternal immune system (Monk, 2015). Many imprinted genes are expressed in the human placenta and play a central role in the development and function of the organ. For instance paternally expressed *MEST* is thought to play a role in angiogenesis in human trophoblast tissue and deciduas, and growth factor receptor-bound protein 10 (*GRB10*) is maternally expressed in cytotrophoblast (Frost and Moore, 2010).

Several studies focusing on the role of placenta in adverse pregnancy outcomes such as intrauterine growth restriction (IUGR), preeclampsia, and LBW have observed aberrant methylation levels or loss of methylation loss of imprinting (LOI) at imprinted loci in the placenta (Rancourt *et al.*, 2013; Janssen *et al.*, 2016). For example, disruption of *IGF2* DMR0 methylation has been reported in IUGR human placentas (Diplas *et al.*, 2009). Abnormal global DNA methylation and *H19* gene promoter methylation were also observed in human preeclamptic placentas (Gao *et al.*, 2011). Altered methylation patterns in the placenta may affect gene expression programs in many cell types leading to abnormal placentation and adverse birth outcome (Fig. 1) (Knerr *et al.*, 2004; Chavan-Gautam *et al.*, 2011). Therefore, understanding the regulation of imprinted genes may help comprehend human placental pathophysiology.

Identification of imprinted genes and the mechanisms controlling their function have been subjects of intense study over recent decades. Although imprinted genes comprise a small subset of protein-coding genes in the human genome, they have been shown to be essential to fetal growth and neurodevelopment (Marques *et al.*, 2008; Doria *et al.*, 2010; Piedrahita, 2011; Patten *et al.*, 2016). Animal studies have shown that they also play an important role in placental adaptive responses to external stimuli (Sandovici *et al.*, 2012). As we learn more about imprinted genes, it has become clear that they are critical for numerous processes such as differentiation, pluripotency, and metabolism (Janssen *et al.*, 2016). They are involved in many aspects of development including placental establishment and growth, embryogenesis, postnatal adaptations, cell proliferation, and adult behaviour (Lim *et al.*, 2012; Janssen *et al.*, 2015; Yang *et al.*, 2015). Broader roles for placentally expressed imprinted genes are seen in other organs through animal studies showing roles in the development of metabolically important organs, including the pancreas, liver, fat, pituitary, and hypothalamus (Charalambous *et al.*, 2007; Lambertini *et al.*, 2012). Imprinted genes function in various ways to regulate mammalian development. Paternally expressed genes such as pleomorphic adenoma gene like 1 (*PLAGL1*), *MEST*, *IGF2*, *PEG10*, and *KCNQ1OT1* favour fetal growth, whereas maternally expressed genes such as *H19*, pleckstrin homolog-like domain (*PHLDA2*, family A, member 2) and cyclin-dependent kinase inhibitor 1C (*CDKN1C*) are growth inhibitory genes.

The imprinted genes that are in focus in this review, *IGF2/H19*, *MEST*, and *PEG10* control embryonic and placental growth. Inappropriate DNA methylation of these genes is related to increased probability of early spontaneous abortion and severe fetal defects such as abnormal growth, LBW, or preeclampsia (Diplas *et al.*, 2009; Doria *et al.*, 2010; Eggermann *et al.*, 2014; Liang *et al.*, 2014; Kappil *et al.*, 2015b; Russo *et al.*, 2016). In the following chapters, we will discuss how DNA methylation patterns of these genes can be affected by stressors with consequences on fetal development and birth weight (Chen *et al.*, 2014; Vidal *et al.*, 2014b; Vangeel *et al.*, 2015; Mansell *et al.*, 2016).

Regulation, expression, and function of IGF2/H19 imprinted genes

Two of the most intensively studied imprinted genes, *IGF2* and *H19*, represent two oppositely expressed and functionally antagonistic genes located adjacent to each other in part of the same imprinted domain at the short arm of chromosome 11 (11p15.5). They share the same ICR, and *IGF2* is paternally expressed, while *H19* maternally expressed (Adkins *et al.*, 2010; Biliya and Bulla, 2010; Koukoura *et al.*, 2011; Moore *et al.*, 2015). *IGF2* is located upstream of *H19* and encodes a growth hormone that plays a major role in regulating fetal development. *H19* encodes a nonprotein-coding RNA that exerts its action mainly or exclusively as an RNA transcript. *IGF2* and *H19* share common enhancers, located downstream of *H19*; the activity of which is regulated by a DMR upstream of the *H19* gene. Imprinted expression of these two genes is mediated by methylation patterns at several different DMRs that dictate long-range interactions between enhancers and promoters (Moore *et al.*, 2015; Mansell *et al.*, 2016). The *IGF2/H19* ICR contains a methylation-sensitive chromatin insulator, which is responsible for controlling the expression of both genes (Fig. 3). In the paternal allele, *H19* is methylated, and binding of the CTCF insulator protein is blocked, thus inactivating *H19* and promoting *IGF2* expression (Marques *et al.*, 2008; Cordeiro *et al.*, 2014; Moore *et al.*, 2015; Mansell *et al.*, 2016). The IGF2 protein binds to insulin-like growth factor 1 receptor (encoded by *IGF1R*), a kinase activator that promotes cell survival and proliferation and consequently promotes fetal growth. Conversely in the maternal allele, *H19* is unmethylated, allowing the CTCF to bind to the DMR. This prevents access of *IGF2* to the common enhancers, thus inhibiting *IGF2* and promoting *H19* expression. When CTCF is bound, it allows *H19* to produce miR-675, which has been shown to restrain growth during gestation and cause downregulation of *IGF1R* (Mansell *et al.*, 2016).

IGF2 acts as a mitogenic growth factor that promotes differentiation and metabolism. *IGF2* is a part of the IGF axis and regulates the

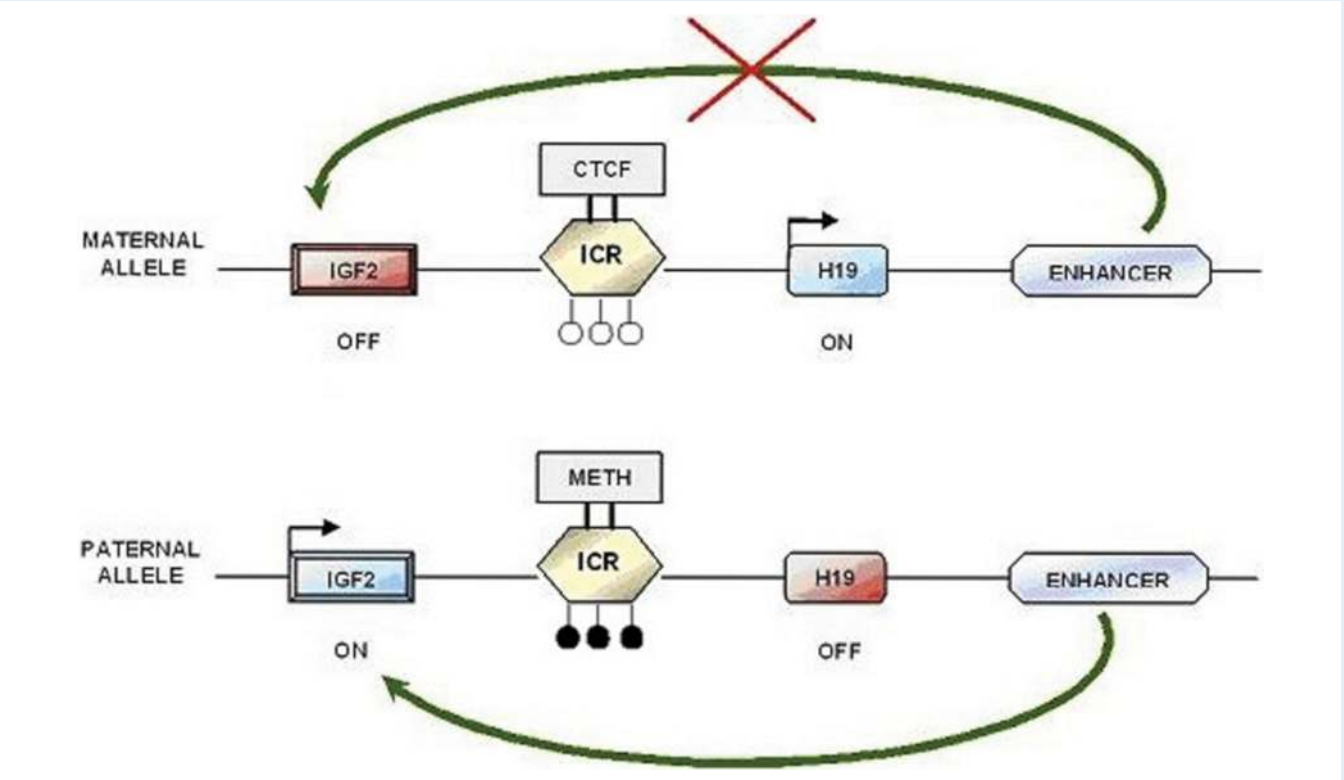

Figure 3 Structure and epigenetic state of the imprinted *IGF2/H19* domain on the maternal and paternal genome. On the maternal chromosome, the *H19* gene and the adjacent ICR are unmethylated (white lollipops) allowing *H19* expression and the binding of the insulator CTCF, thus preventing the enhancer to access the *IGF2* gene and silencing its expression. In the presence of ICR methylation on the paternal allele (black lollipops), binding of CTCF is prevented, allowing the enhancer activity to reach *IGF2*, thus effectively promoting its expression. Enhancer activity is limited to the unmethylated *IGF2* gene and the methylated *H19* gene remains silenced.

postnatal growth of somatic tissues, including the brain (Huang *et al.*, 2012; Perkins *et al.*, 2012). Later in life, changes in the IGF signalling pathway result in altered body fat composition. Lower circulating IGF2 levels have been associated with increased risk of obesity (Huang *et al.*, 2012). *IGF2* together with *H19* have been implicated in the control of placental and embryonic growth through cell proliferation and apoptosis (Ying *et al.*, 2010; Huang *et al.*, 2012; Liu *et al.*, 2012; Vidal *et al.*, 2014a). *H19* is physically and functionally linked to the *IGF2* gene. It is expressed in endoderm and mesoderm tissues during fetal life and is strongly downregulated after birth (Marques *et al.*, 2008; Yu *et al.*, 2009). *H19* regulates embryo development and growth, differentiation of the placenta and is highly expressed in the intermediate trophoblast and cytotrophoblast cells in human placental specimens. However, its precise role in the regulation of fetal development is not yet fully understood (Yu *et al.*, 2009; Koukoura *et al.*, 2011). On the other hand, *IGF2* is expressed across the syncytium to stimulate proliferation and survival of the underlying cytotrophoblast. This is a determining factor in the expansion of the placental syncytium and the generation of a sufficient exchange barrier to facilitate the oxygen and nutritional needs of the growing fetus (Harris *et al.*, 2011). Although there is some conflicting evidence regarding levels of *IGF2* in IUGR placentas (Apostolidou *et al.*, 2007; Antonazzo *et al.*, 2008), the majority of human studies have shown that reduced *IGF2* placental expression is associated with IUGR (McMinn *et al.*, 2006a; Guo *et al.*, 2008; Cordeiro *et al.*, 2014).

IGF2/H19 and birth weight variation

Most studies on *IGF2/H19* gene expression and methylation levels to date have focused on IUGR, since these genes are strong candidates for birth weight variation (Adkins *et al.*, 2010; St-Pierre *et al.*, 2012). LBW may function as a marker of adult health. For instance, LBW neonates are at augmented risk of coronary heart disease, type II diabetes, stroke, obesity, and some adult cancers (Liu *et al.*, 2012). While IGF2 serum levels and mRNA expression levels have been positively linked to infant birth weight in various studies, the findings regarding *IGF2/H19* DMR methylation and birth weight are not consistent. Such disparities, particularly relating to methylation and expression, are not scarce in the literature. However, recent studies positively correlate both *IGF2/H19* methylation and expression with birth weight variation, indicating the role of these genes in fetal development (Table I).

Experimental evidence has shown that for every 1% decrease in methylation of the *IGF2* DMR located upstream of exon 3, there is a two-fold increase in *IGF2* transcription, theoretically equivalent to what would be observed if the *IGF2* maternal allele was aberrantly active (Murphy *et al.*, 2012). This association was observed in women smokers compared to nonsmokers. It is presently unclear how small methylation shifts at imprinted DMRs alter the imprinting status of the related genes and affect developmental outcomes and risk of chronic diseases. Disturbances in *IGF2* and *H19* imprinted genes are known to cause IDs, such as Beckwith–Wiedemann (BWS) and Silver–

Table I Studies investigating the relationship between IGF2/H19 and birth weight.

Study	Genes/Tissues studied	BW relation	Results/Conclusions
Kappil et al., 2015b	Expression and methylation of imprinted genes. Placental tissue	Yes	Identified known and novel imprinted genes (*IGF2/H19, MEST*) related to birth weight. Expression of imprinted genes is crucial for appropriate fetal growth
Bouwland-Both et al., 2013	*IGF2* and *H19* methylation. Umbilical cord blood in SGA and controls	Yes	Lower *IGF2* DNA methylation in SGA children. *IGF2* and *H19* methylation related to fetal and infant growth
Wu et al., 2013	*IGF2* methylation. Nervous tissue, lung tissue, placental tissue from stillborn fetuses	Yes	Increased *IGF2* DRM0 methylation was positively linked to increased risk of neural tube defects
St-Pierre et al., 2012	*IGF2/H19* methylation and *IGF2* expression. Placental tissue and umbilical cord blood	Yes	Higher methylation of *IGF2* DMR0 and DMR2 was linked to higher infant birth weight and decreased *IGF2* mRNA expression. *IGF2/H19* DNA methylation as a modulator of fetal growth and birth weight that links birth weight and fetal metabolic programming of late onset obesity
Hoyo et al., 2012	*IGF2* methylation and protein concentrations. Umbilical cord blood	Yes	*IGF2* hypomethylation resulted in increased *IGF2* transcription and higher birth weight/ *IGF2* DNA methylation is functionally relevant to the production of *IGF2*
Koukoura et al., 2011	*IGF2* expression and methylation. Placentas from FGR and normal pregnancies	No	Significant loss of imprinting in growth restricted placentas. Non-significant decrease in *IGF2* mRNA levels not related to birth weight
Tobi et al., 2011	*IGF2* DNA methylation. Peripheral blood in SGA and control cases	No	No association between *IGF2* DMR methylation and SGA
Diplas et al., 2009	*IGF2, H19, MEST, PEG10* expression. Normal and IUGR human placentas	Yes	*IGF2* downregulation, *PEG10* upregulation and loss of *IGF2/H19* imprinting in placentas of children with IUGR. Regulation of imprinted genes in placental development plays an important role in determining fetal growth
Guo et al., 2008	*IGF2* and *H19* expression and methylation. Placental tissue and umbilical cord blood in SGA and control cases	No	*IGF2* placenta hypomethylation, but no significant differences between SGA children and normal controls. *IGF2* mRNA decreased in SGA placentas. Human 11p15 imprinting cluster regulates growth of placental and fetal tissues
Apostolidou et al., 2007	*IGF2, MEST* expression and methylation. Parental blood and term placentas	No	No correlation between *IGF2* and *MEST* levels and birth weight and no loss of imprinting

BW; Birth Weight, SGA; Small for Gestational Age, FGR; Fetal Growth Restriction

Russell (SRS) syndromes (Russo et al., 2016). Combining current data, it appears that abnormal *IGF2/H19* methylation and/or expression influences birth weight and affects fetal development and health.

Expression and function of MEST and PEG10

Apart from *IGF2* and *H19*, *MEST* and *PEG10* imprinted genes also have a broad developmental effect on fetal growth and birth weight variation. *MEST* is a paternally expressed imprinted gene that has recently been implicated in adipogenesis (Karbiener et al., 2015). It is located on chromosome 7q32, and it is involved in the control of embryonic and placental growth (Zechner et al., 2010; Huntriss et al., 2013). *MEST* encodes an a/b-hydrolase family of proteins and is expressed in the mesoderm of developing embryos (Vidal et al., 2014a). There are two different *MEST* isoforms. Isoform 1 appears to be expressed in a monoallelic fashion in most tissues like brain, skeletal muscle, kidney, adrenal gland, tongue, heart, skin, and placenta (Riesewijk et al., 1997; McMinn et al., 2006b). Initial reports described isoform 2 as being biallelic in blood lymphocytes (Kosaki et al., 2000). Subsequent reports have indicated that in certain tissues, including breast and placenta, isoform 2 may in fact be imprinted (Pedersen et al., 2002; McMinn et al., 2006b; Nelissen et al., 2013). Isoform 2 has been observed to be preferentially paternally expressed in fetal placenta, kidney, and fibroblast lines, but it is biallelically expressed in other fetal tissues and lymphoblastoid cell lines (Nakabayashi et al., 2002). Epigenetic regulation of *MEST* has been related to male infertility and poor sperm parameters (Houshdaran et al., 2007; Marques et al., 2008; Hammoud et al., 2010; Poplinski et al., 2010). It may also contribute to obesity predisposition throughout life and other metabolic disorders. It is scientifically supported that there is a positive correlation between *MEST* methylation percentage in chorionic villus and rates of early spontaneous abortion: the higher the methylation percentage, the greater the chance of early miscarriage (Zheng et al., 2011a).

The imprinted gene *PEG10* was first identified on the basis of its location in an imprinted domain on human chromosome 7q21. It is paternally expressed and maternally silenced (Ono et al., 2001; Chen et al., 2012) and is a functional retrotransposon derived gene but contains no long terminal repeat sequences (Smallwood et al., 2003;

Lux *et al.*, 2010). *PEG10* is expressed in a wide variety of human tissues, such as brain, lung, testis, kidney, and placenta. It is suggested to display a functional role in growth-promoting activities and human placenta formation. *PEG10* affects trophoblast proliferation, differentiation, and invasion (Metsalu *et al.*, 2014; Chen *et al.*, 2015) and is highly expressed in the placenta at ~11–12 weeks of gestation, implying that it is a requirement for placental development towards the end of the first trimester. Moreover, clinical studies have reported aberrant *PEG10* expression in multiple pregnancy complications, such as spontaneous miscarriages, fetal death, IUGR, and preeclampsia, indicating that *PEG10* is a critical player during gestation (Liang *et al.*, 2008; Doria *et al.*, 2010; Liang *et al.*, 2014). *PEG10*, together with *PHLDA2* and *CDKN1C*, control placental hormone production, including placental lactogens, known to induce physiological changes in pregnant women (John, 2013). However, its precise role in the regulation of gestation and in placental development is still poorly understood (Chen *et al.*, 2015).

Role of MEST and PEG10 in growth control

Recent data indicates that *MEST* and *PEG10* are important factors in regulating somatic differentiation and size at birth. According to Kappil *et al.*, *MEST* has the strongest association with fetal growth, with its increased expression linked to large for gestational age infants (Kappil *et al.*, 2015b). Increased *MEST* methylation and lower expression levels among small for gestational age (SGA) infants were also observed. Another study reported decreased *MEST* expression in IUGR placentas compared to normal term placentas, although this was not accompanied by changes in DNA methylation within a *MEST* DMR (McMinn *et al.*, 2006a). Further, the first study to quantitatively survey the expression levels of the vast majority of imprinted genes in human placenta found *PEG10*, among other imprinted genes, to be upregulated in IUGR placentas, compared to normal pregnancies (Diplas *et al.*, 2009; Piedrahita, 2011). In a recent study, *PEG10* was found to be significantly downregulated in umbilical cord samples of LBW babies. The reduced expression correlated inversely with increased DMR methylation likely occurring at the normally unmethylated paternal allele. The authors also suggested a role for *PEG10* in regulating human fetal growth, perhaps through an effect on placenta development. Finally, their data demonstrate that even modest changes in *PEG10* expression levels are associated with significant changes in human prenatal growth (Lim *et al.*, 2012). Combining all this data, it seems that normal expression of *MEST* and *PEG10* is essential for fetal growth and disturbances in their regulation associate with birth weight variation, which should be elucidated further in future studies.

Imprinted genes in disease

The importance of genomic imprinting is reflected by the numerous IDs and other diseases caused by aberrant DNA methylation (epimutation) or other disruptions of imprinted genes. IDs are a group of congenital diseases characterized by molecular changes in imprinted genes and adverse clinical features associated with growth, development, and metabolism (Table II). Typically, disturbances of imprinted genes may alter their regulation (epigenetic mutation) and expression but rarely their genomic sequences (genetic mutation). Types of disruptions found in IDs vary, but the majority of them are associated with uniparental disomy (UPD), chromosomal imbalances (duplication and deletion), and genomic mutations (Ishida and Moore, 2013; Eggermann *et al.*, 2015). Epimutation at ICRs is frequent in IDs and could occur after a defect is introduced either during gametogenesis (causing failure of imprint erasure and re-establishment), or post-fertilization due to unsuccessful imprint maintenance. There are also studies implying that IDs are more frequent in children conceived through ART than in spontaneous conceptions (Manipalviratn *et al.*, 2009; Hiura *et al.*, 2012; Tee *et al.*, 2013; Hiura *et al.*, 2014; Mussa *et al.*, 2017).

Table II Basic characteristics of IDs.

General findings
1. Non-mendelian inheritance
2. Environmental contribution
Typical molecular findings
1. (Epi)mutations
2. Multi-locus methylation defects
Overlapping clinical features
1. Cognitive impairment
2. Aberrant growth, asymmetry
3. Developmental delay
4. Facial features, etc

ID; imprinted diseases

Although IDs are rare, with a collective prevalence less than one per 12 000 births (Manipalviratn *et al.*, 2009), they are often severe, highlighting the necessity for normal expression of this small subset of imprinted genes for normal human development (Table III). For example, accurate DNA methylation of the *IGF2/H19* locus is indispensable for appropriate human growth, and IDs caused by disruption of DNA methylation in this region are associated with phenotypes of altered growth (Huang *et al.*, 2012). Up to now, human genetic diseases linked to various IDs include BWS, SRS, Prader–Willi (PWS) and Angelman (AS) syndromes (Rabinovitz *et al.*, 2012; Choufani *et al.*, 2013; Azzi *et al.*, 2014), transient neonatal diabetes mellitus type 1 (Temple and Shield, 2010) and pseudohypoparathyroidism type Ib (Mantovani, 2011). Furthermore, DNA methylation changes in ICRs of imprinted genes have been implicated in many other diseases (Table IV). The functional outcome in each ID is the unbalanced expression of imprinted genes and often primarily growth and/or neurological development defects, but the clinical phenotypes are diverse and depend on the parental allele affected by the mutation. Despite limited information about aetiology, the molecular defects behind most known IDs are well understood as they are associated with molecular disruptions in specific loci. Undoubtedly, the number of IDs will increase in the future as we learn more about imprinted genes and their role in physiology.

To conclude, imprinted genes are essential to placental and fetal development, involving a variety of processes. Their disruption can lead to IDs and other diseases with molecular and clinical consequences. *IGF2/H19*, *MEST*, and *PEG10* imprinted genes seem to be regulatory

Table III General features and molecular changes in so far identified IDs.

Disorders	Affected imprinted genes	Characteristics
Beckwith-Wiedemann syndrome	*IGF2, CDKN1C, KCNQ1OT1* (11p.15.5)	Overgrowth, tumour and cancer predisposition, macroglossia, etc
Silver-Russell syndrome	*IGF2/H19*, *CDKN1C* MEST, GRB10 upd(7) mat (7q32, 11p15)	Asymmetry, growth failure, adult-onset diseases, facial features, etc
Prader-Willi syndrome	Various imprinted genes on chromosome 15, upd(15)mat (15q11-q13)	Hypotonia, infertility, mental disorders, etc
Angelman syndrome	*UBE3A,* upd(15)pat (15q11-q13)	Abnormal behaviour with excessive laughter, intellectual disability, ataxia, etc
Pseudohypoparathyroidism	Maternal transmission of inactive *GNAS* mutations, upd(20) pat (20q13.2)	Resistance to parathyroid hormone, obesity, osteodystrophy
Transient Neonatal Diabetes Mellitus Type 1	*PLAGL1, HYMAI*, paternal duplication of 6q24, upd(6) pat (6q24)	Diabetes, congenital abnormalities
Temple syndrome [upd(14)mat]	(14q32)	Growth retardation, hypotonia, obesity, mental disorders, etc
Kagami-Ogata syndrome [upd(14)pat]	(14q32)	Polyhydramnios, developmental delay, placentomegaly, intellectual disability

Upd()mat; uniparental disomy (chromosome affected) maternally inherited Upd()pat; uniparental disomy (chromosome affected) paternally inherited

Table IV Diseases caused by methylation changes in ICRs of imprinted genes.

Study	Disorder	Imprinted locus affected
Astuti et al., 2005	Neuroblastoma	*DLK1-MEG3* imprinted domain
Kuerbitz et al., 2002	Acute myeloblastic leukemia	Hypermethylation of the imprinted *NNAT* locus
Moon et al., 2010	Uterine leiomyoma	Overexpression of *PEG1/MEST*
Ribarska et al., 2014	Prostate cancer	Deregulation of an imprinted gene network
Cui et al., 2002/ Ito et al., 2008	Colorectal carcinoma	Hypomethylation of *H19* and *IGF2/IGF2* DMR0 hypomethylation
Pedersen et al., 1999	Breast cancer	*PEG1/MEST* loss of imprinting
Murphy et al., 2006/ Feng et al., 2008	Ovarian cancer	*IGF2/H19* epigenetic alterations/*ARHI* and *PEG3* down-regulation
Wallace et al., 2010	Diabetes	*DLK1-MEG3* imprinted region
Marsit et al., 2012	Neurobehavioral development	Deregulation of an imprinted gene network
Perkins et al., 2012	Obesity	Methylation of IGF2/H19 locus
Liang et al., 2014/ Doria et al., 2010	Pregnancy failure and complications	Downregulation of *PEG10*/Different expression of *IGF2*, *PEG10*, *CDKLN1C*, *PHLDA2*
Smolarek et al., 2010	Hypertension	Global DNA methylation changes
Movassagh et al., 2010	Cardiovascular disease	Differential DNA methylation

factors during fetal growth and development, but further studies are needed to decipher their role.

Effect of stressors on imprinting

In general, epigenetic mechanisms can be influenced by various external factors ranging from depression, cigarette smoke, and diet to toxic chemicals (Hoyo *et al.*, 2014; Kappil *et al.*, 2015a; Vidal *et al.*, 2015). Epigenetic mechanisms play key roles in many cellular processes and are absolutely critical for development, differentiation, and adaptation to the environment, and thereby their disruption may contribute to disease risk throughout life. Intrauterine life is a sensitive window of development during which external factors can influence organ development, homeostasis, and epigenetic processes, including DNA methylation, permanently altering epigenetic patterns and subsequent development (Ollikainen *et al.*, 2010; Antonelli *et al.*, 2017). During early life stages, development shows high plasticity and many biological systems are susceptible to environmental stressors (Harris and Seckl, 2011; Griffiths and Hunter 2014). According to the fetal programming theory, exposures during fetal development and early life have more profound effects on the establishment and/or maintenance of epigenetic marks than exposures in adulthood. This may lead to permanently altered disease susceptibility (King *et al.*, 2015). Adverse

conditions during gestation may induce LOI that does not manifest phenotypically or functionally at birth, due to developmental plasticity, but results in chronic diseases decades later (Ollikainen *et al.*, 2010). Herein, we aim to evaluate the effect of three important environmental stressors (mode of conception, maternal prenatal psychological stress and chemical exposure) on fetal development and imprinting (Fig. 1).

Genomic imprinting and mode of conception

The use of ART procedures is steadily increasing worldwide; >7 million infants have been born so far. However, many questions remain unanswered concerning the long-term health consequences of ART. A series of investigations dating from 2002 have suggested that ART-born children carry a potential higher risk of chromosomal aberrations, perinatal complications, IUGR, pre-term birth (PTB), LBW, IDs, and birth defects (Cox *et al.*, 2002; Hansen *et al.*, 2002; Schieve *et al.*, 2002; Gicquel *et al.*, 2003; Lambert, 2003; Ceelen *et al.*, 2008; Lim *et al.*, 2009; Manipalviratn *et al.*, 2009; Sakka *et al.*, 2010; Savage *et al.*, 2011; Davies *et al.*, 2012; Pandey *et al.*, 2012; Wen *et al.*, 2012; Kochanski *et al.*, 2013; Pinborg *et al.*, 2013a; Hyrapetian *et al.*, 2014; Mussa *et al.*, 2017). The causes of the poorer perinatal outcome in ART-conceived children are probably multifactorial and are still under investigation (Pinborg *et al.*, 2013b).

The periconceptional period is subject to genome-wide epigenetic reprogramming including imprinting and therefore is crucial for proper development and future health (Gillman, 2005). Major epigenetic events take place in the embryo both during pre-implantation development and post-implantation when imprints in the PGCs in the embryo are reset. Periconception is therefore a dynamic period of reprogramming that could be sensitive to environmental stressors leading to epigenetic disturbances (Reik *et al.*, 2001; Jirtle and Skinner, 2007; van Montfoort *et al.*, 2012; Huntriss *et al.*, 2013; Desplats, 2015). Manipulations and processes during ART, including ovarian stimulation, *in-vitro* maturation of gametes, isolation and handling of gametes, *in-vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and *in-vitro* culture of early embryos represent artificial interventions, which coincide with critical windows for epigenetic reprogramming and imprinting (Fig. 2). These procedures expose the developing epigenome to stress factors that could affect the establishment and maintenance of genomic imprints and thereby influence implantation, establishment of placenta, and fetal development (Maher *et al.*, 2003; Horsthemke and Ludwig, 2005; Haaf, 2006; Santos *et al.*, 2010; van Montfoort *et al.*, 2012; El Hajj and Haaf, 2013; Anifandis *et al.*, 2015; Canovas *et al.*, 2017). Oocyte imprints are acquired during follicle growth and maturation, and ART procedures employ hormones to artificially stimulate the growth and maturation of supernumerary ovarian follicles, which could carry improper methylation marks. Further, these hormones may function as potential imprinting disruptors, affecting oocyte and embryo imprint acquisition (Sato *et al.*, 2007; Santos *et al.*, 2010; Obata *et al.*, 2011; Denomme and Mann, 2012; Anifandis *et al.*, 2015).

Concerns have also been raised regarding the impact of extended embryo culture time and embryo cryopreservation on methylation patterns and imprinting, since these procedures are employed during the crucial period of the first embryo divisions. *In-vitro* culture conditions, culture media composition, and day of embryo transfer (namely how many days the embryos are cultured) are very important factors that may have a negative impact on the ART offspring and exert alterations in embryo metabolism and DNA imprinting (Dumoulin *et al.*, 2010; Källén *et al.*, 2010; Ibala-Romdhane *et al.*, 2011; Sazonova *et al.*, 2011; Fernando *et al.*, 2012; Nelissen *et al.*, 2012; Nelissen *et al.*, 2013; Kleijkers *et al.*, 2014; Shi *et al.*, 2014). Suboptimal embryo culture conditions expose embryos to environmental stress (subtle temperature variations in the incubators, exogenous chemical compounds, light exposure, O_2 and CO_2 levels, etc) and can generate morphologically normal but epigenetically compromised blastocysts that fail to implant (El Hajj and Haaf, 2013; Anifandis *et al.*, 2015; Canovas *et al.*, 2017).

Cryopreservation of gametes and embryos has become increasingly important in recent years and has raised questions regarding the safety and long-term consequences of these procedures. Studies comparing children born after frozen embryo transfer (FET) with those born after fresh embryo transfer have revealed that the neonatal clinical outcomes appear to be similar or even improved after FET, especially regarding LBW, SGA, and PTB (Wennerholm *et al.*, 2009; Pelkonen *et al.*, 2010; Pinborg *et al.*, 2010; Wennerholm *et al.*, 2013; Berntsen *et al.*, 2019). The reason for these improved outcomes with FET is still not clear. It might be associated with the absence of ovarian stimulation, which may affect endometrial receptivity and the implantation of the embryo into the uterus during fresh IVF cycles. Another possible explanation is that cryopreservation enables only the good quality embryos to survive (Pinborg *et al.*, 2013a). On the contrary, poorer clinical outcomes appeared when comparing FET with naturally conceived embryos, probably due to patient-related factors or ART aspects (Pelkonen *et al.*, 2010; Pinborg *et al.*, 2010; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013). Finally, accumulating data show that FET singletons have a higher risk of macrosomia and being large for gestational age compared with fresh transferred embryos (Pelkonen *et al.*, 2010; Pinborg *et al.*, 2010; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013), as well as with the general population (Pinborg *et al.*, 2010; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013; Pinborg *et al.*, 2014). Future research should elucidate possible cryoinjuries caused by ice crystals and any potential cytotoxic effects of cryoprotectants on embryo quality, and clarify whether long-term storage of oocytes and embryos is safe and does not cause adverse methylation alterations.

However, the impact of ART on DNA methylation cannot easily be evaluated because patients under ART treatments may differ both genetically and demographically from the general population; usually, they have lower fertility and increased reproductive loss rates. They also encounter different fertility problems, which may contribute variously to imprinting (Chiba *et al.*, 2013; El Hajj and Haaf, 2013; Hiura *et al.*, 2014; Pinborg *et al.*, 2016). Furthermore, ART protocols are not harmonized and therefore vary between clinics. Due to this variation and the inability to control for all the variables involved, large cohort studies are needed to assess whether a causal relationship between ART and increased prevalence of imprinting defects exists. No definitive conclusions have been drawn yet, since it remains unresolved whether the use of ART treatments or the underlying subfertility per se is the causal factor of the higher prevalence of complications and epimutations in ART-conceived children (Zhu *et al.*, 2006; Doornbos *et al.*, 2007;

El Hajj and Haaf, 2013; Pinborg et al., 2013a; Pinborg et al., 2013b; Hyrapetian et al., 2014; Källén et al., 2010; Simpson, 2014; Berntsen et al., 2019). In summary, the literature concerning the safety of these techniques is too limited to draw any firm conclusions regarding health outcomes.

The effect of ART on imprinted gene regulation

Although current knowledge on the mechanisms controlling fetal development is still limited, it seems that acquisition of imprinting errors during placental and early embryo development may predispose to diseases and gene expression abnormalities in the offspring (Nelissen et al., 2011; Zheng et al., 2011b; Shi et al., 2014; Monk, 2015). Stability of DNA methylation and expression of imprinted genes have been intensively investigated in placenta, oocytes, and embryos in relation to ART processes. Altered gene expression was observed in placental tissue of IVF/ICSI patients and several biological pathways playing a role in metabolism, immune response, and transmembrane signalling were upregulated (Nelissen et al., 2014). It is assumed that these differences can potentially affect fetal development and lead to an increased risk for late-onset diseases. Specific epigenetic changes observed at DMRs in placentas and umbilical cord blood of ART newborns suggest that the conditions during fertility treatments may affect DNA methylation and lead to IDs (Pinborg et al., 2016; Canovas et al., 2017). Such epigenetic disruptions were first recorded in a number of studies focusing on the impact of ART on the regulation of imprinted genes and the risk of developing BWS, AS, and SRS IDs (Cox et al., 2002; DeBaun et al., 2003; Maher et al., 2003; Kagami et al., 2007; Lim et al., 2009; Manipalviratn et al., 2009; Hiura et al., 2012; Hiura et al., 2014; Mussa et al., 2017). However, a small increase in the incidence of IDs after ART cannot easily be evaluated because the prevalence of these diseases is very low in the general population (El Hajj and Haaf, 2013; Uyar and Seli, 2014). This could explain why some studies have failed to support a link between IDs and ART procedures (Doornbos et al., 2007; Odom and Segars, 2010; Chiba et al., 2013; Vermeiden and Bernardus, 2013; Uyar and Seli, 2014; Pinborg et al., 2016). Many studies, however, have still found support for a trend of increased imprinting defects in ART-conceived children (meta-analysis of Cortessis et al., 2018; Berntsen et al., 2019). These data call for caution and further studies.

As far as imprinted genes are concerned, several studies performed in oocytes, preimplantation fresh or frozen embryos, peripheral blood, umbilical cord blood, amniotic membranes, chorionic villi, and placenta samples have presented contradictory data about the effect of ART on the regulation of *IGF2/H19, MEST*, and *PEG10* among other imprinted genes (van Montfoort et al., 2012; Uyar and Seli, 2014). Some studies have observed disrupted patterns of DNA methylation and/or gene expression in cord blood, chorionic villi, placenta (Turan et al., 2010; Zechner et al., 2010; Nelissen et al., 2013; Nelissen et al., 2014; Sakian et al., 2015), and preimplantation embryos (Huntriss et al., 2013; Shi et al., 2014) compared to respective samples of spontaneously conceived children. However, others have reported no detectable alterations in DNA methylation and gene expression after ART treatments (Wong et al., 2011; Zheng et al., 2011a; Zheng et al., 2011b; Oliver et al., 2012; Puumala et al., 2012; Camprubí et al., 2013; El Hajj et al., 2017). Furthermore, although a few studies have revealed small differential DNA methylation or altered expression of a subset of imprinted genes (including *PEG10* and *MEST*) in cord blood, peripheral blood, and placental samples, they have concluded that the general imprinting and expression pattern were stable in ART-conceived children (Gomes et al., 2009; Tierling et al., 2010; Feng et al., 2011; Shi et al., 2011; Rancourt et al., 2012; Vincent et al., 2016).

Two genome-wide studies (Katari et al., 2009; Melamed et al., 2015) identified a number of affected imprinted genes in cord blood and placental samples of IVF children, but due to small sample sizes these results need to be interpreted with caution. A meta-analysis of 18 studies showed an association between ART and IDs compared to children conceived naturally but reported no evidence of DNA methylation changes in imprinted genes (Lazaraviciute et al., 2014). However, in many cases the amount of data was heterogeneous and limited; thus, more controlled studies are needed. Finally, a recent study comparing human blastocysts derived from nonvitrified embryos with vitrified ones demonstrated that vitrification on Day 3 did not significantly alter the DNA methylation pattern of the *IGF2/H19* DMR (Derakhshan-Horeh et al., 2016), suggesting that cryopreservation does not affect imprinting of this locus. In summary, the present state of knowledge does not allow for solid conclusions regarding the mode of conception and fetal genomic imprinting. Further studies are needed to decipher the consequences of ART interventions on DNA methylation, IDs, and regulation of imprinted genes. Since imprints are maintained not only by DNA methylation but also by histone modifications and long noncoding RNAs, these mechanisms should also be considered as a possible explanation to differing results between studies. As the number of people seeking ART treatments will probably continue to rise in the years to come, long-term evaluation of children conceived through ART methods is necessary for understanding the potential risks and the impact of these technologies on birth outcomes and long-term health in the offspring.

Maternal psychological stress and offspring health

There is now an extensive body of literature from human studies showing significant associations between maternal prenatal psychological stress, fetal development, and postnatal health (Khashan et al., 2009; Harville et al., 2010; Dancause et al., 2012; King et al., 2012; Dancause et al., 2013; Cao-Lei et al., 2014; Lee, 2014; Walder et al., 2014; Harville et al., 2015; Yong Ping et al., 2015; Saulnier and Brolin, 2015; Liu et al., 2016; Cai et al., 2017; Isgut et al., 2017; Van den Bergh et al., 2017; MacKinnon et al., 2018). Prenatal traumatic exposures that have been linked to altered child outcome vary from severe traumas, such as bereavement (Khashan et al., 2008), to mild events, such as daily hassles. They also include exposure to acute external disasters (Laplante et al., 2008), the September 11 attacks (Yehuda et al., 2005), Chernobyl (Huizink et al., 2008), natural disasters (Kinney et al., 2008; Laplante et al., 2008) and war (Kleinhaus et al., 2013). However, it is difficult to adjust for confounders in the context of natural disasters, since, for example, stress and malnutrition often occur simultaneously under these circumstances and are inter-related (Lucassen et al., 2013).

Various studies report that maternal psychological stress during gestation, such as symptoms of anxiety and/or depression, can lead to fetal growth restriction, PTB, and LBW (Graignic-Philippe et al., 2014). It can also cause adverse health outcomes later in life, including susceptibility to metabolism-related diseases, neurodevelopmental delay, learning difficulties, behavioural problems, cognitive impair-

ment, cardiovascular and respiratory diseases, neural tube defects, and depressive symptoms (Rondo *et al.*, 2003; Van den Bergh *et al.*, 2005; Grote *et al.*, 2010; Tegethoff *et al.*, 2010; Zhu *et al.*, 2010; Class *et al.*, 2011; Grigoriadis *et al.*, 2013; Ding *et al.*, 2014; Guxens *et al.*, 2014; Slykerman *et al.*, 2015; Janssen *et al.*, 2016; Lee *et al.*, 2016; Gentile, 2017; Lee *et al.*, 2017). The effects have been shown to continue at least until early adulthood (Pearson *et al.*, 2013; O'Donnell *et al.*, 2014; Capron *et al.*, 2015; Kingsbury *et al.*, 2016), while further long-term studies are still being conducted.

Mechanisms mediating stress effects on fetal development

The biological mechanisms through which maternal psychological stress affects the developing fetus are not yet fully understood. It is suggested that maternal–fetal psychological stress transfer is a combination of numerous transfer mechanisms that may act together in a synergistic way (Fig. 4). One possible underlying mechanism concerns glucocorticoids secreted by the mother's HPA axis, which plays a fundamental role in the regulation of homeostasis and stress response (van Bodegom *et al.*, 2017; McGowan and Matthews, 2018). Development of the HPA axis and brain sections involved in its regulation is tremendous during the prenatal period and continues after birth. Therefore, environmental insults can have fundamental effects on the fetal developing brain. Many studies have focused on concentrations of cortisol, the end product of the HPA axis, as a marker of stress and anxiety (Bergman *et al.*, 2010; Davis and Sandman, 2010; Rothenberger *et al.*, 2011). Maternal prenatal exposure to stressful events can result in increased transplacental transfer of maternal circulating glucocorticoids to the fetal compartment (Kingsbury *et al.*, 2016). The placenta is involved in HPA development through the activity of placental-expressed genes that regulate the cortisol pathway. There are documented associations between prenatal psychological stress and altered placental function both in animal models (Mairesse *et al.*, 2007; Jensen Pena *et al.*, 2012) and in humans (O'Donnell *et al.*, 2012; Blakeley *et al.*, 2013; Reynolds *et al.*, 2015), which may lead to adverse infant outcomes. Glucocorticoids are essential for fetal development and maturation of vital organs and tissues during pregnancy, and normally ~3% of the maternal cortisol is transferred to the fetal circulation (Stirrat *et al.*, 2018). However, excessive exposure to the maturing human brain can impair normal development and dysregulate the hippocampus and the HPA axis, with long-term consequences for offspring's neurobehavioural and cardiometabolic health (Reynolds *et al.*, 2013a, 2013b; Glover, 2014; Appleton *et al.*, 2015; Provençal and Binder, 2015; Vaiserman, 2015; Kingsbury *et al.*, 2016; Lin *et al.*, 2017). Furthermore, prenatal exposure to exogenous glucocorticoids can alter genomic programming indirectly by altering placental transport and subsequently the availability of different methyl donors (O'Neill *et al.*, 2014). Malnutrition and *in-utero* lack of methyl donors may modify fetal DNA methylation patterns and lead to functional alterations throughout the life course, as mentioned above. The fetus is protected from high levels of maternal cortisol by molecular mechanisms in the placenta, such as the activation of the barrier enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) and the *NR3C1* gene, that prevent excess catecholamines and glucocorticoids from reaching the fetus. However, their function may be downregulated under psychological stress (O'Donnell *et al.*, 2012; Cottrell *et al.*, 2014; Togher *et al.*, 2014).

In addition to cortisol, there is emerging evidence that several other factors released during acute maternal psychological stress, such as catecholamines, cytokines, and serotonin, act together in a synergistic manner and can transmit maternal psychological stress to the fetus (Rakers *et al.*, 2017). For example, serotonin produced by human placenta is critical for fetal brain development (Bonnin *et al.*, 2011). It has been suggested that stress-induced changes in serotonin levels could contribute to fetal programming and brain development leading to long-lasting mental health outcomes (Bonnin *et al.*, 2011; Blakeley *et al.*, 2013; St-Pierre *et al.*, 2016).

To conclude, the mechanisms that underlie fetal programming by maternal psychological stress have not yet been clarified. Accumulating evidence has well established that the impact of maternal psychological stress on child outcomes does not depend solely on genetic factors. In a study by Rice *et al.*, IVF babies who were genetically related to their mother were compared with babies born after gamete/embryo donation (Rice *et al.*, 2010). This study showed an association between maternal psychological stress in pregnancy and conduct disorder in both groups. This observation supports the idea that the association between psychological stress and child conduct disorder is not based on genetics only but is also affected by the environment. Furthermore, studies assessing the consequences of natural or man-made disasters, such as the Canadian Ice Storm and/or terror attacks, on child outcomes have proved, by assessing levels of objective stress, that the effects are not caused by pre-existing maternal problems or genetic influences (Yehuda *et al.*, 2005; Huizink *et al.*, 2008; Laplante *et al.*, 2008; Kinney *et al.*, 2008; Kleinhaus *et al.*, 2013; Saulnier and Brolin, 2015).

Impact of prenatal psychological stress on imprinted genes

In-utero exposure to maternal psychological stress could result in epigenome alterations, which further impact on the adaptation and physiology of the offspring throughout childhood and even adulthood (Nemoda *et al.*, 2015; Cao-Lei *et al.*, 2017). Imprinted genes may respond to *in-utero* maternal psychological stress through changes in their epigenetic patterns and affect infant growth and neurobehavioural development (Green *et al.*, 2015; Isgut *et al.*, 2017). It is possible that aberrant placental imprinted gene expression results in impaired placental function and mediates the association between maternal psychological stress and adverse infant outcomes. Several studies provide preliminary evidence that maternal mental health during pregnancy can result in differential methylation levels of imprinted genes in the offspring (Liu *et al.*, 2012; St-Pierre *et al.*, 2012; Chen *et al.*, 2014; Ding *et al.*, 2014; Non *et al.*, 2014; Vidal *et al.*, 2014b; Vangeel *et al.*, 2015; Janssen *et al.*, 2016; Mansell *et al.*, 2016). For example, it has been shown that maternal prenatal depression and anxiety can have a widespread effect on genome-wide DNA methylation of newborns (Non *et al.*, 2014). Maternal depressed mood during pregnancy and birth weight were examined in a study of LBW newborns. It was observed that infants from mothers with depression had significantly higher cord blood DNA methylation levels at the *MEG3* DMR, while LBW infants had lower methylation at the *IGF2* and *PLAGL1* DMRs (Liu *et al.*, 2012). Cord blood methylation levels of *MEST* were positively correlated with maternal self-reported psychological stress during pregnancy in a recent study of 79 mother–infant pairs (Vidal *et al.*, 2014b). In addition, offspring *IGF2* and *H19* methylation has been shown to be influenced by maternal mental health. Increased cord blood and placental methylation of the ICR located upstream of *H19* has been reported in infants born to mothers with high

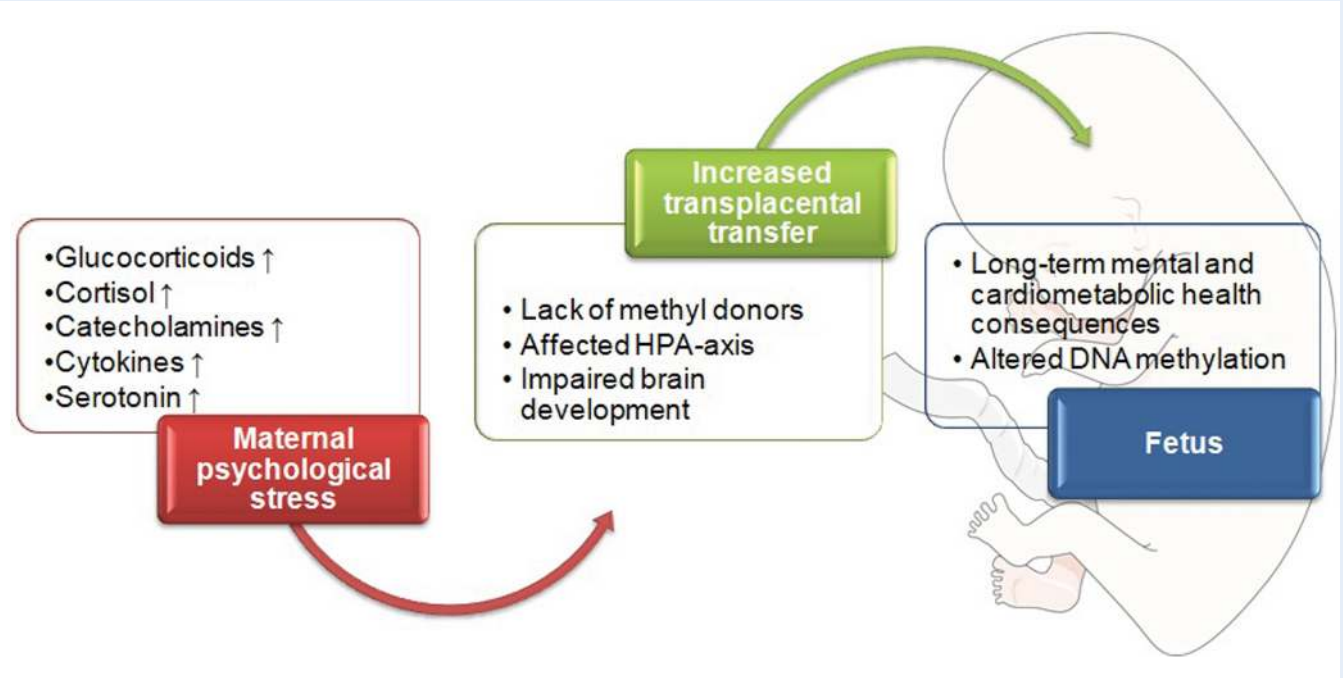

Figure 4 Possible mechanisms of maternal–fetal psychological stress transfer. Maternal prenatal psychological stress can result in increased transplacental transfer of maternal circulating glucocorticoids to the fetal compartment, which can lead to impaired hippocampus and HPA axis development with long-term consequences for the offspring's neurobehavioural and cardiometabolic health. The availability of methyl donors can also be affected and result in altered DNA methylation patterns. Other factors released during acute maternal psychological stress, such as catecholamines, cytokines, and serotonin act together and can transmit maternal psychological stress to the fetus. These changes can impact on brain development and have long-lasting mental health outcomes for the fetus.

perceived stress and anxiety (Chen *et al.*, 2014), whereas decreased methylation of the *IGF2* DMR0 has also been found (Vangeel *et al.*, 2015). Contrary to Chen *et al.*'s findings (2014), Mansell *et al.* (2016) provided compelling evidence of an association between maternal anxiety and decreased *IGF2/H19* ICR methylation at the major CpG sites (Mansell *et al.*, 2016). Interestingly, these data confirm and strengthen previous reports that link maternal anxiety and decreased infant *IGF2/H19* ICR methylation with an increased risk of PTB and LBW (St-Pierre *et al.*, 2012; Ding *et al.*, 2014). Although there are still limited studies focusing on maternal psychological stress and regulation of imprinted genes, the abovementioned findings suggest a relationship between maternal prenatal psychological stress and adverse birth outcomes mediated through impaired imprinted gene methylation.

Environmental chemicals and imprinting

In addition to psychological stress, chemical stress can affect fetal development, DNA methylation, and imprinting. Exposure to industrial chemicals is ubiquitous. For example, polychlorinated biphenyls, organochloride pesticides, fluoroalkyl compounds, phenols, polybrominated diphenyl ethers, and perchlorate can be found in virtually all Americans including pregnant mothers (Crinnion, 2010; Woodruff *et al.*, 2011). The fetus is directly exposed too, since chemicals can pass to umbilical cord blood, fetal tissues, and amniotic fluid (Morello-Frosch *et al.*, 2016; Mamsen *et al.*, 2019).

Documented associations between gestational chemical stress and adverse fetal growth are many. For example, tobacco smoke, air pollution, and heavy metals in ground water associate with smaller birth weight in cohort studies (Abbott and Winzer-Serhan, 2012; Pedersen et al., 2013; Edwards, 2014), as do maternal serum levels of dichlorodiphenyldichloroethylene, polychlorinated biphenyls, and perfluorooctane sulfonate (Casas *et al.*, 2015; Robledo *et al.*, 2015; Lenters *et al.*, 2016). Dysregulation of DNA methylation and genomic imprinting could be one mechanism connecting chemical stress to lower birth weight. For example, Zhao *et al.* have shown that maternal urinary levels of certain phthalates associate with significant changes in global DNA methylation as well as to specific changes in methylation of *IGF2* gene in human placenta and infant birth weight (Zhao *et al.*, 2015, 2016). Phthalates are a large group of synthetic industrial chemicals used for instance in food wrappings, personal care products and children's toys. More than 8 billion kg of phthalates are used worldwide each year and humans are repeatedly and continuously exposed (Heudorf *et al.*, 2007; Högberg *et al.*, 2008).

Another chemical with documented effects on DNA methylation, imprinting, and birth weight is bisphenol A (BPA), a high production volume industrial chemical used in the manufacture of plastics and resins. In humans, concentration of BPA in placenta correlates negatively with birth weight and positively with global DNA methylation: higher BPA levels in placenta associate with lower birth weight and increased DNA methylation levels (Troisi *et al.*, 2014; Nahar *et al.*, 2015). The effects can be mimicked in mice, where exposure to low

level BPA during preconception and early gestation leads to abnormal placental growth and dysregulation of genomic imprinting of *Igf2* in particular in the placenta (Susiarjo *et al.*, 2013). Alarmingly, BPA affects imprinting already during oocyte growth as well. Treatment of mouse ovarian follicles with low level BPA during *in-vitro* follicle growth leads to hypo-methylation of maternally imprinted genes as well as a decrease in histone methylation marks in the oocytes (Trapphoff *et al.*, 2013). Dysregulated imprinting in germ line could lead to transgenerational inheritance of the defective phenotype, emphasizing the importance of environmental factors in health and disease on the individual level as well as on a population level.

In summary, several lines of evidence show that mode of conception, maternal psychological stress during pregnancy and chemical exposure represent environmental stressors that can affect fetal development and postnatal health. Although these factors have different mechanisms of action, they are often inter-related, affect epigenetic mechanisms, and commonly affect women of reproductive age. There is preliminary data supporting that these stressors can cause also disruptions in the DNA methylation patterns of imprinted genes. However, this hypothesis needs to be further investigated.

Discussion

Genomic imprinting has a central role in human development and is essential to placental functions that regulate normal fetal growth. Genes that are subject to genomic imprinting are expressed in a parent-of-origin-specific manner and may influence fetal growth through effects on placental function and nutrient metabolism. Dysregulation of imprinted gene methylation has been repeatedly proposed as a key molecular mechanism linking developmental exposures with adverse health outcomes later in life. DNA methylation can be regarded as an epigenetic memory of previous exposures and its alterations have consequences in subsequent growth, development, and behaviour. Abnormal birth weight predisposes to altered postnatal growth trajectory and disease susceptibility, demonstrating that *in-utero* fetal development is a period of primary importance in defining the health in adulthood. Several recent studies have elucidated the role of imprinted genes, including *IGF2*, *H19*, *MEST*, and *PEG10,* in determining birth weight and growth, and found support for the importance of genomic imprinting in fetal development. Impaired methylation and expression of imprinted genes are associated with adverse fetal growth, PTB, and birth weight variation. Given the multitude of genetic and environmental factors known to affect DNA methylation during *in-utero* development, dysregulation of imprinted genes provides a plausible mechanism through which genes and environment interact to affect fetal growth.

The literature indicates that imprinted genes are responsive to environmental factors during specific windows of development, suggesting the potential of these marks to serve as environmental sensors. Maternal prenatal psychological stress is one of the factors appearing to influence imprinted gene methylation, along with smoking, antidepressant use, and exposure to chemicals. Exposure to environmental chemicals has been connected to fetal growth restriction in several cohort studies, and evidence from animals demonstrate that environmentally relevant exposure levels can affect establishment of imprints in the placenta and germ line. As chemicals are an unavoidable part of life in modern society, the impact of exposures on human genomic imprinting and subsequent health should be carefully investigated. The role of the prenatal environment on cognitive and emotional development of the fetus has become clearer in recent years, and effects of maternal mental health on the structure and function of the infant HPA axis in particular have been implicated. Exposure to stressors during gestation has a major impact on fetal growth and can lead to long-term diseases in offspring, causing physical and mental health problems.

The role of ART in epigenetic stability is under investigation. Methylation differences observed in ART-conceived children have usually been within the normal variation range; however, IDs are still reported at higher rates. This could suggest that additional imprinting mechanisms should be considered. The results indicate that ART can have effects on the epigenome of the offspring. Furthermore the variations in ART protocols used in different clinics complicate the interpretation of these studies and may lead to inaccurate conclusions. Large international databases should be compiled in order to enable better studies. It is necessary to understand health risks and underlying molecular mechanisms of ART interventions, for the purpose of increasing the safety of these techniques and enable couples contemplating ART to be fully informed about the possible health consequences.

To conclude, many factors can affect the DNA methylation profile in general as well as the methylation patterns on imprinted genes, which could constitute an adaptation mechanism to environmental conditions including stressors that can lead to adverse birth outcome and increased risk of disease (Fig. 1). Data suggesting a causal role for imprinting in common diseases such as cancer, diabetes, and neurodevelopmental perturbations are increasing, highlighting the broad impact of imprinted genes in human health. Deeper understanding of the role of imprinted genes and their regulation in fetal development will allow development of strategies to prevent or ameliorate these effects and provide early effective interventions for preventing poor health outcomes.

Acknowledgements

None declared.

Authors' roles

All authors contributed to the conception and design of the review article. M.A. and P.D. undertook the literature search and drafted the article. P.D., K.C., G.G., B.T., M.S., and A.L. critically revised the manuscript and suggested helpful corrections. All authors approved the final version of the review to be submitted.

Funding

No funding was used for this project.

Conflict of interest

None declared.

References

Abbott LC, Winzer-Serhan UH. Smoking during pregnancy: lessons learned from epidemiological studies and experimental studies using animal models. *Crit Rev Toxicol* 2012;**42**:279–303.

Adkins RM, Somes G, Morrison LC, Hill JB, Watson EM, Magann EF, Krushkai J. Association of birth weight with polymorphisms in the *IGF-2*, *H19* and *IGF-2R* genes. *Pediatr Res* 2010;**68**:429–434.

Anifandis G, Messini CI, Dafopoulos K, Messinis IE. Genes and conditions controlling mammalian pre- and post-implantation embryo development. *Curr Genomics* 2015;**16**:32–46.

Antonazzo P, Alvino G, Cozzi V, Grati FR, Tabano S, Sirchia S, Miozzo M, Cetin I. Placental *IGF2* expression in normal and intrauterine growth restricted (IUGR) pregnancies. *Placenta* 2008;**29**: 99–101.

Antonelli MC, Pallarés ME, Ceccatelli S, Spulber S. Long-term consequences of prenatal stress and neurotoxicants exposure on neurodevelopment. *Prog Neurobiol* 2017;**155**:21–35.

Apostolidou S, Abu-Amero S, O'Donoghue K, Frost J, Olafsdottir O, Chavele KM, Whittaker JC, Loughna P, Stanier P, Moore GE. Elevated placental expression of the imprinted *PHLDA2* gene is associated with low birth weight. *J Mol Med* 2007;**85**:379–387.

Appleton AA, Lester BM, Armstrong DA, Lesseur C, Marsit CJ. Examining the joint contribution of placental *NR3C1* and *HSD11B2* methylation for infant neurobehavior. *Psychoneuroendocrinology* 2015;**52**:32–42.

Astuti D, Latif F, Wagner K, Gentle D, Cooper WN, Catchpoole D, Grundy R, Ferguson-Smith AC, Maher ER. Epigenetic alteration at the DLK1-GTL2 imprinted domain in human neoplasia: analysis of neuroblastoma, phaeochromocytoma and Wilms' tumour. *Br J Cancer* 2005;**92**:1574–1580.

Azzi S, Abi Habib W, Netchine I. Beckwith–Wiedemann and Russell–Silver syndromes: from new molecular insights to the comprehension of imprinting regulation. *Curr Opin Endocrinol Diabetes Obes* 2014;**21**:30–38.

Bergman K, Glover V, Sarkar P, Abbott DH, O'Connor TG. *In utero* cortisol and testosterone exposure and fear reactivity in infancy. *Horm Behav* 2010;**57**:306–312.

Berntsen S, Söderström-Anttila V, Wennerholm UB, Laivuori H, Loft A, Oldereid NB, Romundstad LB, Bergh C, Pinborg A. The health of children conceived by ART: 'the chicken or the egg?'. *Hum Reprod Update* 2019;**25**:137–158.

Biliya S, Bulla LA. Genomic imprinting: the influence of differential methylation in the two sexes. *Exp Biol Med (Maywood)* 2010;**235**:139–147.

Blakeley PM, Capron LE, Jensen AB, O'Donnell KJ, Glover V. Maternal prenatal symptoms of depression and down regulation of placental monoamine oxidase A expression. *J Psychosom Res* 2013;**75**:341–345.

Bonnin A, Goeden N, Chen K, Wilson ML, King J, Shih JC, Blakely RD, Deneris ES, Levitt P. A transient placental source of serotonin for the fetal forebrain. *Nature* 2011;**472**:347–350.

Bouwland-Both M, van Mil NH, Stolk L, Eilers PH, Verbiest MM, Heijmans BT, Tiemeier H, Hofman A, Steegers EA, Jaddoe VW *et al.* DNA methylation of *IGF2* DMR and *H19* is associated with fetal and infant growth: the generation R study. *PLoS One* 2013;**8**: e81731.

Cai D, Zhu Z, Sun H, Qi Y, Xing L, Zhao X, Wan Q, Su Q, Li H. Maternal PTSD following exposure to the Wenchuan earthquake is associated with impaired mental development of children. *PLoS One* 2017;**12**:e0168747.

Camprubí C, Iglesias-Platas I, Martin-Trujillo A, Salvador-Alarcon C, Rodriguez MA, Barredo DR, Court F, Monk D. Stability of genomic imprinting and gestational-age dynamic methylation in complicated pregnancies conceived following assisted reproductive technologies. *Biol Reprod* 2013;**50**:1–9.

Canovas S, Ross PJ, Kelsey G, Coy P. DNA methylation in embryo development: epigenetic impact of ART (assisted reproductive technologies). *Bioessays* 2017;**39**:e1700106.

Cao-Lei L, de Rooij SR, King S, Matthews SG, Metz GAS, Roseboom TJ, Szyf M. Prenatal stress and epigenetics. *Neurosci Biobehav Rev* 2017; pii: S0149-7634(16)30726-6. doi: https://doi.org/10.1016/j.neubiorev.2017.05.016.

Cao-Lei L, Massart R, Suderman MJ, Machnes Z, Elgbeili G, Laplante DP, Szyf M, King S. DNA methylation signatures triggered by prenatal maternal stress exposure to a natural disaster: Project Ice Storm. *PLoS One* 2014;**9**:e107653.

Capron L, Glover V, Pearson R, Evans J, O'Connor TG, Stein A, Murphy SE, Ramchandani P. Associations of maternal and paternal depression and anxiety with offspring anxiety disorder at age 18 years. *J Affect Disord* 2015;**187**:20–26.

Carless MA, Kulkarni H, Kos MZ, Charlesworth J, Peralta JM, Göring HH, Curran JE, Almasy L, Dyer TD, Comuzzie AG *et al.* Genetic effects on DNA methylation and its potential relevance for obesity in Mexican Americans. *PLoS One* 2013;**8**:e73950.

Caruso M, Evangelista M, Parolini O. Human term placental cells: phenotype, properties and new avenues in regenerative medicine. *Int J Mol Cell Med* 2012;**1**:64–74.

Casas M, Nieuwenhuijsen M, Martínez D, Ballester F, Basagaña X, Basterrechea M, Chatzi L, Chevrier C, Eggesbø M, Fernandez MF *et al.* Prenatal exposure to PCB-153, p,p′-DDE and birth outcomes in 9000 mother-child pairs: exposure–response relationship and effect modifiers. *Environ Int* 2015;**74**:23–31.

Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Wall HA. Cardiometabolic differences in children born after *in vitro* fertilization: follow up study. *J Clin Endocrinol Metab* 2008;**93**:1682–1688.

Charalambous M, da Rocha ST, Ferguson-Smith AC. Genomic imprinting, growth control and the allocation of nutritional resources: consequences for postnatal life. *Curr Opin Endocrinol Diabetes Obes* 2007;**14**:3–12.

Chavan-Gautam P, Sundrani D, Pisal H, Nimbargi V, Mehendale S, Joshi S. Gestation-dependent changes in human placental global DNA methylation levels. *Mol Reprod Dev* 2011;**78**:150.

Chen H, Sun M, Liu J, Tong C, Meng T. Silencing of paternally expressed gene 10 inhibits trophoblast proliferation and invasion. *PLoS One* 2015;**10**:e0144845.

Chen H, Sun M, Zhao G, Liu J, Gao W, Si S, Meng T. Elevated expression of *PEG10* in human placentas from preeclamptic pregnancies. *Acta Histochem* 2012;**114**:589–593.

Chen J, Li Q, Rialdi A, Mystal E, Ly J, Finik J, Davey T, Lambertini L, Nomura Y. Influences of maternal stress during pregnancy on the epi/genome: comparison of placenta and umbilical cord blood. *J Depress Anxiety* 2014;**3**:152.

Chiba H, Hiura H, Okae H, Miyauchi N, Sato F, Sato A, Arima T. DNA methylation errors in imprinting disorders and assisted reproductive technology. *Pediatr Int* 2013;**55**:542–549.

Choufani S, Shuman C, Weksberg R. Molecular findings in Beckwith–Wiedemann syndrome. *Am J Med Genet C Semin Med Genet* 2013;**163C**:131–140.

Class QA, Lichtenstein P, Langstrom N, D'Onofrio BM. Timing of prenatal maternal exposure to severe life events and adverse pregnancy outcomes: a population study of 2.6 million pregnancies. *Psychosom Med* 2011;**73**:234–241.

Cordeiro A, Neto AP, Carvalho F, Ramalho C, Dória S. Relevance of genomic imprinting in intrauterine human growth expression of *CDKN1C*, *H19*, *IGF2*, *KCNQ1* and *PHLDA2* imprinted genes. *J Assist Reprod Genet* 2014;**31**:1361–1368.

Cortessis VK, Azadian M, Buxbaum J, Sanogo F, Song AY, Sriprasert I, Wei PC, Yu J, Chung K, Siegmund KD. Comprehensive meta-analysis reveals association between multiple imprinting disorders and conception by assisted reproductive technology. *J Assist Reprod Genet* 2018;**35**:943–952.

Cottrell EC, Seckl JR, Holmes MC, Wyrwoll CS. Foetal and placental 11beta-HSD2: a hub for developmental programming. *Acta Physiol (Oxf)* 2014;**210**:288–295.

Cox GF, Bürger J, Lip V, Mau UA, Sperling K, Wu BL, Horsthemke B. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet* 2002;**71**:162–164.

Crinnion WJ. The CDC fourth national report on human exposure to environmental chemicals: what it tells us about our toxic burden and how it assist environmental medicine physicians. *Altern Med Rev* 2010;**15**:101–109.

Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of *H19* and *IGF2*. *Cancer Res* 2002;**62**:442–446.

Dancause KN, Laplante DP, Fraser S, Brunet A, Ciampi A, Schmitz N, King S. Prenatal exposure to a natural disaster increases risk for obesity in 51/2-year-old children. *Pediatr Res* 2012;**71**:126–131.

Dancause KN, Veru F, Andersen RE, Laplante DP, King S. Prenatal stress due to a natural disaster predicts insulin secretion in adolescence. *Early Hum Dev* 2013;**89**:773–776.

Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, Chan A. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012;**366**:1803–1813.

Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev* 2010;**81**:131–148.

DeBaun MR, Niemitz EL, Feinberg AP. Association of *in vitro* fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of *LIT1* and *H19*. *Am J Hum Genet* 2003;**72**:156–160.

Denomme MM, Mann MR. Genomic imprints as a model for the analysis of epigenetic stability during assisted reproductive technologies. *Reproduction* 2012;**144**:393–409.

Derakhshan-Horeh M, Abolhassani F, Jafarpour F, Moini A, Karbalaie K, Hosseini SM, Nasr-Esfahani MH. Vitrification at Day3 stage appears not to affect the methylation status of H19/IGF2 differentially methylated region of in vitro produced human blastocysts. *Cryobiology* 2016;**73**:168–174.

Desplats P. Perinatal programming of neurodevelopment: epigenetic mechanisms and the prenatal shaping of the brain. *Adv Neurobiol* 2015;**10**:335–361.

Ding XX, Wu YL, Xu SJ, Zhu RP, Jia XM, Zhang SF, Huang K, Zhu P, Hao JH, Tao FB. Maternal anxiety during pregnancy and adverse birth outcomes: a systematic review and meta-analysis of prospective cohort studies. *J Affect Disord* 2014;**159**:103–110.

Diplas AI, Lambertini L, Lee MJ, Sperling R, Lee YL, Wetmur J, Chen J. Differential expression of imprinted genes in normal and IUGR human placentas. *Epigenetics* 2009;**4**:235–240.

Doornbos M, Maas SM, McDonell J, Veermedien JPW, Hennekam RCM. Infertility, assisted reproduction technologies and imprinting disturbances: a Dutch study. *Hum Reprod* 2007;**22**:2476–2480.

Doria S, Sousa M, Fernandes S, Ramalho C, Brandao O, Matias A, Barros A, Carvalho F. Gene expression pattern of *IGF2*, *PHLDA2*, *PEG10* and *CDKN1C* imprinted genes in spontaneous miscarriages or fetal deaths. *Epigenetics* 2010;**5**:444–450.

Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD, Geraedts JP *et al.* Effect of *in vitro* culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;**25**:605–612.

Edwards M. Fetal death and reduced birth rates associated with exposure to lead-contaminated drinking water. *Environ Sci Technol* 2014;**48**:739–746.

Eggermann T, Binder G, Brioude F, Maher ER, Lapunzina P, Cubellis MV, Bergadá I, Prawitt D, Begemann M. *CDKN1C* mutations: two sides of the same coin. *Trends Mol Med* 2014;**20**:614e622.

Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tümer Z, Monk D, Mackay DJ, Grønskov K, Riccio A, Linglart A *et al.* Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin Epigenetics* 2015;**7**:123.

El Hajj N, Haaf T. Epigenetic disturbances in *in vitro* cultured gametes and embryos: implications for human assisted reproduction. *Fertil Steril* 2013;**99**:632–641.

El Hajj N, Haertle L, Dittrich M, Denk S, Lehnen H, Hahn T, Schorsch M, Haaf T. DNA methylation signatures in cord blood of ICSI children. *Hum Reprod* 2017;**32**:1761–1769.

Feng C, Tian S, Zhang Y, He J, Zhu XM, Zhang D, Sheng JZ, Huang HF. General imprinting status is stable in assisted reproduction-conceived offspring. *Fertil Steril* 2011;**96**:1417–1423.

Feng W, Marquez RT, Lu Z, Liu J, Lu KH, Issa JP, Fishman DM, Yu Y, Bast RC Jr. Imprinted tumor suppressor genes *ARHI* and *PEG3* are the most frequently down-regulated in human ovarian cancers by loss of heterozygosity and promoter methylation. *Cancer* 2008;**112**:1489–1502.

Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus non blastocyst transfer in assisted reproductive technology. *Fertil Steril* 2012;**97**:579–584.

Frost J, Moore G. The importance of imprinting in the human placenta. *PLoS Genet* 2010;**6**:e1001015.

Gao WL, Li D, Xiao ZX, Liao QP, Yang HX, Li YX, Ji L, Wang YL. Detection of global DNA methylation and paternally imprinted *H19* gene methylation in preeclamptic placentas. *Hypertens Res* 2011;**34**:655–661.

Gentile S. Untreated depression during pregnancy: short- and long-term effects in offspring. A systematic review. *Neuroscience* 2017;**342**:154–166.

Gicquel C, Gaston V, Mandelbaum J, Siffroi JP, Flahault A, Le Bouc Y. *In vitro* fertilization may increase the risk of Beckwith–Wiedemann syndrome related to the abnormal imprinting of the *KCN1OT* gene. *Am J Hum Genet* 2003;**72**:1338–1341.

Gillman MW. Developmental origins of health and disease. *N Engl J Med* 2005;**353**:1848–1850.

Gkountela S, Li Z, Vincent JJ, Zhang KX, Chen A, Pellegrini M, Clark AT. The ontogeny of cKIT$^+$ human primordial germ cells proves to be a resource for human germ line reprogramming, imprint erasure and *in vitro* differentiation. *Nat Cell Biol* 2013;**15**:113–122.

Gkountela S, Zhang KX, Shafiq TA, Liao W-W, Hargan-Calvopiña J, Chen P-Y, Clark AT. DNA demethylation dynamics in the human prenatal germline. *Cell* 2015;**161**:1425–1436.

Glover V. Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. *Best Pract Res Clin Obstet Gynaecol* 2014;**28**:25–35.

Gomes MV, Huber J, Ferriani RA, Amaral Neto AM, Ramos ES. Abnormal methylation at the *KvDMR1* imprinting control region in clinically normal children conceived by assisted reproductive technologies. *Mol Hum Reprod* 2009;**15**:471–477.

Graignic-Philippe R, Dayan J, Chokron S, Jacquet AY, Tordjman S. Effects of prenatal stress on fetal and child development: a critical literature review. *Neurosci Biobehav Rev* 2014;**43**:137–162.

Green BB, Kappil M, Lambertini L, Armstrong DA, Guerin DJ, Sharp AJ, Lester BM, Chen J, Marsit CJ. Expression of imprinted genes in placenta is associated with infant neurobehavioral development. *Epigenetics* 2015;**10**:834–841.

Griffiths BB, Hunter RG. Neuroepigenetics of stress. *Neuroscience* 2014;**275**:420–435.

Grigoriadis S, VonderPorten EH, Mamisashvili L, Tomlinson G, Dennis CL, Koren G, Steiner M, Mousmanis P, Cheung A, Radford K *et al.* The impact of maternal depression during pregnancy on perinatal outcomes: a systematic review and meta-analysis. *J Clin Psychiatry* 2013;**74**:e321–e341.

Grote NK, Bridge JA, Gavin AR, Melville JL, Iyengar S, Katon WJ. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight and intrauterine growth restriction. *Arch Gen Psychiatry* 2010;**67**:1012–1024.

Guo F, Yan L, Guo H, Li L, Hu B, Zhao Y, Yong J, Hu Y, Wang X, Wei Y *et al.* The transcriptome and DNA methylome landscapes of human primordial germ cells. *Cell* 2015;**161**:1437–1452.

Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, Yan J, Ren X, Lin S, Li J *et al.* The DNA methylation landscape of human early embryos. *Nature* 2014;**511**:606–610.

Guo L, Choufani S, Ferreira J, Smith A, Chitayat D, Shuman C, Uxa R, Keating S, Kingdom J, Weksberg R. Altered gene expression and methylation of the human chromosome 11 imprinted region in small for gestational age (SGA) placentae. *Dev Biol* 2008;**320**:79–91.

Guxens M, Sonnenschein-van der Voort AM, Tiemeier H, Hofman A, Sunyer J, de Jongste JC, Jaddoe VW, Duijts L. Parental psychological distress during pregnancy and wheezing in preschool children: the generation R study. *J Allergy Clin Immunol* 2014;**133**:59–67.e1–12.

Haaf T. Methylation dynamics in the early mammalian embryo: implications of genome reprogramming defects for development. *Curr Top Microbiol Immunol* 2006;**310**:13–22.

Hammoud SS, Purwar J, Pflueger C, Cairns BR, Carrell DT. Alterations in sperm DNA methylation patterns at imprinted loci in two classes of infertility. *Fertil Steril* 2010;**94**:1728–1733.

Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and *in vitro* fertilization. *N Engl J Med* 2002;**346**:725–730.

Harris A, Seckl J. Glucocorticoids, prenatal stress and the programming of disease. *Horm Behav* 2011;**59**:279–289.

Harris LK, Crocker IP, Baker PN, Aplin JD, Westwood M. *IGF2* actions on trophoblast in human placenta are regulated by the insulin-like growth factor 2 receptor, which can function as both a signaling and clearance receptor. *Biol Reprod* 2011;**84**:440–446.

Harville E, Xiong X, Buekens P. Disasters and perinatal health: a systematic review. *Obstet Gynecol Surv* 2010;**65**:713–728.

Harville EW, Giarratano G, Savage J, Barcelona de Mendoza V, Zotkiewicz T. Birth outcomes in a disaster recovery environment: New Orleans women after Katrina. *Matern Child Health J* 2015;**19**:2512–2522.

Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. *Int J Hyg Environ Health* 2007;**210**:623–634.

Hiura H, Okae H, Chiba H, Miyauchi N, Sato F, Sato A, Arima T. Imprinting methylation errors in ART. *Reprod Med Biol* 2014;**13**:193–202.

Hiura H, Okae H, Miyauchi N, Sato F, Sato A, Van De Pette M, John RM, Kagami M, Nakai K, Soejima H *et al.* Characterization of DNA methylation errors in patients with imprinting disorders conceived by assisted reproduction technologies. *Hum Reprod* 2012;**27**:2541–2548.

Högberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, Filipsson AF, Jansson B, Johansson N, Appelgren M *et al.* Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. *Environ Health Perspect* 2008;**116**:334–339.

Horsthemke B, Ludwig M. Assisted reproduction: the epigenetic perspective. *Hum Reprod Update* 2005;**11**:473–482.

Houshdaran S, Cortessis VK, Siegmund K, Yang A, Laird PW, Sokol RZ. Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. *PLoS One* 2007;**2**:e1289.

Hoyo C, Daltveit AK, Iversen E, Benjamin-Neelon SE, Fuemmeler B, Schildkraut J, Murtha AP, Overcash F, Vidal AC, Wang F *et al.* Erythrocyte folate concentrations, CpG methylation at genomically imprinted domains and birth weight in a multiethnic newborn cohort. *Epigenetics* 2014;**9**:1120–1130.

Hoyo C, Fortner K, Murtha AP, Schildkraut JM, Soubry A, Demark-Wahnefried W, Jirtle RL, Kurtzberg J, Forman MR, Overcash F *et al.* Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (*IGF2*), plasma IGF2 and birth weight. *Cancer Causes Control* 2012;**23**:635–645.

Huang JM, Kim J. DNA methylation analysis of the mammalian *PEG3* imprinted domain. *Gene* 2009;**442**:18–25.

Huang RC, Galati JC, Burrows S, Beilin LJ, Li X, Pennell CE, van Eekelen J, Mori TA, Adams LA, Craig JM. DNA methylation of the *IGF2/H19*

imprinting control region and adiposity distribution in young adults. *Clin Epigenetics* 2012;**4**:21.

Huizink AC, Bartels M, Rose RJ, Pulkkinen L, Eriksson CJ, Kaprio J. Chernobyl exposure as stressor during pregnancy and hormone levels in adolescent offspring. *J Epidemiol Community Health* 2008;**62**:e5.

Huntriss J, Picton HM. Epigenetic consequences of assisted reproduction and infertility on the human preimplantation embryo. *Hum Fertil (Camb)* 2008;**11**:85–94.

Huntriss JD, Hemmings KE, Hinkins M, Rutherford AJ, Sturmey RG, Elder K, Picton HM. Variable imprinting of the *MEST* gene in human preimplantation embryos. *Eur J Hum Genet* 2013;**21**:40–47.

Hyrapetian M, Loucaides EM, Sutcliffe AG. Health and disease in children born after assistive reproductive therapies (ART). *J Reprod Immunol* 2014;**106**:21–26.

Ibala-Romdhane S, Al-Khtib M, Khoueiry R, Blachère T, Guérin JF, Lefèvre A. Analysis of *H19* methylation in control and abnormal human embryos, sperm and oocytes. *Eur J Hum Genet* 2011;**19**:1138–1143.

Isgut M, Smith AK, Reimann ES, Kucuk O, Ryan J. The impact of psychological distress during pregnancy on the developing fetus: biological mechanisms and the potential benefits of mindfulness interventions. *J Perinat Med* 2017;**45**:999–1011.

Ishida M, Moore GE. The role of imprinted genes in humans. *Mol Aspects Med* 2013;**34**:826–840.

Ito Y, Koessler T, Ibrahim AE, Rai S, Vowler SL, Abu-Amero S, Silva AL, Maia AT, Huddleston JE, Uribe-Lewis S *et al.* Somatically acquired hypomethylation of *IGF2* in breast and colorectal cancer. *Hum Mol Genet* 2008;**17**:2633–2643.

Janssen AB, Capron LE, O'Donnell K, Tunster SJ, Ramchandani PG, Heazell AEP, Glover V, John RM. Maternal prenatal depression is associated with decreased placental expression of the imprinted gene *PEG3*. *Psychol Med* 2016;**46**:2999–3011.

Janssen AB, Tunster SJ, Savory N, Holmes A, Beasley J, Parveen SAR, Penketh RJA, John RM. Placental expression of imprinted genes varies with sampling site and mode of delivery. *Placenta* 2015;**36**:790–795.

Jensen Pena C, Monk C, Champagne FA. Epigenetic effects of prenatal stress on 11β-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. *PLoS One* 2012;**7**:e39791.

Jiang X, Yan J, West AA, Perry CA, Malysheva OV, Devapatla S, Pressman E, Vermeylen F, Caudill MA. Maternal choline intake alters the epigenetic state of fetal cortisol-regulating genes in humans. *FASEB J* 2012;**26**:3563–3574.

Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet* 2007;**8**:253–262.

John RM. Epigenetic regulation of placental endocrine lineages and complications of pregnancy. *Biochem Soc Trans* 2013;**41**:701–709.

Kagami M, Nagai T, Fukami M, Yamazawa K, Ogata T. Silver–Russell syndrome in a girl born after *in vitro* fertilization: partial hypermethylation at the differentially methylated region of *PEG1/MEST*. *J Assist Reprod Genet* 2007;**24**:131–136.

Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Blastocyst versus cleavage stage transfer in *in vitro* fertilization: differences in neonatal outcome? *Fertil Steril* 2010;**94**:1680–1683.

Kappil M, Lambertini L, Chen J. Environmental influences on genomic imprinting. *Curr Environ Health Rep* 2015a;**2**:155–162.

Kappil MA, Green BB, Armstrong DA, Sharp AJ, Lambertini L, Marsit CJ, Chen J. Placental expression profile of imprinted genes impacts birth weight. *Epigenetics* 2015b;**10**:842–849.

Karbiener M, Glantschnig C, Pisani DF, Laurencikiene J, Dahlman I, Herzig S, Amri EZ, Scheideler M. Mesoderm-specific transcript (MEST) is a negative regulator of human adipocyte differentiation. *Int J Obes (Lond)* 2015;**39**:1733–1741.

Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, Gaughan JP, Coutifaris C, Sapienza C. DNA methylation and gene expression differences in children conceived *in vitro* or *in vivo*. *Hum Mol Genet* 2009;**18**:3769e78.

Kerjean A, Dupont JM, Vasseur C, Le Tessier D, Cuisset L, Páldi A, Jouannet P, Jeanpierre M. Establishment of the paternal methylation imprint of the human *H19* and *MEST/PEG1* genes during spermatogenesis. *Hum Mol Genet* 2000;**9**:2183–2187.

Khashan AS, Abel KM, McNamee R, Pedersen MG, Webb RT, Baker PN, Kenny LC, Mortensen PB. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. *Arch Gen Psychiatry* 2008;**65**:146–152.

Khashan AS, McNamee R, Abel KM, Mortensen PB, Kenny LC, Pedersen MG, Webb RT, Baker PN. Rates of preterm birth following antenatal maternal exposure to severe life events: a population-based cohort study. *Hum Reprod* 2009;**24**:429–437.

King K, Murphy S, Hoyo C. Epigenetic regulation of newborns' imprinted genes related to gestational growth: patterning by parental race/ethnicity and maternal socioeconomic status. *J Epidemiol Community Health* 2015;**69**:639–647.

King S, Dancause K, Turcotte-Tremblay AM, Veru F, Laplante DP. Using natural disasters to study the effects of prenatal maternal stress on child health and development. *Birth Defects Res C Embryo Today* 2012;**96**:273–288.

Kingsbury M, Weeks M, MacKinnon N, Evans J, Mahedy L, Dykxhoorn J, Colman I. Stressful life events during pregnancy and offspring depression: evidence from a prospective cohort study. *J Am Acad Child Adolesc Psychiatry* 2016;**55**:709–716.e2.

Kinney DK, Miller AM, Crowley DJ, Huang E, Gerber E. Autism prevalence following prenatal exposure to hurricanes and tropical storms in Louisiana. *J Autism Dev Disord* 2008;**38**:481–488.

Kleijkers SH, van Montfoort AP, Smits LJ, Viechtbauer W, Roseboom TJ, Nelissen EC, Coonen E, Derhaag JG, Bastings L, Schreurs IE *et al.* IVF culture medium affects post-natal weight in humans during the first 2 years of life. *Hum Reprod* 2014;**29**:661–669.

Kleinhaus K, Harlap S, Perrin M, Manor O, Margalit-Calderon R, Opler M, Friedlander Y, Malaspina D. Prenatal stress and affective disorders in a population birth cohort. *Bipolar Disord* 2013;**15**:92–99.

Knerr I, Huppertz B, Weigel C, Dötsch J, Wich C, Schild RL, Beckmann MW. Endogenous retroviral syncytin: compilation of experimental research on syncytin and its possible role in normal and disturbed human placentogenesis. *Mol Hum Reprod* 2004;**10**:581–588.

Kochanski A, Merritt TA, Gadzinowski J, Jopek A. The impact of assisted reproductive technologies on the genome and epigenome of the newborn. *J Neonatal Perinatal Med* 2013;**6**:101–108.

Kosaki K, Kosaki R, Craigen WJ, Matsuo N. Isoform-specific imprinting of the human *PEG1/MEST* gene. *Am J Hum Genet* 2000;**66**:309e12.

Koukoura O, Sifakis S, Soufla G, Zaravinos A, Apostolidou S, Jones A, Widschwendter M, Spandidos DA. Loss of imprinting and aberrant

methylation of *IGF-2* in placentas from pregnancies complicated with fetal growth restriction. *Int J Mol Med* 2011;**28**:481–487.

Kuerbitz SJ, Pahys J, Wilson A, Compitello N, Gray TA. Hypermethylation of the imprinted *NNAT* locus occurs frequently in pediatric acute leukemia. *Carcinogenesis* 2002;**23**:559–564.

Kültz D. Molecular and evolutionary basis of the cellular stress response. *Annu Rev Physiol* 2005;**67**:225–257.

Lambert RD. Safety issues in assisted reproductive technology: aetiology of health problems in singleton ART babies. *Hum Reprod* 2003;**18**:1987–1991.

Lambertini L, Marsit CJ, Sharma P, Maccani M, Ma Y, Hu J, Chen J. Imprinted gene expression in fetal growth and development. *Placenta* 2012;**33**:480–486.

Laplante DP, Brunet A, Schmitz N, Ciampi A, King S. Project Ice Storm: prenatal maternal stress affects cognitive and linguistic functioning in 5 1/2-year-old children. *J Am Acad Child Adolesc Psychiatry* 2008;**47**:1063–1072.

Lazaraviciute G, Kauser M, Bhattacharya S, Haggarty P, Bhattacharya S. A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously. *Hum Reprod Update* 2014;**20**:840–852.

Lee A, Mathilda Chiu YH, Rosa MJ, Jara C, Wright RO, Coull BA, Wright RJ. Prenatal and postnatal stress and asthma in children: temporal- and sex-specific associations. *J Allergy Clin Immunol* 2016;**138**:740–747.e3.

Lee AG, Chiu YM, Rosa MJ, Cohen S, Coull BA, Wright RO, Morgan WJ, Wright RJ. Association of prenatal and early childhood stress with reduced lung function in 7-year-olds. *Ann Allergy Asthma Immunol* 2017;**119**:153–159.

Lee C. Intergenerational health consequences of *in utero* exposure to maternal stress: evidence from the 1980 Kwangju uprising. *Soc Sci Med* 2014;**119**:284–291.

Lenters V, Portengen L, Rignell-Hydbom A, Jönsson BA, Lindh CH, Piersma AH, Toft G, Bonde JP, Heederik D, Rylander L *et al.* Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. *Environ Health Perspect* 2016;**124**:365–372.

Lewis A, Reik W. How imprinting centres work. *Cytogenet Genome Res* 2006;**113**:81–89.

Liang XY, Chen X, Jin YZ, Chen XO, Chen QZ. Expression and significance of the imprinted gene *PEG10* in placenta of patients with preeclampsia. *Genet Mol Res* 2014;**13**:10607–10614.

Liang XY, Liu XQ, Ding YB, Chen XM, Wang YX. Genetic imprinted gene *PEG10* expression in deciduas from inevitable abortion. *Yi Chuan* 2008;**30**:735–740.

Lillycrop KA, Burdge GC. The effect of nutrition during early life on the epigenetic regulation of transcription and implications for human diseases. *J Nutrigenet Nutrigenomics* 2011;**4**:248–260.

Lim AL, Ferguson-Smith AC. Genomic imprinting effects in a compromised *in utero* environment: implications for a healthy pregnancy. *Semin Cell Dev Biol* 2010;**21**:201e208.

Lim AL, Ng S, Leow SC, Choo R, Ito M, Chan YH, Goh SK, Tng E, Kwek K, Chong YS *et al.* Epigenetic state and expression of imprinted genes in umbilical cord correlates with growth parameters in human pregnancy. *J Med Genet* 2012;**49**:689–697.

Lim D, Bowdin SC, Tee L, Kirby GA, Blair E, Fryer A, Lam W, Oley C, Cole T, Brueton LA *et al.* Clinical and molecular genetic features of Beckwith–Wiedemann syndrome associated with assisted reproductive technologies. *Hum Reprod* 2009;**24**:741–747.

Lin Y, Xu J, Huang J, Jia Y, Zhang J, Yan C, Zhang J. Effects of prenatal and postnatal maternal emotional stress on toddlers' cognitive and temperamental development. *J Affect Disord* 2017;**207**:9–17.

Liu GT, Dancause KN, Elgbeili G, Laplante DP, King S. Disaster-related prenatal maternal stress explains increasing amounts of variance in body composition through childhood and adolescence: Project Ice Storm. *Environ Res* 2016;**150**:1–7.

Liu Y, Murphy SK, Murtha AP, Fuemmeler BF, Schildkraut J, Huang Z, Overcash F, Kurtzberg J, Jirtle R, Iversen ES *et al.* Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. *Epigenetics* 2012;**7**:735–746.

Lucassen PJ, Naninck EF, van Goudoever JB, Fitzsimons C, Joels M, Korosi A. Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. *Trends Neurosci* 2013;**36**:621–631.

Lux H, Flammann H, Hafner M, Lux A. Genetic and molecular analyses of PEG10 reveal new aspects of genomic organization, transcription and translation. *PLoS One* 2010;**5**:1–16.

MacKinnon N, Kingsbury M, Mahedy L, Evans J, Colman I. The association between prenatal stress and externalizing symptoms in childhood: evidence from the Avon Longitudinal Study of Parents and Children. *Biol Psychiatry* 2018;**83**:100–108.

Maher ER, Brueton LA, Bowdin SC, Luharia A, Cooper W, Cole TR, Macdonald F, Sampson JR, Barratt CL, Reik W *et al.* Beckwith–Wiedemann syndrome and assisted reproduction technology (ART). *J Med Genet* 2003;**40**:62–64.

Mairesse J, Lesage J, Breton C, Breant B, Hahn T, Darnaudery M, Dickson SL, Seckl J, Blondeau B, Vieau D *et al.* Maternal stress alters endocrine function of the feto-placental unit in rats. *Am J Physiol Endocrinol Metab* 2007;**292**:E1526–E1533.

Mamsen LS, Björvang RD, Mucs D, Vinnars MT, Papadogiannakis N, Lindh CH, Andersen CY, Damdimopoulou P. Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. *Environ Int* 2019;**124**:482–492.

Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. *Fertil Steril* 2009;**91**:305–315.

Mansell T, Novakovic B, Meyer B, Rzehak P, Vuillermin P, Ponsonby AL, Collier F, Burgner D, Saffery R, Ryan J *et al.* The effects of maternal anxiety during pregnancy on *IGF2/H19* methylation in cord blood. *Transl Psychiatry* 2016;**6**:e765.

Mantovani G. Clinical review: pseudohypoparathyroidism: diagnosis and treatment. *J Clin Endocrinol Metab* 2011;**96**:3020–3030.

Marques CJ, Costa P, Vaz B, Carvalho F, Fernandes S, Barros A, Sousa M. Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia. *Mol Hum Reprod* 2008;**14**:67–73.

Marques CJ, João Pinho M, Carvalho F, Bièche I, Barros A, Sousa M. DNA methylation imprinting marks and DNA methyltransferase expression in human spermatogenic cell stages. *Epigenetics* 2011;**6**:1354–1361.

Marsit CJ, Lambertini L, Maccani MA, Koestler DC, Houseman EA, Padbury JF, Lester BM, Chen J. Placenta-imprinted gene

expression association of infant neurobehavior. *J Pediatr* 2012;**160**: 854–860.

McCullough LE, Mendez MA, Miller EE, Murtha AP, Murphy SK, Hoyo C. Associations between prenatal physical activity, birth weight and DNA methylation at genomically imprinted domains in a multiethnic newborn cohort. *Epigenetics* 2015;**10**:597–606.

McGowan PO, Matthews SG. Prenatal stress, glucocorticoids, and developmental programming of the stress response. *Endocrinology* 2018;**159**:69–82.

McKee SE, Reyes TM. Effect of supplementation with methyl-donor nutrients on neurodevelopment and cognition: considerations for future research. *Nutr Rev* 2018;**76**:497–511.

McMinn J, Wei M, Sadovsky Y, Thaker HM, Tycko B. Imprinting of *PEG1/MEST* isoform 2 in human placenta. *Placenta* 2006b;**27**:119–126.

McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, Weksberg R, Thaker HM, Tycko B. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. *Placenta* 2006a;**27**:540–549.

Melamed N, Choufani S, Wilkins-Haug LE, Koren G, Weksberg R. Comparison of genome-wide and gene-specific DNA methylation between ART and naturally conceived pregnancies. *Epigenetics* 2015;**10**:474–483.

Metsalu T, Viltrop T, Tiirats A, Rajashekar B, Reimann E, Kõks S, Rull K, Milani L, Acharya G, Basnet P *et al*. Using RNA sequencing for identifying gene imprinting and random monoallelic expression in human placenta. *Epigenetics* 2014;**9**:1397–1409.

Monk D. Genomic imprinting in the human placenta. *Am J Obstet Gynecol* 2015;**213**:S152–S162.

Moon YS, Park SK, Kim HT, Lee TS, Kim JH, Choi YS. Imprinting and expression status of isoforms 1 and 2 of *PEG1/MEST* gene in uterine leiomyoma. *Gynecol Obstet Invest* 2010;**70**:120–125.

Moore GE, Ishida M, Demetriou C, Al-Olabi L, Leon LJ, Thomas AC, Abu-Amero S, Frost JM, Stafford JL, Chaoqun Y *et al*. The role and interaction of imprinted genes in human fetal growth. *Philos Trans R Soc Lond B Biol Sci* 2015;**370**: 20140074.

Morello-Frosch R, Cushing LJ, Jesdale BM, Schwartz JM, Guo W, Guo T, Wang M, Harwani S, Petropoulou SE, Duong W *et al*. Environmental chemicals in an urban population of pregnant women and their newborns from San Francisco. *Environ Sci Technol* 2016;**50**:12464–12472.

Moss TJ, Wallrath LL. Connections between epigenetic gene silencing and human disease. *Mutat Res* 2007;**618**:163–174.

Movassagh M, Choy MK, Goddard M, Bennett MR, Down TA, Foo RS. Differential DNA methylation correlates with differential expression of angiogenic factors in human heart failure. *PLoS One* 2010;**5**: e8564.

Murphy SK, Adigun A, Huang Z, Overcash F, Wang F, Jirtle RL, Schildkraut JM, Murtha AP, Iversen ES, Hoyo C. Gender-specific methylation differences in relation to prenatal exposure to cigarette smoke. *Gene* 2012;**494**:36–43.

Murphy SK, Huang Z, Wen Y, Spillman MA, Whitaker RS, Simel LR, Nichols TD, Marks JR, Berchuck A. Frequent *IGF2/H19* domain epigenetic alterations and elevated *IGF2* expression in epithelial ovarian cancer. *Mol Cancer Res* 2006;**4**:283–292.

Mussa A, Molinatto C, Cerrato F, Palumbo O, Carella M, Baldassarre G, Carli D, Peris C, Riccio A, Ferrero GB. Assisted reproductive techniques and risk of Beckwith–Wiedemann syndrome. *Pediatrics* 2017;**140**:e20164311.

Nahar MS, Liao C, Kannan K, Harris C, Dolinoy DC. *In utero* bisphenol A concentration, metabolism, and global DNA methylation across matched placenta, kidney, and liver in the human fetus. *Chemosphere* 2015;**124**:54–60.

Nakabayashi K, Bentley L, Hitchins MP, Mitsuya K, Meguro M, Minagawa S, Bamforth JS, Stanier P, Preece M, Weksberg R *et al*. Identification and characterization of an imprinted antisense RNA (MESTIT1) in the human *MEST* locus on chromosome 7q32. *Hum Mol Genet* 2002;**11**:1743–1756.

Nelissen EC, Dumoulin JC, Busato F, Ponger L, Eijssen LM, Evers JL, Tost J, van Montfoort AP. Altered gene expression in human placentas after IVF/ICSI. *Hum Reprod* 2014;**29**:2821–2831.

Nelissen EC, Dumoulin JC, Daunay A, Evers JL, Tost J, van Montfoort AP. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA methylation level at the *H19* and *MEST* differentially methylated regions. *Hum Reprod* 2013;**28**:1117–1126.

Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, Land JA, Evers JL, Dumoulin JC. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. *Hum Reprod* 2012;**27**:1966–1976.

Nelissen EC, van Montfoort AP, Dumoulin JC, Evers JL. Epigenetics and the placenta. *Hum Reprod Update* 2011;**17**:397–417.

Nemoda Z, Massart R, Suderman M, Hallett M, Li T, Coote M, Cody N, Sun ZS, Soares CN, Turecki G *et al*. Maternal depression is associated with DNA methylation changes in cord blood T lymphocytes and adult hippocampi. *Transl Psychiatry* 2015;**5**:e545.

Non AL, Binder AM, Barault L, Rancourt RC, Kubzansky LD, Michels KB. DNA methylation of stress-related genes and LINE-1 repetitive elements across the healthy human placenta. *Placenta* 2012;**33**:183–187.

Non AL, Binder AM, Kubzansky LD, Michels KB. Genome-wide DNA methylation in neonates exposed to maternal depression, anxiety, or SSRI medication during pregnancy. *Epigenetics* 2014;**9**:964–972.

O'Donnell KJ, Bugge Jensen A, Freeman L, Khalife N, O'Connor TG, Glover V. Maternal prenatal anxiety and downregulation of placental 11β-HSD2. *Psychoneuroendocrinology* 2012;**37**:818–826.

O'Donnell KJ, Glover V, Holbrook JD, O'Connor TG. Maternal prenatal anxiety and child brain-derived neurotrophic factor (*BDNF*) genotype: effects on internalizing symptoms from 4 to 15 years of age. *Dev Psychopathol* 2014;**26**:1255–1266.

Obata Y, Hiura H, Fukuda A, Komiyama J, Hatada I, Kono T. Epigenetically immature oocytes lead to loss of imprinting during embryogenesis. *J Reprod Dev* 2011;**57**:327–334.

Odom LN, Segars J. Imprinting disorders and assisted reproductive technology. *Curr Opin Endocrinol Diabetes Obes* 2010;**17**:517–522.

Okae H, Chiba H, Hiura H, Hamada H, Sato A, Utsunomiya T, Kikuchi H, Yoshida H, Tanaka A, Suyama M. Genome-wide analysis of DNA methylation dynamics during early human development. *PLoS Genet* 2014;**10**:e1004868.

Oliver VF, Miles HL, Cutfield WS, Hofman PL, Ludgate JL, Morison IM. Defects in imprinting and genome-wide DNA methylation are not common in the *in vitro* fertilization population. *Fertil Steril* 2012;**97**:147–153.e7.

Ollikainen M, Smith KR, Joo EJ, Ng HK, Andronikos R, Novakovic B, Abdul Aziz NK, Carlin JB, Morley R, Saffery R *et al*. DNA methylation

analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum Mol Genet* 2010;**19**:4176–4188.

O'Neill RJ, Vrana PB, Rosenfeld CS. Maternal methyl supplemented diets and effects on offspring health. *Front Genet* 2014;**5**:289.

Ono R, Kobayashi S, Wagatsuma H, Aisaka K, Kohda T, Kaneko-Ishino T, Ishino F. A retrotransposonderived gene, *PEG10*, is a novel imprinted gene located on human chromosome 7q21. *Genomics* 2001;**73**:232–237.

Pandey S, Maheshwari A, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Fertil Steril* 2012;**97**: 1331–1337.

Patten MM, Cowley M, Oakey RJ, Feil R. Regulatory links between imprinted genes: evolutionary predictions and consequences. *Proc Biol Sci* 2016;**283**. doi:https://doi.org/10.1098/rspb.2015.2760

Pauwels S, Ghosh M, Duca RC, Bekaert B, Freson K, Huybrechts I, A S Langie S, Koppen G, Devlieger R, Godderis L. Dietary and supplemental maternal methyl-group donor intake and cord blood DNA methylation. *Epigenetics* 2017;**12**:1–10.

Pearson R, Fernyhough C, Bentall RP, Evans J, Heron J, Joinson C, Stein A, Lewis G. Association between maternal depressogenic cognitive style during pregnancy and offspring cognitive style 18 years later. *Am J Psychiatry* 2013;**170**:434–441.

Pedersen IS, Dervan P, McGoldrick A, Harrison M, Ponchel F, Speirs V, Isaacs JD, Gorey T, McCann A. Promoter switch: a novel mechanism causing biallelic *PEG1/MEST* expression in invasive breast cancer. *Hum Mol Genet* 2002;**11**:1449e53.

Pedersen IS, Dervan PA, Broderick D, Harrison M, Miller N, Delany E, O'Shea D, Costello P, McGoldrick A, Keating G. Frequent loss of imprinting of *PEG1/MEST* in invasive breast cancer. *Cancer Res* 1999;**59**:5449–5451.

Pedersen M, Giorgis-Allemand L, Bernard C, Aguilera I, Andersen AM, Ballester F, Beelen RM, Chatzi L, Cirach M, Danileviciute A *et al.* Ambient air pollution and low birthweight: a European cohort study (ESCAPE). *Lancet Respir Med* 2013;**1**:695–704.

Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, Martikainen H, Tiitinen A, Hartikainen AL. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. *Hum Reprod* 2010;**25**:914–923.

Perkins E, Murphy SK, Murtha AP, Schildkraut J, Jirtle RL, Demark-Wahnefried W, Forman MR, Kurtzberg J, Overcash F, Huang Z *et al. Insulin-like growth factor 2/H19* methylation at birth and risk of overweight and obesity in children. *J Pediatr* 2012;**161**:31–39.

Piedrahita JA. The role of imprinted genes in fetal growth abnormalities. *Birth Defects Res A Clin Mol Teratol* 2011;**91**:682–692.

Pinborg A, Aaris Henningsen AK, Malchau S, Loft A. Congenital anomalies after assisted reproductive technology. *Fertil Steril* 2013a;**99**:327–332.

Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? *Hum Reprod* 2014;**29**:618–627.

Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. *Fertil Steril* 2010;**94**:1320–1327.

Pinborg A, Loft A, Romundstad LB, Wennerholm UB, Söderström-Anttila V, Bergh C, Aittomäki K. Epigenetics and assisted reproductive technologies. *Acta Obstet Gynecol Scand* 2016;**95**:10–15.

Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, Nygren KG, Hazekamp J, Bergh C. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013b;**19**:87–104.

Poplinski A, Tüttelmann F, Kanber D, Horsthemke B, Gromoll J. Idiopathic male infertility is strongly associated with aberrant methylation of *MEST* and *IGF2/H19* ICR1. *Int J Androl* 2010;**33**:642–649.

Provençal N, Binder EB. The effects of early life stress on the epigenome: from the womb to adulthood and even before. *Exp Neurol* 2015;**268**:10–20.

Puumala SE, Nelson HH, Ross JA, Nguyen RH, Damario MA, Spector LG. Similar DNA methylation levels in specific imprinting control regions in children conceived with and without assisted reproductive technology: a cross-sectional study. *BMC Pediatr* 2012;**12**:33.

Rabinovitz S, Kaufman Y, Ludwig G, Razin A, Shemer R. Mechanisms of activation of the paternally expressed genes by the Prader–Willi imprinting center in the Prader–Willi/Angelman syndromes domains. *Proc Natl Acad Sci U S A* 2012;**109**:7403–7408.

Rakers F, Rupprecht S, Dreiling M, Bergmeier C, Witte OW, Schwab M. Transfer of maternal psychosocial stress to the fetus. *Neurosci Biobehav Rev* 2017; pii: S0149-7634(16)30719-9. doi:https://doi.org/10.1016/j.neubiorev.2017.02.019

Rancourt RC, Harris HR, Barault L, Michels KB. The prevalence of loss of imprinting of *H19* and *IGF2* at birth. *FASEB J* 2013;**27**: 3335–3343.

Rancourt RC, Harris HR, Michels KB. Methylation levels at imprinting control regions are not altered with ovulation induction or *in vitro* fertilization in a birth cohort. *Hum Reprod* 2012;**27**:2208–2216.

Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 2001;**293**:1089–1093.

Reynolds RM, Jacobsen GH, Drake AJ. What is the evidence in humans that DNA methylation changes link events *in utero* and later life disease? *Clin Endocrinol (Oxf)* 2013a;**78**:814–822.

Reynolds RM, Labad J, Buss C, Ghaemmaghami P, Räikkönen K. Transmitting biological effects of stress *in utero*: implications for mother and offspring. *Psychoneuroendocrinology* 2013b;**38**:1843–1849.

Reynolds RM, Pesonen AK, O'Reilly JR, Tuovinen S, Lahti M, Kajantie E, Villa PM, Laivuori H, Hamalainen E, Seckl JR *et al.* Maternal depressive symptoms throughout pregnancy are associated with increased placental glucocorticoid sensitivity. *Psychol Med* 2015;**45**: 2023–2030.

Ribarska T, Goering W, Droop J, Bastian KM, Ingenwerth M, Schulz WA. Deregulation of an imprinted gene network in prostate cancer. *Epigenetics* 2014;**9**:704–717.

Rice F, Harold GT, Boivin J, van den Bree M, Hay DF, Thapar A. The links between prenatal stress and offspring development and psychopathology: disentangling environmental and inherited influences. *Psychol Med* 2010;**40**:335–345.

Riesewijk AM, Hu L, Schulz U, Tariverdian G, Hoglund P, Kere J, Ropers HH, Kalscheuer VM. Monoallelic expression of human PEG1/MEST

is paralleled by parent specific methylation in fetuses. *Genomics* 1997;**42**:236–244.

Robins JC, Marsit CJ, Padbury JF, Sharma SS. Endocrine disruptors, environmental oxygen, epigenetics and pregnancy. *Front Biosci (Elite Ed)* 2011;**3**:690–700.

Robledo CA, Yeung E, Mendola P, Sundaram R, Maisog J, Sweeney AM, Barr DB, Louis GM. Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. *Environ Health Perspect* 2015;**123**:88–94.

Rondo PH, Ferreira RF, Nogueira F, Ribeiro MC, Lobert H, Artes R. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur J Clin Nutr* 2003;**57**:266–272.

Rothenberger SE, Resch F, Doszpod N, Moehler E. Prenatal stress and infant affective reactivity at 5 months of age. *Early Hum Dev* 2011;**87**:129–136.

Russo S, Calzari L, Mussa A, Mainini E, Cassina M, Di Candia S, Clementi M, Guzzetti S, Tabano S, Miozzo M *et al.* A multi-method approach to the molecular diagnosis of overt and borderline 11p15.5 defects underlying silver–Russell and Beckwith–Wiedemann syndromes. *Clin Epigenetics* 2016;**8**:23.

Sakian S, Louie K, Wong EC, Havelock J, Kashyap S, Rowe T, Taylor B, Ma S. Altered gene expression of *H19* and *IGF2* in placentas from ART pregnancies. *Placenta* 2015;**36**:1100–1105.

Sakka SD, Loutradis D, Kanaka-Gantenbein C, Margeli A, Papastamataki M, Papassotiriou I, Chrousos GP. Absence of insulin resistance and low-grade inflammation despite early metabolic syndrome manifestations in children born after *in vitro* fertilization. *Fertil Steril* 2010;**94**:1693–1699.

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

Santos F, Hyslop L, Stojkovic P, Leary C, Murdoch A, Reik W, Stojkovic M, Herbert M, Dean W. Evaluation of epigenetic marks in human embryos derived from IVF and ICSI. *Hum Reprod* 2010;**25**:2387–2395.

Sato A, Otsu E, Negishi H, Utsunomiya T, Arima T. Aberrant DNA methylation of imprinted loci in superovulated oocytes. *Hum Reprod* 2007;**22**:26–35.

Saulnier DD, Brolin K. A systematic review of the health effects of prenatal exposure to disaster. *Int J Public Health* 2015;**60**:781–787.

Savage T, Peek J, Hofman PL, Cutfield WS. Childhood outcomes of assisted reproductive technology. *Hum Reprod* 2011;**26**:2392–2400.

Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Factors affecting obstetric outcome of singletons born after IVF. *Hum Reprod* 2011;**26**:2878–2288.

Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after *in vitro* fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012;**27**:1343–1350.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;**346**:731–737.

Shi X, Chen S, Zheng H, Wang L, Wu Y. Abnormal DNA methylation of imprinted loci in human preimplantation embryos. *Reprod Sci* 2014;**21**:978–983.

Shi X, Ni Y, Zheng H, Chen S, Zhong M, Wu F, Xia R, Luo Y. Abnormal methylation patterns at the *IGF2/H19* imprinting control region in phenotypically normal babies conceived by assisted reproductive technologies. *Eur J Obstet Gynecol Reprod Biol* 2011;**158**:52–55.

Simpson JL. Birth defects and assisted reproductive technologies. *Semin Fetal Neonatal Med* 2014;**19**:177–182.

Slykerman RF, Thompson J, Waldie K, Murphy R, Wall C, Mitchell EA. Maternal stress during pregnancy is associated with moderate to severe depression in 11-year-old children. *Acta Paediatr* 2015;**104**:68–74.

Smallwood A, Papageorghiou A, Nicolaides K, Alley MKR, Jim A, Nargund G, Ojha K, Campbell S, Banerjee S. Temporal regulation of the expression of syncytin (HERV-W), maternally imprinted *PEG10* and *SGCE* in human placenta. *Biol Reprod* 2003;**69**:286–293.

Smallwood SA, Tomizawa S-I, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat Genet* 2011;**43**:811–814.

Smith ZD, Chan MM, Humm KC, Karnik R, Mekhoubad S, Regev A, Eggan K, Meissner A. DNA methylation dynamics of the human preimplantation embryo. *Nature* 2014;**511**:611–615.

Smolarek I, Wyszko E, Barciszewska AM, Nowak S, Gawronska I, Jablecka A, Barciszewska MZ. Global DNA methylation changes in blood of patients with essential hypertension. *Med Sci Monit* 2010;**16**:CR149–CR155.

Soubry A, Murphy S, Huang Z, Murtha A, Schildkraut J, Jirtle R, Wang F, Kurtzberg J, Demark-Wahnefried W, Forman M *et al.* The effects of depression and use of antidepressive medicines during pregnancy on the methylation status of the *IGF2* imprinted control regions in the offspring. *Clin Epigenetics* 2011;**3**:2.

Stirrat LI, Sengers BG, Norman JE, Homer NZM, Andrew R, Lewis RM, Reynolds RM. Transfer and metabolism of cortisol by the isolated perfused human placenta. *J Clin Endocrinol Metab* 2018;**103**:640–648.

St-Pierre J, Hivert MF, Perron P, Poirier P, Guay SP, Brisson D, Bouchard L. *IGF2* DNA methylation is a modulator of newborn's fetal growth and development. *Epigenetics* 2012;**7**:1125–1132.

St-Pierre J, Laurent L, King S, Vaillancourt C. Effects of prenatal maternal stress on serotonin and fetal development. *Placenta* 2016;**48**:S66–S71.

Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol a exposure disrupts genomic imprinting in the mouse. *PLoS Genet* 2013;**9**:e1003401.

Tang WWC, Dietmann S, Irie N, Leitch HG, Floros VI, Bradshaw CR, Hackett JA, Chinnery PF, Surani MAA. Unique gene regulatory network resets the human germline epigenome for development. *Cell* 2015;**161**:1453–1467.

Tee L, Lim DH, Dias RP, Baudement MO, Slater AA, Kirby G, Hancocks T, Stewart H, Hardy C, Macdonald F *et al.* Epimutation profiling in Beckwith–Wiedemann syndrome: relationship with assisted reproductive technology. *Clin Epigenetics* 2013;**5**:23.

Tegethoff M, Greene N, Olsen J, Meyer AH, Meinlschmidt G. Maternal psychosocial adversity during pregnancy is associated with length of gestation and offspring size at birth: evidence from a population-based cohort study. *Psychosom Med* 2010;**72**:419–426.

Temple IK, Shield JP. 6q24 transient neonatal diabetes. *Rev Endocr Metab Disord* 2010;**11**:199–204.

Tierling S, Souren NY, Gries J, Loporto C, Groth M, Lutsik P, Neitzel H, Utz-Billing I, Gillessen-Kaesbach G, Kentenich H *et al.* Assisted reproductive technologies do not enhance the variability of DNA methylation imprints in human. *J Med Genet* 2010;**47**: 371–376.

Tobi EW, Heijmans BT, Kremer D, Putter H, Delemarre-van de Waal HA, Finken MJ, Wit JM, Slagboom PE. DNA methylation of *IGF2*, *GNASAS*, *INSIGF* and *LEP* and being born small for gestational age. *Epigenetics* 2011;**6**:171–176.

Togher KL, O'Keeffe MM, Khashan AS, Gutierrez H, Kenny LC, O'Keeffe GW. Epigenetic regulation of the placental HSD11B2 barrier and its role as a critical regulator of fetal development. *Epigenetics* 2014;**9**:816–822.

Trapphoff T, Heiligentag M, El Hajj N, Haaf T, Eichenlaub-Ritter U. Chronic exposure to a low concentration of bisphenol A during follicle culture affects the epigenetic status of germinal vesicles and metaphase II oocytes. *Fertil Steril* 2013;**100**:1758–1767.

Troisi J, Mikelson C, Richards S, Symes S, Adair D, Zullo F, Guida M. Placental concentrations of bisphenol A and birth weight from births in the southeastern U.S. *Placenta* 2014;**35**:947–952.

Turan N, Katari S, Gerson LF, Chalian R, Foster MW, Gaughan JP, Coutifaris C, Sapienza C. Inter- and intra-individual variation in allele-specific DNA methylation and gene expression in children conceived using assisted reproductive technology. *PLoS Genet* 2010;**6**:e1001033.

Uyar A, Seli E. The impact of assisted reproductive technologies on genomic imprinting and imprinting disorders. *Curr Opin Obstet Gynecol* 2014;**26**:210–221.

Vaiserman AM. Epigenetic programming by early-life stress: evidence from human populations. *Dev Dyn* 2015;**244**:254–265.

van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the hypothalamic–pituitary–adrenal axis by early life stress exposure. *Front Cell Neurosci* 2017;**11**.

van den Bergh BR, Mulder EJ, Mennes M, Glover V. Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. *Neurosci Biobehav Rev* 2005;**29**:237–258.

van den Bergh RH, van den Heuvel MI, Lahti M, Braeken M, de Rooij SR, Entringer S, Hoyer D, Roseboom T, Räikkönen K, King S *et al.* Prenatal developmental origins of behavior and mental health: the influence of maternal stress in pregnancy. *Neurosci Biobehav Rev* 2017; pii: S0149-7634(16)30734-5. doi:https://doi.org/10.1016/10.1016/j.neubiorev.2017.07.003.

van Montfoort AP, Hanssen LL, de Sutter P, Viville S, Geraedts JP, de Boer P. Assisted reproduction treatment and epigenetic inheritance. *Hum Reprod Update* 2012;**18**:171–197.

Vangeel EB, Izzi B, Hompes T, Vansteelandt K, Lambrechts D, Freson K, Claes S. DNA methylation in imprinted genes *IGF2* and *GNASXL* is associated with prenatal maternal stress. *Genes Brain Behav* 2015;**14**:573–582.

Vermeiden JP, Bernardus RE. Are imprinting disorders more prevalent after human *in vitro* fertilization or intracytoplasmic sperm injection? *Fertil Steril* 2013;**99**:642–651.

Vidal AC, Benjamin Neelon SE, Liu Y, Tuli AM, Fuemmeler BF, Hoyo C, Murtha AP, Huang Z, Schildkraut J, Overcash F *et al.* Maternal stress, preterm birth and DNA methylation at imprint regulatory sequences in humans. *Genet Epigenet* 2014b;**6**:37–44.

Vidal AC, Henry NM, Murphy SK, Oneko O, Nye M, Bartlett JA, Overcash F, Huang Z, Wang F, Mlay P *et al.* *PEG1/MEST* and *IGF2* DNA methylation in CIN and in cervical cancer. *Clin Transl Oncol* 2014a;**16**:266–272.

Vidal AC, Semenova V, Darrah T, Vengosh A, Huang Z, King K, Nye MD, Fry R, Skaar D, Maguire R *et al.* Maternal cadmium, iron and zinc levels, DNA methylation and birth weight. *BMC Pharmacol Toxicol* 2015;**16**:20.

Vincent RN, Gooding LD, Louie K, Chan Wong E, Ma S. Altered DNA methylation and expression of *PLAGL1* in cord blood from assisted reproductive technology pregnancies compared with natural conceptions. *Fertil Steril* 2016;**106**:739–748.e3.

Walder DJ, Laplante DP, Sousa-Pires A, Veru F, Brunet A, King S. Prenatal maternal stress predicts autism traits in $6^{1}/_{2}$ year-old children: Project Ice Storm. *Psychiatry Res* 2014;**219**:353–360.

Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG. The imprinted *DLK1-MEG3* gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. *Nat Genet* 2010;**42**:68–71.

Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y, Liu J, Hu Z. Birth defects in children conceived by *in vitro* fertilization and intracytoplasmic sperm injection: a meta-analysis. *Fertil Steril* 2012;**97**: 1331–1337.

Wennerholm UB, Henningsen AKA, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. *Hum Reprod* 2013;**28**:2545–2553.

Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, Selbing A, Loft A. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod* 2009;**24**:2158–2172.

White CR, MacDonald WA, Mann MR. Conservation of DNA methylation programming between mouse and human gametes and preimplantation embryos. *Biol Reprod* 2016;**95**:61.

Wong EC, Hatakeyama C, Robinson WP, Ma S. DNA methylation at *H19/IGF2* ICR1 in the placenta of pregnancies conceived by *in vitro* fertilization and intracytoplasmic sperm injection. *Fertil Steril* 2011;**95**:2524–2526.e1-3.

Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ Health Perspect* 2011;**119**:878–885.

Wu L, Wang L, Shangguan S, Chang S, Wang Z, Lu X, Zhang Q, Wang J, Zhao H, Wang F *et al.* Altered methylation of *IGF2* DMR0 is associated with neural tube defects. *Mol Cell Biochem* 2013;**380**: 33–42.

Yang B, Damaschke N, Yao T, McCormick J, Wagner J, Jarrard D. Pyrosequencing for accurate imprinted allele expression analysis. *J Cell Biochem* 2015;**116**:1165–1170.

Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of post-traumatic stress disorder in babies of mothers exposed to the world trade center attacks during pregnancy. *J Clin Endocrinol Metab* 2005;**90**:4115–4118.

Ying W, Li FJ, Wei SW, Li WL. Genomic imprinting status of *IGF-2* and *H19* in placentas of fetal growth restriction patients. *J Genet* 2010;**89**:213–216.

Yong Ping E, Laplante DP, Elgbeili G, Hillerer KM, Brunet A, O'Hara MW, King S. Prenatal maternal stress predicts stress reactivity at $2^{1}/_{2}$ years of age: the Iowa flood study. *Psychoneuroendocrinology* 2015;**56**:62–78.

Yu L, Chen M, Zhao D, Yi P, Lua L, Han J, Zheng X, Zhou Y, Li L. The *H19* gene imprinting in normal pregnancy and pre-eclampsia. *Placenta* 2009;**30**:443–447.

Zechner U, Pliusch G, Schneider E, Hall NE, Tresch A, Shufaro Y, Seidmann L, Coerdt W, Mueller A, Haaf T. Quantitative methylation analysis of developmentally important genes in human pregnancy losses after ART and spontaneous conception. *Mol Hum Reprod* 2010;**16**:704–713.

Zhao Y, Chen J, Wang X, Song Q, Xu HH, Zhang YH. Third trimester phthalate exposure is associated with DNA methylation of growth-related genes in human placenta. *Sci Rep* 2016;**6**:33449.

Zhao Y, Shi HJ, Xie CM, Chen J, Laue H, Zhang YH. Prenatal phthalate exposure, infant growth and global DNA methylation of human placenta. *Environ Mol Mutagen* 2015;**56**: 286–292.

Zheng H, Shi X, Wang L, Wu Y, Chen S, Zhang L. Study of DNA methylation patterns of imprinted genes in children born after assisted reproductive technologies reveals no imprinting errors: a pilot study. *Exp Ther Med* 2011b;**2**:751–755.

Zheng HY, Shi XY, Wu FR, Wu YQ, Wang LL, Chen SL. Assisted reproductive technologies do not increase risk of abnormal methylation of *PEG1/MEST* in human early pregnancy loss. *Fertil Steril* 2011a;**96**:84–89.

Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ* 2006;**333**:679.

Zhu P, Tao F, Hao J, Sun Y, Jiang X. Prenatal life events stress: implications for preterm birth and infant birthweight. *Am J Obstet Gynecol* 2010;**203**:34.e1–34.e8.