Journal of Genetics, Vol. 96, No. 3, July 2017, pp. 465–482
DOI 10.1007/s12041-017-0788-5

REVIEW ARTICLE

The placental gateway of maternal transgenerational epigenetic inheritance

S. PURNIMA SAILASREE, SURABHI SRIVASTAVA* and RAKESH K. MISHRA*

CSIR - Centre for Cellular and Molecular Biology, Hyderabad 500 007, India
*For correspondence. E-mail: Surabhi Srivastava, ssurabhi@ccmb.res.in; Rakesh K. Mishra, mishra@ccmb.res.in.

Received 26 September 2016; accepted 10 November 2016; published online 4 July 2017

Abstract. While much of our understanding of genetic inheritance is based on the genome of the organism, it is becoming clear that there is an ample amount of epigenetic inheritance, which though reversible, escapes erasing process during gametogenesis and goes on to the next generation. Several examples of transgenerational inheritance of epigenetic features with potential impact on embryonic development and subsequent adult life have come to light. In placental mammals, the placenta is an additional route for epigenetic information flow. This information does not go through any meiotic reprogramming and is, therefore, likely to have a more profound influence on the organism. This also has the implication of providing epigenetic instructions for several months, which is clearly a maternal advantage. Although less well-known, there is also an impact of the embryo in emitting genetic information to the maternal system that remains well beyond the completion of the pregnancy. In this review, we discuss several factors in the context of the evolution of this mammal-specific phenomenon, including genomic imprinting, micromosaicism, and assisted reproduction. We also highlight how this kind of inheritance might require attention in the modern lifestyle within the larger context of the evolutionary process.

Keywords. placenta; epigenetics; transgenerational inheritance; mammals; foetus; imprinting.

Introduction

Epigenetic factors are only recently being investigated as a global and dynamic way used by the genome to respond to environmental cues, and the mechanisms by which this regulation is fine-tuned are not yet understood. Some of the responses to environmental cues are passed on to the progeny as adaptations of the present generation, distinct from the faithful transmission of DNA sequence information. Epigenetic modifications present the genome with a mechanism to propagate changes that are not in the germplasm and have been introduced during the life of the parent. DNA methylation, histone modifications, chromatin packaging, and miRNA expression changes are a few of the best known epigenetic mechanisms as summarized in appendix. Somatic cells faithfully maintain and transmit epigenetic marks between successive rounds of cell division. Typically, strict mechanisms exist to ensure the faithful transmission of epigenetic information during mitosis, when chromatin undergoes condensation and decondensation for replication. Transgenerational inheritance assumes significance because, unlike in mitosis, the genome is largely reprogrammed between successive generations. This is achieved during gametogenesis as well as in the embryonic stage prior to implantation (Feng *et al.* 2010). This provides a window during which the epigenetic profile is being reset for the genome to factor in environmental effects to tweak gene expression in the next generation. Germline transmission has the potential for subtle, yet significant alterations to be brought about through epigenetic mechanisms, and these are increasingly gaining significance in the context of human disease.

The beginning of this century saw a new understanding emerge of how environmental effects can harm health in more ways than simply through carcinogenic and toxic activity at the cellular level. Studying the origins of disease now includes investigating the effects of epigenetic mechanisms on genome regulation, starting with preconception and foetal influences, and continuing into childhood and adulthood. Several studies indicate that some epigenetic changes occurring in the germline have the potential to be transmitted to the offspring. This has been defined as transgenerational inheritance and can be considered as the burden of the epigenetic inheritance passed on from the parental generation to the offspring. In this light, investigation of epigenetic mechanisms in the

context of environmental contaminants and lifestyle stressors becomes crucial. Although alterations in both the male and female gametes have the potential to be inherited, in this review we focus especially on transmission through the mother through exposure to the *in utero* environment.

The placenta is formed transiently to support foetal development and defines the interactions of the foetus within the maternal environment. As such, the placental epigenetic landscape is likely to be highly dynamic and complex to support the rapid transcriptional changes required during the gestational period, after which the placenta is discarded. Recent studies have focussed on placental epigenetic mechanisms and their role in healthy foetal development as well as diseased states, the so-called developmental origins of health and disease (DOHaD). The impact of the *in utero* environment on the foetus is reviewed completely elsewhere (Babenko *et al.* 2015; Fernandez-Twinn *et al.* 2015), but not much is known about the effects that are inherited not just by the growing embryo but also by its subsequent progeny through transgenerational transmission. In recent years, mounting evidence suggests that the effects of *in utero* epigenetic changes go beyond influencing the mother and the child; they are carried forward through the adulthood of the child as well as into its germline, to the detriment of successive generations.

The widespread use of next generation sequencing (NGS) technologies (Buermans and den Dunnen 2014) has led to a new emerging field of studying placental transgenerational effect by investigating changes in chromatin state. Investigating placental epigenetic mechanisms has already revealed a surprising potential of the foetal genome to sample and adapt to the environment for maximum postnatal survival success. However, this also means that exposure to a harmful environment can have long term consequences on health and future fitness. Therefore, it becomes relevant to analyse the effects of environmental exposure on developmentally inherited epigenetic adaptations and their transmission.

The lasting influence that the previous generations have on an individual's wellbeing is a relatively unexplored field of study and the mechanisms by which nongenetic effects are inherited are yet to be understood. In this review, we focus on the placenta–foetal connection in the context of information transmission through epigenetic mechanisms.

Placenta as an organ

Placenta: a historical and evolutionary perspective

The evolution of the placenta conferred mammals with the advantage of *in utero* embryonic development. The placenta as an organ has intrigued many scholars since the time of Aristotle, when it was used for medication, cosmetics and other purposes. The placenta is a transient, flattened circular organ in the uterus of pregnant eutherian mammals that connects the vascular system of the mother with that of the foetus. The mode of establishing this connection is not the same in all mammals and there are many unique features amongst different eutherians. Afrotherians, the first placental mammals, appeared to have had a choriovitelline extension to provide nourishment to the growing foetus (O'Leary *et al.* 2013; Buckley 2015). The significant advantage this offered to the survival of the offspring probably paved the way for greater diversification. Subsequently, various modifications of the placenta have arisen, with humans showing the haemochorial placenta (table 1).

The invasive haemochorial placental type in humans and the great apes has two aspects namely, haemorrhages in the mothers, and effects on foetal growth and survival (Abrams and Rutherford 2011). The formation of this transient organ is a result of an array of genes that have a link to its functional significance. The phylogenetic origin of some of these genes has been studied in an effort to understand its origin. For example, the syncytin genes are derivatives of the retroviral envelope genes. These genes have membrane fusogenic properties and are essential for the formation of the syncytiotrophoblast layer in the placenta at the maternal and foetal interface (Dupressoir *et al.* 2012). The placental growth factor gene (PGF) belongs to the growth factor gene family of VEGF genes (such as FGF) that play a role in angiogenesis and vasculogenesis across organ systems and in foetal development (Cross *et al.* 2003).

Table 1. The placenta is classified into diverse groups.

Basis	Type 1	Type 2	Type 3	Type 4
Shape	Diffuse (horse, pigs)	Cotyledonary (ruminants)	Discoid (human, apes, rodents)	Zonary (dog, cat)
Layers attached	Epitheliochorial (ruminants)	Endotheliochorial (carnivores)	Hemochorial (human, apes rodents)	
Epithelial point of contact	Chorioallontoic (horse, pigs, ruminants)	Choriovitelline (rodents and lagomorphs)		

Table 2. Basic functions of placenta.

Role	Importance
Nutrition	Supply of micronutrients and macronutrients for foetal development
Excretion	Removal of waste products and cellular debris
Signalling	Communication between the mother and foetus depending on the stage and need for growth and development
Immunity	Protection for the foetus and filtering of harmful microbes
Aeration	Supply of oxygen to the foetus
Hormone secretion	Secretion of prolactin for milk production, oxytocin for milk let down
Sampling the environment	Transmission of information of in utero conditions in the form of epigenetic memory that sets foetal gene expression

The placenta and foetus are thus linked to each other in the mother's womb not only through a physical connection but also because of genetic mechanisms. In fact, the expression of some placental genes varies depending upon the sex of the foetus (Sood *et al.* 2006). The early placenta depends on the trophoblast layer for development; placental and foetal birth weight ratios indicate a clear genetic link between foetal and placental defects (Watkins *et al.* 2015). Hence, a thorough investigation of placental regulation is extremely significant in the context of understanding foetal health.

Functions of placenta

The word 'placenta' comes from the Roman word 'cake'. It is a derivative of the trophectoderm cells present in the outer rim of the blastocyst stage embryo and forms a barrier between the maternal and the foetal blood supply. The placenta itself can be divided into layers based on point of contact, with each layer playing a definite role in maintaining the foetus. The overall function of the placenta is to provide nutrition to the growing embryo, however the multiple layers regulate a diverse array of functions (table 2). Being fetomaternal in origin and extraembryonic in nature, the placenta is influenced and regulated both by the maternal and the foetal genomes. This link is lost immediately after birth and the placenta is thus transient in its presence. During the gestation period, dynamic epigenetic mechanisms orchestrate its gene expression profiles to allow tight regulation of placental function. The significant role played by the placenta is increasingly being revealed by investigating the epigenetic mechanisms involved in its complex regulation. Studying the human placenta during pregnancy is difficult, and inferences from studies in model organisms are confounded by the fact that while a single placenta is present in humans, many other mammals have more than one foetus per gestation and, thereby, more than one placenta.

Nevertheless, a large number of studies over the last few years have dissected the role of placental function on foetal development and its wellbeing as a neonate and into adulthood. The foetus is influenced by many factors including the nutritional status of the mother (Wildman *et al.* 2006), diseases that have affected the mother during pregnancy (Adams Waldorf and McAdams 2013) and exposure to toxins, teratogens and pathogens, including the recent Zika virus (Plessinger and Woods 1993; Sachdeva *et al.* 2009; Adibi *et al.* 2016). The intensity of these influences also varies depending on the stage of gestation at which the stress was imposed on the mother. These are discussed in detail in the following sections along with their lingering effects on the child and on the succeeding generations.

Developmental role of placenta

The growth of the placenta is interlinked with foetal growth from the moment of implantation of the embryo in the uterus of the female. As mentioned above, the placenta grows as an extra embryonic tissue and is fetomaternal in origin. The placenta grows depending on the needs of the foetus as per the stage of gestation. The functional role of placenta varies depending on the stage of foetal growth linked to the demands of the foetus but not according to the maternal environment. During the primary stages of implantation, the placenta acts as an endocrine gland by secreting cortisone, prolactin, human chorionic gonadotropin, etc., which are required for foetal growth and also equip the foetus with these hormones (Burton and Fowden 2015). The functional role of the placenta is analogous to those of the lung, pituitary, liver and other vital organs, prior to their organogenesis in the foetus.

The placenta also plays a role in immune suppression to allow implantation and the growth of what is essentially foreign tissue in the uterus without causing rejection. This immunity suppression of the mother to permit the growth of the foetus is localized to the foetal development zone but not to the rest of the immune system functions. The defence mechanisms of the mother, in fact, confer resistance to both the mother and the foetus during pregnancy. The placenta is selectively permeable to the immunoglobulins carried from the mother into the foetus (Pentsuk and van der Laan 2009; Palmeira *et al.* 2012) and this, sometimes, is carried as memory cells for certain antigens in the baby. This is the first form of acquired passive immunity in the growing foetus, received from the mother through the placenta, and can thus be considered as one of the first forms of crosstalk between the mother and foetus. The immunity acquired from the mother is unique even amongst siblings born to the same mother through the differential influence of the placenta in each case.

As the foetus grows in size, due to cellular differentiation and development of the various organelles, the womb of the mother is stretched. The effect of this stretching will be perceived by the mother, so the placenta secretes antiinflammatory substances to the maternal body to suppress the pain (Kawakatsu *et al.* 2013). This is essential for continued growth of the foetus without severe discomfort to the mother. As the growing foetus increases in size, it requires greater nourishment, for which the placenta signals for a higher metabolic rate of the mother (Diaz *et al.* 2014). After the formation of the organs of the foetus, the functional role of the placenta becomes restricted and the hormones needed for such restriction are synthesised by the placenta. During foetal development, the placenta monitors the demands of the foetus, such as differential needs of nutrition and oxygen supply, and sends signals to the mother. Placental deregulation can thus have disastrous effects on foetal growth. Intrauterine growth restriction (IUGR) is a complication encountered due to the unregulated growth restriction of the mother on the foetus (Chen *et al.* 2015), which lowers the nourishment sent to the foetus by the placenta.

Signalling through placenta

Placental signalling through the mTOR pathway is critical for foetal growth–inhibition of placental mTOR is associated with IUGR implicated in maternal nutrient restriction while overactivation is observed in obese women and is associated with large-sized babies (Jansson *et al.* 2013). The placenta thus not only carries nutrients from the mother to the foetus, but also signals the needs of the foetus to the mother through hormonal regulation. The placenta functions as an endocrine gland and produces many cytokines and hormones such as progesterone, which reduces the effect of foetal antigens, thereby minimizing the chance of maternal rejection at the time of foetal implantation (Guleria and Sayegh 2007). The precursor of progesterone, 17α-hydroxy progesterone, is also a vital indicator of foetal wellbeing. High levels of this hormone are an indicator of congenital adrenal hyperplasia, involving the activation of the adrenal gland and foetal sex hormone deregulation leading to infertility (Nagamani *et al.* 1978). Deficiency of the hormone, on the other hand, leads to sexually unique phenotypes (Yanase *et al.* 1992). Progesterone is also produced by the placenta later in the pregnancy and may help avoid ovarian cancer (Han *et al.* 2013). Progesterone probably has the same regulatory effect on cell proliferation in the placenta, helping to regulate placental growth in the context of foetal growth during the late stages of pregnancy.

High levels of cortisol are essential for the proper growth of the foetus and this is produced by the mother during the early stages of pregnancy (Davis and Sandman 2010). The placenta converts cortisol into cortisone through the action of 11β-hydroxyl steroid dehydrogenase. The converted cortisone serves to elevate blood pressure, reduce inflammation of maternal tissues, and set the pituitary axis in the foetus (Yang 1997). Prolactin, a hormone secreted by the placenta during the final stages of gestation, signals to the maternal mammary glands to produce milk for feeding the neonate. The hormonal functions of the placenta end with the production of oxytocin needed for the delivery of the foetus. The role of this hormone is to initiate contractions in the uterus and activate the mammary glands for the letdown of milk.

Other maternal hormones influence the placenta without being produced by it, such as IGF-I, insulin, and leptin that increase supply of nutrients through the placenta, thereby governing foetal growth (Jansson and Powell 2007; Baumann *et al.* 2014). Exposure to maternal androgen in polycystic ovary syndrome (PCOS) pregnancies has been associated with mood disorders in offspring and with changes in amygdala and hippocampal signalling, causing anxiety-like behaviour and excess androgen in adult females (Hu *et al.* 2015). The excess androgen could act in a fashion reminiscent of the progesterone effect and produce variegated phenotypes. The transgenerational impact of androgen excess in the future pregnancies of affected females needs to be assessed, but the observation that increased maternal stress affects foetal wellbeing (Diego *et al.* 2006) indicates the potential for PCOS effects to be indirectly transmitted to subsequent generations.

Production of maternal inflammatory cytokines and placental inflammation associated with obesity and gestational diabetes (Challier *et al.* 2008) affects placental function and nutrient transport. This may be associated with a propensity for disease and metabolic disorders in the adult life of the offspring. Subnormal environments, such as hypoxic condition of the foetus (Spungin *et al.* 1987) are communicated to the mother through cross talk but defects in maternal supply are not compensated by the placenta; rather they are carried as a memory in the foetus in the form of developmental defects or malnourishment effects. It is increasingly being realized that such effects can manifest as postnatal or adult disorders at later stages of life.

Placenta and foetus: dynamic connections and exchanges

Placenta is a vital organ for intrauterine foetal development. A healthy and functionally active placenta plays a major role in conception and delivery of the foetus while placental misregulation as seen in placental infarcts, preeclampsia, placenta previa, placental abruption, placental acreta etc. is to the detriment of both mother and child, and can lead to IUGR, low birth weight, premature delivery or miscarriage. Preterm birth can be caused by many factors (Varner and Esplin 2005) but there is also a

clear genetic predisposition to this in humans, manifested in the form of previous occurrences. Many of these cases can be averted by gene product therapies, namely administration of immunoglobulin IgG (Diejomaoh *et al.* 2015), which has been shown to be present in both healthy and pathologically determined pregnancy failures (Palmeira *et al.* 2012). Insulin resistance also appears to be preponderant in cases of low foetal birth weight (Jahan *et al.* 2009), inherited due to maternal genes or due to factors during pregnancy and manifested through the placenta.

The exchange that takes place between the placenta and the foetus is very complex (figure 1). The foetus is entirely dependent on the mother's resources and there is no extra nourishment available from the placenta to the foetus. This kind of interaction is both beneficial to the foetus, due to the availability of nourishment from the mother, and harmful as it is not only the excess but also the shortage that is communicated. The influence of maternal deficit is generally more harmful as the effects of maternal deficiencies can lead to metabolic and growth related problems in the foetus. Vitamins function as precursors to major metabolic processes. Subtle changes in the levels of vitamins can cause harmful effects in the neonate or even manifest in adulthood: scurvy, bow leg syndrome, night blindness due to the activation or inactivation of specific genes. For example, vitamin A deficiency affects the *TNF-α* gene leading to cell necrosis (Antipatis *et al.* 2002). Besides nutritional deficits, pregnant women exposed to pollutants and toxins can transmit their harmful effects to the developing foetus. The effects of such environmental changes, as well as genome regulation changes, e.g., at imprinted genes are discussed in the following sections.

Placental influence on foetal development

Placenta is a highly dynamic organ that is under the constant influence of the mother from the point of formation. The nourishment provided to the foetus through the placenta depends on the dietary patterns of the mother and environmental conditions. The influences accrued during development, the so-called effects of 'foetal programming', can have short term influence on the foetus affecting its growth and development as well as long lasting consequences during its adulthood and sometimes even on the progeny in the next generations. One of the most popular examples of this kind of programming includes the predisposition for type 2 diabetes, as explained by the thrifty gene and thrifty phenotype hypotheses (Hales and Barker 1992). These states that certain 'thrifty' genes evolved because they conferred a survival advantage by allowing deposition of fat reserves during periods of abundance for utilization during famines. But in the context of plentiful food supply in the current human society these genes have become detrimental, causing diabetes, obesity and the related spectrum of diseases. Similarly, placental mechanisms developed to adapt to limited intrauterine food supplies through foetal programming now correlate with coronary diseases, stroke and metabolic syndromes later in life.

Epigenetic mechanisms regulate foetal programming by modifying gene expression, e.g., by DNA methylation or demethylation leading to gene silencing or activation, respectively. Such changes occur in the womb of the mother due to her dietary pattern (Gabory *et al.* 2011) and the impairment of the supply of nutrition through the placenta (Lof *et al.* 2005). They are heritable in the offspring (Holness and Sugden 2006) and transmitted transgenerationally in rodent experiments (Gheorghe *et al.* 2010). Exposure to the external environment through placental sampling also leads to epigenetic alterations during gestation. The extent of the effect of environmental exposure faced by the mother on the next generation(s) through the placenta is still unclear, although emerging data suggests that it has a critical influence on the health and wellbeing of individuals not just at birth but throughout their lifetime, including the late onset and age related disease spectrum (Gluckman *et al.* 2007). Contributing factors experienced by the mother that can lead to undesirable consequences can be as diverse as diet, environmental toxins and pollutants such as heavy metals, stress and lifestyle issues. These are briefly discussed here in the context of the placental influence on transgenerational inheritance.

Maternal nutrition and diet

Not only does the nutrition taken by the mother during gestation directly impact the foetus but the overall maternal health, malnutrition or obesity also influence the growth of the foetus (Leddy *et al.* 2008). The placenta acts as a conduit to transport nutrients to the developing foetus and, hence, controls nutrient availability that governs growth. Apart from direct effects, the link between maternal malnutrition or obesity and subsequent metabolic disorders found in the child is one of the key contributors towards DOHaD.

Maternal hormones regulated by the placenta govern supply and thus directly dictate the extent of foetal growth, with specific growth hormones like IGF1 and insulin fluctuating from normal physiological levels in cases of IUGR at one end and obesity or diabetes at the other (Holmes *et al.* 1997; Jansson *et al.* 2008). The placenta is a key player in determining foetal health as a direct consequence of maternal health. It is also believed to play a role in information transmission from the preconception stage exposure of the foetus (Gronowitz *et al.* 1987). In mammalian species that bear multiple offspring from a single pregnancy, the nutritional distribution from the mother to each foetus is even more complex. In the case of single offspring in a litter, the nutrition source is a single placenta,

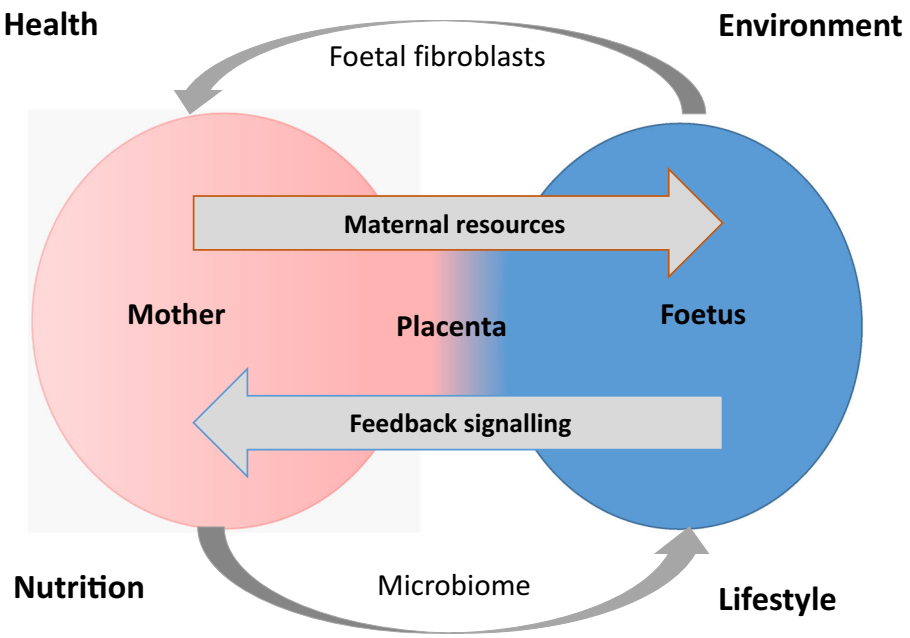

Figure 1. Fetomaternal interaction occurs through the placenta. Diagrammatic representation of the information flow across the placenta which forms a closed unit of exchange between the mother and the foetus affected by external factors.

but with an increase in litter size, the numbers of placenta match the litter size leaving the maternal resources to be divided amongst them. In suboptimal *in utero* environment caused by maternal malnutrition, decreased blood flow or hypoxia, resources need to be reallocated through placental nutrient sensing through mechanisms such as placental downregulation of transplacental transporters of amino acids, glucose and lipids (Diaz *et al.* 2014). IUGR babies are known to have depleted lipid stores (Padoan *et al.* 2004) with possible consequences in adult life. In humans, the glucose transporter GLUT1, encoded by the *SLC2A1* gene, is unaffected in the placenta, while amino acid transporters show reduced activity if placental blood flow is decreased (Jansson *et al.* 1993). However, *SLC2A1* expression drops with altitude-induced hypoxia (Zamudio *et al.* 2006).

Examples of known maternal nutrition related foetal health issues include folate deficiencies, long known to be important for preventing neural tube, heart and placental defects. Folate deficiency found in mouse models has recently been shown to cause congenital malformation defects that persists up to five generations probably by a transgenerational mechanism. The 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) gene that encodes the methionine synthase reductase enzyme possesses a hypomorphic phenotype in the F_4 generation of female mice and likely gets transmitted through epigenetic mechanisms from the maternal grandparent bearing a similar phenotype (Padmanabhan *et al.* 2013). The placenta has receptors for many nutrients including folate and transmits signals depending on the maternal nutrition (Farkas *et al.* 2013). Nutritionally deficient mothers produce children affected by malnutrition, as was seen during the 'Hunger winter' in Amsterdam and western Holland in November 1944–45 when only low calorie diet supply was available for pregnant and lactating women. This led to changes in the health of children born during this period by the time they reached adulthood (El Hajj *et al.* 2014). The ill-effects were underscored by epigenetic changes such as the hypomethylation of *Igf2*, an imprinted growth factor gene (Heijmans *et al.* 2008).

Several studies have pointed out that maternal body composition has detrimental effects on the growing foetus (Bell and Ehrhardt 2002) but what is less understood are the subtle effects caused due to maternal nutrition deficiency on foetal development (Godfrey 2002; Nelissen *et al.* 2011). Triplets and twin studies in primates have helped to address this question. The physiological effect of nutrient supply on triplets (litter size of three) has been compared to single pregnancies in marmoset monkeys; the titration of hormonal levels of insulin was checked among the triplets, revealing that each of the triplets showed a value that was not comparable to the average of the three (Rutherford 2009).

Toxic exposure through placenta

With increasing realization of the effects of man-made pollution on human health, researchers are trying to address the interaction between the environment and the unborn child through the sampling provided by the placenta. Many lines of evidence indicate that just as maternal nutrition and health impact the adult life of the offspring, exposure to harmful toxins also reprogrammes the foetus

in a manner that can impact it and its succeeding generations.

Many agents used in agriculture including pesticides have a lot of side effects on future generations, though many of these effects may not be global due to the usage of different compounds in different areas in the world and the cumulative effect of multiple exposures. The use of pesticides in agricultural fields has been shown to affect pregnant females living in close proximity, with defects documented in the cranial growth and head circumference in newborns (Petit *et al.* 2010). The transgenerational effect of pesticide exposure has been studied using a mix of permethrin and DEET injected into rodents at gestation days 8–14 (Manikkam *et al.* 2012). A clear phenotypic effect was seen in the reproductive organs of both male and female progeny up to the F_3 generation. Air pollution involves exposure of the respiratory tract to nonnatural gaseous emissions such as ozone and nitrogen dioxide due to industries, vehicle emissions etc. Exposure of pregnant women to these pollutants during the first trimester of gestation creates oxidative stress in the developing foetus that can result in preterm birth (Olsson *et al.* 2012). The effects of preterm birth are felt most dramatically on neural development and the child may have ensuing neurological problems (Ment and Vohr 2008). Further studies in this direction are needed since an understanding of the effects of similar exposure at different gestational stages is missing.

Different toxins have different effects on the foetus depending on the stage of development. Alterations in DNA methylation patterns in placenta have been noticed to occur due to maternal smoking resulting in exposure of the foetus to tobacco smoke (Maccani and Maccani 2015). For example, RUNX3, a transcription factor gene associated with cellular differentiation, is differentially methylated upon exposure to tobacco smoke from the mother. Runx3 is known to transcriptionally attenuate a chemokine receptor CCR7 through TGF-β mediated pathway which has been associated with asthma like aetiology (Fainaru *et al.* 2005). First trimester exposure is also associated with effects on the mental development of the foetus. The exact link between the exposure and the frequency with which it occurs or the mechanism involved in this phenomenon is as yet unexplained. These kinds of lacunae need to be filled since relevant interventions during pregnancy can go a long way towards preventing problems in early childhood as well as adulthood.

Microbiome of foetus: derivation and influence from mother

The microbiome (microorganisms that symbiotically share our body) of an individual is unique and defines the phenotype and response to the environment. Studies indicate that the placental microbiome is similar to the gut microbiome of the foetus (Satokari *et al.* 2009). This implies that the maternal microbiome is inherited by the progeny and hence encompasses the maternal phenotype. Other studies have elucidated that the placental microbiome is unique and most similar to the oral microbiome indicating that the microbiota of the foetus may have been established through the placenta from the oral cavity of the mother (Stout *et al.* 2013; Aagaard *et al.* 2014). The derivation of the microbiome in the neonate from the maternal microbiota may probably give the advantage of metabolising milk. This thus constitutes the first microbial colony to be established in the human body. The origin of foetal microbiome is expected to additionally include sources from the vaginal microbiome, mammary gland microbiome and placental microbiome (Romano-Keeler and Weitkamp 2015). The specific role the placental microbiome plays in the developing foetus and its postbirth effects are still under scrutiny (Guttmacher *et al.* 2014), however since it is now clear that the microbiome is passed on along the maternal line, it can be considered a part of the transgenerational inheritance of an individual.

Indeed, an emerging theory suggests that the increase in Caesarean births in society today are depriving the newer generation from colonization by the maternal microbiome. This, coupled with the increased use of antibiotics and an unhealthy diet and lifestyle, creates a predisposition towards asthma (Russell *et al.* 2012), obesity (Cho *et al.* 2012), and reduced immune regulation in terms of autoimmunity and inflammation (Belkaid and Hand 2014). A study in mice demonstrated that unhealthy diet in pregnant mice caused increased cancer risk and accelerated ageing in the progeny as well as in the F_2 generation, while early exposure to beneficial microbes mitigated the risk of succumbing to transgenerational obesity and cancer (Poutahidis *et al.* 2015).

Genomic imprinting and placenta

The term imprinting was used in the 1930s in relation to behaviour patterning in animals. Genomic imprinting is defined as parental-specific gene expression in diploid cells (Fowden *et al.* 2011; Barlow and Bartolomei 2014) i.e., certain genes are selectively expressed from a single parent owing to restrictions put forth by as yet unclear determinants. Most imprinted genes play an important role during development and are involved in foetal growth. In fact, the first imprinted gene to be understood was *Igf2r*, the receptor for the growth factor IGF-2 (Reik and Lewis 2005). The growth-specific nature of many imprinted genes led to the development of the 'parental conflict' hypothesis, explained below. Changes affecting imprinted genes thus have direct consequences on foetal wellbeing. Changes can be induced in imprinted genes through exposure to environmental stimuli occurring at any stage in the life of an individual, including early foetal development stages. The placental cohorts of certain foetal samples have shown that

the effects of environmental stimuli are transmitted to the next generations. This kind of transgenerational inheritance of epigenetic influence of environmental stimuli on the foetus can even be used as a diagnostic tool (Lambertini 2014).

Maternal and paternal imprinting theories

Genomic imprinting is an evolutionarily advanced mechanism of fine-tuning the contributions of the paternal and maternal genomes. It appears to have coevolved with viviparity, where maternal investment and expenditure per pregnancy are much higher than the paternal contribution. One of the hypotheses in this connection is the 'conflict of the genomes' hypothesis (Iwasa 1998). This states that the reproductive success of the species is ensured by the males focussing on passing on their genes to the maximum number of healthy offspring while the female invests in a long gestation period, and suckling and nurturing of all her young to ensure their survival till reproductive age. The paternal genome thus has a vested interest in promoting foetal intake during gestation while the maternal genome needs to balance the distribution of resources for itself and among multiple potential pregnancies (Kinoshita *et al.* 2008). A large number of imprinted genes are thus expressed in the placenta (Tunster *et al.* 2013), and many of the growth and development genes are maternally imprinted and paternally expressed.

Imprinted genes are remarkably stable and resistant to evolutionary selection pressures, as changes in their monoallelic expression affect dosage detrimentally. They are shielded from the genomewide reprogramming that occurs during fertilization by protection from demethylation and active maintenance of the original imprint (Li *et al.* 2008; Bian and Yu 2014). The placental phenotype is greatly influenced by imprinting, studied best in mice. Imprinting in the gametes can be manipulated by the maternal environment both during the process of fertilization and further during gestation (Gabory *et al.* 2012). This kind of effect was clearly observed when sperms collected from alcohol administered mice were compared to those from normal mice showing that there was an adverse effect on the reproductive performance of the sperms produced in the alcohol-exposure condition (Siervo *et al.* 2015). Further, analysis of offspring born from alcohol administered mice indicated spermatogenesis defects not only in the parent mice but also in the progeny. The litter size of pups born was low and these pups also showed changes in characters related to maleness, such as testis structure, motility and count of sperms at adulthood. Poor-quality oocyte also leads to multiple defects such as poor establishment of pregnancy, poor survival of the foetus, retarded development of the foetus and also adulthood diseases (Krisher 2004). This has been attributed to differential DNA methylation in the germ cells which affects the pattern of cell division that are essential for foetal growth (Smallwood and Kelsey 2012).

Influence of placental imprinted genes in embryonic development and growth

As described above, imprinted genes form a special group that follow distinct mechanisms to escape genomewide reprogramming effects but the role of transgenerational epigenetic mechanisms in this context is not yet clearly defined. There are many genes which are involved in placental growth such as the pluripotency gene for the transcription factor Oct-4 (*Pou5f1*). Its promoter shows distinct methylation pattern in embryonic stem cells versus trophoblast cells with aberrant methylation leading to the formation of smaller placentas in rats (Serman *et al.* 2007).

It is believed that imprinting and mammalian brain development have coevolved with many imprinted genes showing placental/hypothalamic coexpression that coincides with critical developmental periods and feed back into signalling in the maternal adult hypothalamus (Keverne 2014). Many of the placental imprinted genes, such as *Grb10*, *Phlda2*, *p57kip2* (maternally expressed) and *Dlk*, *Gnas*, *Magel2*, *Nnat*, *Necdin*, *Peg1*, *Peg3*, *Zac1* (paternally expressed) are also in fact involved in foetal hypothalamic development. This is interesting as it implies an ability for environmental influences acting on the mother to impact brain development and behaviour in subsequent generations. In line with this hypothesis, it has been seen that a healthy pregnancy and good maternal care are consistent with improved nurturing behaviour in the offspring that later encourages them to provide optimal lactation and nourishment to their own progeny, i.e., the F_2 generation. A classic example along these lines is evidence gained from the Rwandan genocide, where the mother's exposure to maltreatment as a child rather than her posttraumatic stress disorder (PTSD) profile subsequently affected her children in the form of a 'cycle of violence' (Roth *et al.* 2014).

During maternal food deprivation, expression of some genes like *Peg3* (a paternally expressed gene involved in cell proliferation and p53-mediated apoptosis) decreases in the placenta while increasing in the developing foetal hypothalamus, thereby protecting the foetus (and potentially its next generation) from the effects of deprivation (Broad and Keverne 2011). Thus, imprinted genes can help buffer the transmission of harmful effects transgenerationally and many of these changes in the transmission occur due to hypomethylation or hypermethylation at specific loci. DNA methylation has been associated with other brain function linked genes such as *MAP2K1* or *BDNF* resulting in psychosis associated disease aetiologies (Mill *et al.* 2008). Even psychotic diseases such as schizophrenia and bipolar disorder have been associated

Table 3. Key epigenetic features studied in placenta.

Epigenetic mark	Function	Placental effect	References
G9/G9a	Histone methyl transferase for H3K9 methylation	Placental defects due to chorioallontoic fusion	Tachibana *et al.* (2002, 2005)
Ezh2/Ezh12	Polycomb group family protein for H3K27 methylation	Placental defects due to failed chorioallontoic attachment; lethal	Pasini *et al.* (2004)
HDAC sirtuin1	Histone deacetylase associated with HIF-2alpha	Mechanistic link between hypoxia and histone acetylation in placenta	Dioum *et al.* (2009)
HIF factors	Reduced progenitor proliferation	Impaired vascularization of labryrinth	Adelman *et al.* (2000)
Syncitin1	Hypermethylation of ERVW-1	Decreased expression causes placental syndromes	Ruebner *et al.* (2013)
MiRNA	Noncoding RNA	Hypoxia, Preeclampsia (e.g. miRNA210 upregulation)	Yan *et al.* (2016)
LncRNA and piRNA	Noncoding RNAs	Transgenerational silencing of retrotransposons	Kuramochi-Miyagawa *et al.* (2008)

with methylation effects, as seen in monozygotic twins (Dempster *et al.* 2011). Further, an occurrence of later life effects, instead of at birth, has also been observed in monozygotic twins. Discordant monozygotic twins with monochorionic placenta show higher DNA methylation at cell division genes than such twins with dichorionic placenta (Gordon *et al.* 2012). DNA methylation, discussed in the subsequent section, is thus one of the main markers when studying placental transmission. It also marks a large number of developmentally important genes, e.g. the *CNTNAP2* gene, or the 'language gene'. It is hypothesized to be one of the key genes involved in the evolution of the human brain, possibly due to different DNA methylation percentages when the phylogenetic split between monkeys and apes occurred (Schneider *et al.* 2014). The role of the placenta in contributing to epigenetic variation in humans is best addressed using twins, demonstrating variations in DNA methylation profile among neonates from twin births (Gordon *et al.* 2012). Monozygotic twins have a common genetic makeup as well as intrauterine environmental exposure while dizygotic twins share only the latter during development. Twin studies thus make for an excellent model for delineating the role of the placenta in programming the foetal epigenome (Cordova-Palomera *et al.* 2015; Malki *et al.* 2016).

Transgenerational inheritance through the placenta

Epigenetic modifications, as discussed so far, can involve temporal, spatial or global changes in gene function and can be heritable across generations. 'Transgenerational transmission' is the term used to describe those changes that get conveyed to future generations. The placenta is a complex organ which is influenced by a complex set of genes for growth and transcriptional factors, as well as extracellular matrix, cell signalling and various other functional genes. The epigenetic effects on this organ have effects on the phenotype of the offspring sometimes even causing lethality of the embryos (Rossant and Cross 2001). Recent studies have identified a few epigenetic mechanisms associated with placental dysregulation, some of which are listed in table 3.

The effect of placenta-derived factors produced by maternal genes for foetal growth and foeto-maternal immunity (Chu *et al.* 1998) have been known but not much is studied about the epigenetic mechanisms that govern these modulators and their transgenerational effects. Igf2, the first imprinted gene studied, appears to play a key role in placental permeability and transport processes (Coan *et al.* 2008). Upregulation of porforin under hypoxic condition (Iwaki *et al.* 2004) is associated with greater number of natural killer cells, immune system cells that have the ability to invade the placenta (Parham 2004). Changes such as elevated circulating cortisol concentration and increased mean arterial blood pressure have been noticed which is caused due to a reduced supply of food (70% of normal chow) to guinea pig dams in either the first or the second half of the gestation period. The epigenetic effects caused due to the reduced chow were seen to persist through to the F_2 generation (Bertram *et al.* 2008).

Transgenerational epigenetic mechanisms

The embryo changes and resets its gene expression profile by depletion of histone marks postfertilization (Puschendorf *et al.* 2008). Many changes are also brought about by the waves of methylation and demethylation as well as the paternal reprogramming at the time of fertilization and implantation (Oswald *et al.* 2000). As we have seen in the previous sections, foetal programming is regulated by the changes induced during gestation under the influence of a range of factors. Such changes may have evolved due to survival advantages to the foetus in a dynamic and

changing environment, and there is no known correction mechanism to reverse them. Some of the changes that add up to the genome of individuals from the point of conception thus depend on the lifestyle and exposure of their parental generation, which further accrue on to their progeny, and become transgenerational.

Transgenerational epigenetic mechanisms also include the gene expression changes in the offspring that can be associated with preconception exposure in the parents or grandparents. DNA methylation, being the most stable epigenetic mark for propagation, is an ideal candidate to look for transgenerational effects. Approximately 37% of the placental genome contains partially methylated domains (PMDs) otherwise seen only in cancer tissues (Schroeder *et al.* 2013). Maternal nutrition significantly affects the establishment of DNA methylation at metastable epialleles—locations where CpG methylation is established stochastically in the embryo, and maintained through differentiation for propagation in the individual—correlating with environmental conditions that define food availability at the time of conception (Waterland *et al.* 2010). Folate and methionine in the maternal diet act as methyl donors while high homocysteine and cysteine levels correlate with lower DNA methylation levels in the embryo (Dominguez-Salas *et al.* 2014).

A recent study has identified changes in methylation patterns at key cardiovascular and metabolic genes that appear to be associated with low birth weight and hypothesized a correlation between epigenetic regulation and disease risk in later life (Gordon *et al.* 2012). Another recent study revealed the role of altered DNA methylation at genes involved in Wnt signalling and lipid metabolism defects due to placental dysregulation in cases of severe IUGR (Roifman *et al.* 2016). Altered methylation during *in utero* development has also been associated with susceptibility for impaired glucose metabolism and diabetes by multiple studies (e.g., Zheng *et al.* 2014). Changes in expression profiles of insulin signalling and glucose transport genes such as GLUT4 have been shown in undernourished rat foetuses and have been correlated with low birth weight in humans (Ozanne *et al.* 2005).

Maternal and paternal effects

Undernourishment at foetal stages in mice results in underdevelopment in pups leading to a decreased ability to suckle and chew as a neonate (Perez-Torrero *et al.* 2001). This has been linked to malnourishment in the pups leading to poor health in adulthood. Further, it is associated with the prevalence of metabolic disorders in adulthood, as a consequence of the epigenetic modification in the placental supply of nutrients (Maccani and Marsit 2009; Aguilera *et al.* 2010). The foetus adapts itself to the available nutrition, but studies suggest that such adaptations are unhealthy for the adult life of the foetus (Godfrey 2002). For example, when a condition such as hypoxia occurs, foetus adapts itself through placental interference to the lower oxygen levels (Krebs *et al.* 1997; Penninga and Longo 1998; Roh *et al.* 2005). This adaptation to hypoxic conditions results in transmission of the oxygen rich blood more to the foetal brain by both autonomic and metabolic mechanisms. This adaptation by placenta is referred to as 'brain-sparing' adaptation, but it generally results in the growth impairment of different organs (Giussani 2016). A similar kind of effect is seen on the epigenetic profile of human placental trophoblasts when exposed to hypoxic conditions (Yuen *et al.* 2010). The net evolutionary advantage of such foetal adaptations is unclear and most of them may not get fixed for long on the evolutionary time scale.

Not only undernourishment but also exposure to various environmental contaminants including endocrine disrupting chemicals have been demonstrated to have epigenetic effects on the germline and promote disease across several generations (Crews *et al.* 2007). Gestational exposure to vinclozolin, known to have an antiandrogenic effect that alters differentially methylated region (DMR) methylation at imprinted genes (Stouder and Paoloni-Giacobino 2010), causes infertility in male progeny up to the F_4 generation (Anway *et al.* 2005). Although the focus of this review has been on effects through placental transmission, it is worth noting that paternal influence is also significant in terms of long term effects transmitted through the sperm genome. For example, obesity in the father has been shown to be associated with differential gene expression through changes in cell signalling pathways that lead to metabolic disorders and stress in the progeny (Binder *et al.* 2015). Paternal nutritional deficit in mice causes changes in serum glucose levels (Andersen *et al.* 2006) or metabolism (Carone *et al.* 2010) in the progeny. Genotoxic stress such as paternal radiation exposure causes hypomethylation of retrotransposons in the genome of the progeny (Filkowski *et al.* 2010).

Microchimerism

Another form of communication between the mother and the foetus through the placenta involves the reverse transmission of foetal fibroblasts into the maternal plasma (Dawe *et al.* 2007; Miech 2010). Microchimerism is defined as the presence of a small number of cells that do not belong to the host organism and have distinct features of another organism from which they have originated. Foetal microchimerism is a complex term coined to understand the presence of foetal origin fibroblasts in the maternal plasma during gestation of the foetus. The presence of foetal fibroblasts demonstrates a leakiness in the placenta as a filter, which allows the foetus to send signals that affect both placental development and maternal behaviour (Bocheva and Boyadjieva 2011). The foetal fibroblasts act as information carriers to the maternal organs and persist

in the mother even after the delivery of the foetus by their eventual grafting into maternal tissues.

Foetal fibroblasts are believed to lead to changes in the mother which may be transmissible to other offspring in subsequent pregnancies, with long lasting effects influencing the major histocompatability genes postdelivery (Stevens 2016). These long lasting effects are referred to as telegony (Crean *et al.* 2014). Telegony can be explained as those changes in the mother which have been brought about by the first offspring that are transmissible to future siblings. The acceptance of these cells has parallels with tissue grafting with similar issues of immunological rejection (Yusuf and Kliman 2008).

Finally, foetal fibroblasts play a role in a unique mechanism of resistance that confers protective immunity to the mother even after the delivery. Studies have shown that foetal ES cells can confer resistance to breast cancer till 15 years from the last delivery (Mosca *et al.* 2003; Tan *et al.* 2005; Dawe *et al.* 2007). There is a nonlinear relationship between the incidence of breast cancer and the date of last child birth (Albrektsen *et al.* 1995). This implies that there is a possible effect of foetal microchimerism on the mother's immunity to cancer development (Gadi and Nelson 2007; Gadi 2009). Another interesting observation in this context is the increased risk for autoimmune response seen in females who have had abortions, linked to the increased transfer of foetal cells into maternal circulation during the surgical abortion procedure (Miech 2010). It is not yet clear whether the mother imports the foetal cells for healing or if the foetus transfers these cells trying to take over the maternal environment for its own benefit. Such effects are yet to be understood for other diseases, but it is well established that BP, heart rate, and weight of the female during gestation correspond to the foetal demands (Godfrey 2002). Interestingly, certain placental miRNAs (miR-141, miR-149, miR229-5p and miR135b) are also secreted into maternal plasma and have the ability to travel to various maternal tissues, though their concentrations significantly reduce postparturition (Chim *et al.* 2008). It would be useful to explore the therapeutic potential of such fetoplacental phenomena lingering in the adult female body.

Artificial reproductive technology (ART) and its effect on placentation

ART is the mode of choice when there is a problem in the natural fertilization process. Infertility can result from problems ranging from male or female gametes production or their fertilization to issues with the prospective mother in carrying the pregnancy to term, in which case surrogate mothers can play a role. Procedures such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are a boon for infertile couples who otherwise cannot conceive and become parents. However studies in the last few years have explored changes in gene expression patterns in children conceived through such interventions (Katari *et al.* 2009). There is evidence of epigenetic dysregulation brought about due to the ART procedure, which has the potential to contribute to DOHaD as in the case of diabetes or obesity. Studies documenting the role of epigenetic factors in ART have gathered momentum relatively recently and the mouse has been used as a model system to test the effects of super ovulation, *in vitro* culture and embryo transfer (reviewed in van Montfoort *et al.* 2012). Until now, not much is established regarding the causes and effects of the use of this technology. It also remains unknown if such effects are transgenerationally transmitted.

Epigenetic consequences of ART

ART involves collection of sperms and oocytes *in vitro* and creating favourable conditions for fertilization to produce an embryo. After the embryo is cultured, it is transferred into the uterus of the female for conception. Exposure to *in vitro* conditions, such as culture medium faced by the gametes and the embryo is unique as these conditions are not experienced *in vivo* (El Hajj and Haaf 2013). There is thus the fear of introducing epigenetic changes due to even minute factors in media and its nutrient composition. This is especially relevant for imprinted genes, which are very sensitive to environmental stimuli and many of them directly affect foetal growth. The H19/Igf2 locus is known to be modified during various steps of ART such as super ovulation and media changes (Fowden *et al.* 2011). Since imprinted loci escape the genomewide reprogramming in the early embryo, changes at these loci have the potential to get carried over and have transgenerational effects.

It is thus important to conduct a detailed study of the methylation patterns of the gametic and early embryonic genomes in a stepwise pattern during the *in vitro* culture till the blastocyst stage (Mayer *et al.* 2000; Oswald *et al.* 2000). The paternal genome is expressed first during the oocyte fertilization process (Suzuki *et al.* 2014), possibly correlated with the wave of its demethylation at this stage; the maternal genome is not demethylated to the same extent (Santos *et al.* 2002). Changes introduced due to ART in the normal methylation patterns of genes may have implications at various stages of life (Zheng *et al.* 2014). For example, interference with genes performing functions linked to extra embryonic membrane production leads to effects on the placenta (Jaenisch 1997).

ART and foetal defects

ART involves medicating both or either of the gamete donors to collect sperms and ova. Following *in vitro* fertilization, the healthiest and most viable embryos are selected

for implantation. The embryos formed by these procedure, however, can carry some epigenetic changes in their genome owing to the effect of various steps in the protocol (Edwards and Ferguson-Smith 2007) and *in vitro* exposure of the gametes (Kobayashi *et al.* 2009). The use of fresh and frozen embryos can also have an effect on the context of low birth weight, presumably prompting placental adaptations to micronutrient availability from the environment in which the embryo is conditioned (Bloise *et al.* 2014). It has been reported that the frequency of children possessing visually obvious birth defects born after ART is higher than that observed in those born through natural conception (Hansen *et al.* 2002). Drastic defects are generally not viable and result in abortion of the foetus, but many epigenetic changes can be more subtle. For example, epigenetic modifications induced during preimplantation embryo culture affects imprinting genes important for foetal and placental development (Mann *et al.* 2004). A higher incidence of diseases such as Beckwith–Wiedemann syndrome (BWS) and Angelman syndrome caused due to misregulation of imprinted genes has been documented in children born after ART. The incidence of BWS is ~4% in such pregnancies (Odom and Segars 2010). The defects underlying these syndromes can be both genetic and epigenetic in origin, but so far no specific common factors have been identified as being causative of the epigenetic changes brought about due to ART. Some of the factors have been tested in mouse models (Khosla *et al.* 2001) but a wide variety of ART steps and techniques are associated with increased BWS in humans (reviewed in van Montfoort *et al.* 2012).

Optimal uterine environment is essential for establishment and maintenance of embryonic epigenetic patterns (Vickaryous and Whitelaw 2005). There are many factors that lead to a high incidence of placental defects during ART. The process of implantation in ART assisted pregnancies is a key step associated with abnormalities (Choux *et al.* 2015). Implantation involves the invasion of the trophoblast layer by the blastocyst stage embryo into the uterus. Embryos from IVF have fewer trophoblast stem cells (Turan *et al.* 2010) and placental IGF2/H19 levels are reduced in these cases. Overall, methylation differences are seen at 16% of the CpG sites in ART placentas compared to normal placenta (Katari *et al.* 2009). About 26 genes have been documented to have altered gene expression levels in the placenta of ART pregnancies versus normal pregnancy (Zhang *et al.* 2010). Another critical factor is hormone induction of the mother, the effects of which persist until the formation of the placenta. Studies in mouse have shown the deleterious effect this hormonal milieu can have on the foetus and on pregnancy (Mainigi *et al.* 2014). The mechanistic details of these outcomes and their transgenerational potential require further elucidation, though it is difficult to dissect the specific effects of the interventions from effects associated with parental infertility. It is essential to evaluate the epigenetic changes carried by the individual gametes and those that are introduced at each stage of intervention. This will help to understand the key points at which the ART procedure can be altered in order to reduce such effects while successfully granting healthy babies to infertile couples.

Conclusions

Although the placenta has long been recognised as a very important organ that has multiple effects on the foetus, its role in transgenerational inheritance is not yet clear. The impact of the uterine sampling provided by the placenta on the foetus is manifested through epigenetic mechanisms that are dynamically set throughout the gametogenesis, conception and gestation processes. Epigenetic memory further ensures that at least some of these changes are transmitted to the germline of the next generations thus linking health and disease in an individual with the influence of grandparental and great-grandparental generations.

Keeping in view the current focus on DOHaD and transgenerational effects, we have examined epigenetic mechanisms that operate through the placenta and their influence on various aspects of the progeny's development, *in utero* and through birth to adulthood. There are multiple issues that complicate the study of placental inheritance. Owing to the transient nature of this organ, placental studies in humans need to be directed along similar lines such as prenatal diagnosis of the embryo. Twin studies serve as an invaluable resource and need to be utilized to their full potential by performing large scale NGS-based epigenetic investigations on placental tissues collected as after-birth. Importantly, the prenatal or even postnatal diagnosis of possible defects in the placenta can forewarn of possible diseases that the child may be predisposed to during its lifetime. Similarly, artificial reproductive technology, which is increasingly used in human conception and gestation, is associated with placental-specific effects. Although ART has been associated with imprinting disorders, the number of documented cases is not very large and a much larger cohort needs to be identified for statistically significant relationships to be established with epigenetic dysregulation. Effects of environment, diet and lifestyle that are transgenerational similarly need to be documented to a far greater extent than is currently being done. These have implications not only for the current generation but also on future adaptations and changes.

Epigenetic mechanisms have a major role in directing the form and function of the genome. The maternal influences sampled and conveyed by the placenta impact the foetus through epigenetic alterations that translate into altered gene expression profiles (figure 2). Even when the changes in modifications, such as DNA methylation do not directly alter gene expression in the progeny, they could lead to cumulative effects that manifest in successive generations.

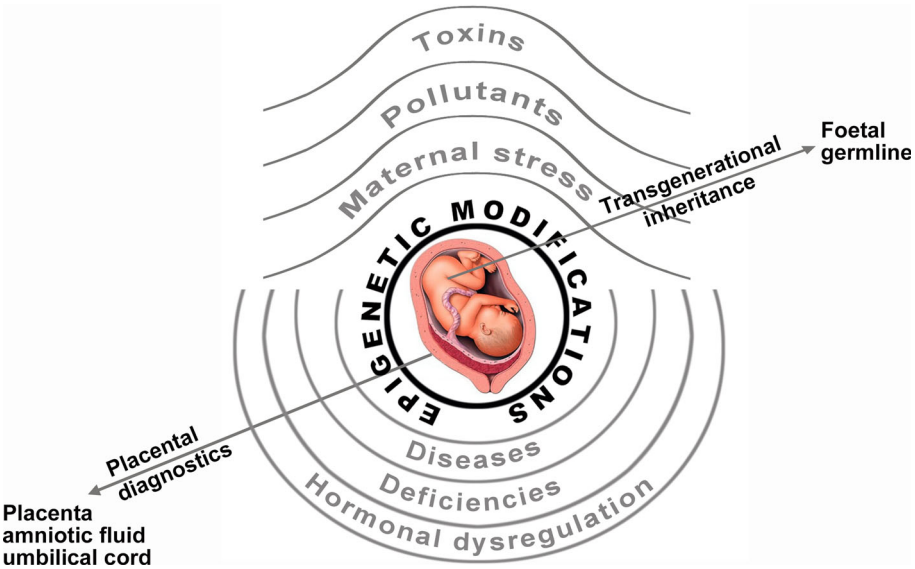

Figure 2. Maternal and environmental influences manifest through epigenetic mechanisms. The influence of the external and internal environment alters the epigenetic landscape which needs to be monitored by effective placental diagnostics. Alterations to the epigenome affect foetal as well as adult health; transmitted to the germline, these effects become transgenerational.

These mechanisms are dynamic enough that direct causes can lead to immediate changes in somatic tissue while epigenetic memory can contribute to heritable changes in each generation via transmission through the germline. Research into placental effects and the transmission and retention of information through this route is thus essential. The recent development of the placenta cord blood bank (Waller-Wise 2011) is a good resource for the scientific community. Further tools in placental diagnostics will go a long way towards understanding some of the issues highlighted in this review.

Appendix

Epigenetic mechanisms govern genome regulation

Histones wrap the DNA around a core octamer to form nucleosomes that generate higher order chromatin folding and architecture to govern access to DNA for transcription machinery and other cellular processes. Chemical modifications of the N-terminals of the histone tails constitute a dynamic mechanism to fine tune gene expression in conjunction with DNA methylation. The methylation of lysine residues of histone H3 has been most extensively studied in the context of transcriptional control; trimethylation of lysine 4 (H3K4me3) and acetylation of lysine 9 (H3K9ac) at gene promoters and acetylation of lysine 27 (H3K27ac) and methylation of lysine 4 (H3K4me) at enhancers are strongly associated with gene activation. Similarly, trimethylation of lysine residues 27 and 9 (H3K27me3 and H3K9me3) promote gene repression through polycomb (PcG) and HP1-heterochromatin mediated mechanisms, respectively. Such modifications are catalyzed by histone acetyltransferases (HATs) / histone deacetylases (HDACs) and histone methyl transferases (HMTs) / histone demethylases (HDMs) that add or remove the acetyl or methyl groups, respectively. The presence of these marks causes recruitment of a diverse array of proteins that ultimately regulate genome activation. There are a large number of histone variants and their modifications associated with genome regulation, and these are now being studied in the light of their inheritance through reproduction (Bunkar *et al.* 2016); only a few of these have been investigated in placental tissues (Nelissen *et al.* 2011).

The methylation of CpG dinucleotides in the genome is crucial in the context of gene expression, especially at CpG islands located near gene promoters. These are mostly unmethylated to allow transcription and their methylation is associated with gene silencing. DNA methylation is brought about by DNA methyltransferases (DNMTs) that are essential for development and survival since DNMT mutants are not viable (Li *et al.* 1992). Demethylation of DNA is also critical during development and involves both 'passive' and 'active' mechanisms to reprogramme the foetal genome (Moore *et al.* 2013) by erasure of parental methylation marks and resetting of transcriptional profiles for differentiation. MicroRNAs (miRNAs) are short noncoding RNAs of approximately 20 nt length. Unlike mRNAs, they do not code for functional proteins but regulate expression of their target genes. Tissue specific miRNAs are increasingly being discovered to be associated with key biological processes. MiRNA expression is linked to DNA methylation levels (Han *et al.* 2007) and methylation mutants show miRNA dysregulation and vice versa (Han *et al.* 2013). MiRNAs also target chromatin remodelling complexes, thus directly and indirectly modulating the setting of histone marks and demonstrating the interconnectedness and redundant nature of epigenetic programmes.

Acknowledgements

This work was supported by funding from Council for Scientific and Industrial Research (CSIR) under project EpiHeD (BSC0118). G. Giridharan is gratefully acknowledged for help with figures.

References

Aagaard K., Ma J., Antony K. M., Ganu R., Petrosino J. and Versalovic J. 2014 The placenta harbors a unique microbiome. *Sci. Transl. Med.* **6**, 237–265.

Adams Waldorf K. M. and McAdams R. M. 2013 Influence of infection during pregnancy on fetal development. *Reproduction* **146**, R151–R162.

Adelman D. M., Gertsenstein M., Nagy A., Simon M. C. and Maltepe E. 2000 Placental cell fates are regulated in vivo by HIF-mediated hypoxia responses. *Genes Dev.* **14**, 3191–3203.

Adibi J. J., Marques Jr E. T., Cartus A. and Beigi R. H. 2016 Teratogenic effects of the Zika virus and the role of the placenta. *Lancet* **387**, 1587–1590.

Aguilera O., Fernandez A. F., Munoz A. and Fraga M. F. 2010 Epigenetics and environment: a complex relationship. *J. Appl. Physiol.* **109**, 243–251.

Albrektsen G., Heuch I., Tretli S. and Kvale G. 1995 Is the risk of cancer of the corpus uteri reduced by a recent pregnancy? A prospective study of 765,756 Norwegian women. *Int. J. Cancer* **61**, 485–490.

Andersen L. B., Harro M., Sardinha L. B., Froberg K., Ekelund U., Brage S. and Anderssen S. A. 2006 Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *Lancet* **368**, 299–304.

Antipatis C., Ashworth C. J., Riley S. C., Hannah L., Hoggard N. and Lea R. G. 2002 Vitamin A deficiency during rat pregnancy alters placental TNF-alpha signalling and apoptosis. *Am. J. Reprod. Immunol.* **47**, 151–158.

Anway M. D., Cupp A. S., Uzumcu M. and Skinner M. K. 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**, 1466–1469.

Babenko O., Kovalchuk I. and Metz G. A. 2015 Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. *Neurosci. Biobehav. Rev.* **48**, 70–91.

Barlow D. P. and Bartolomei M. S. 2014 Genomic imprinting in mammals. *Cold Spring Harb. Perspect. Biol.* **6**, pii. a018382.

Baumann M. U., Schneider H., Malek A., Palta V., Surbek D. V., Sager R. *et al.* 2014 Regulation of human trophoblast GLUT1 glucose transporter by insulin-like growth factor I (IGF-I). *PLoS One* **9**, e106037.

Belkaid Y. and Hand T. W. 2014 Role of the microbiota in immunity and inflammation. *Cell* **157**, 121–141.

Bell A. W. and Ehrhardt R. A. 2002 Regulation of placental nutrient transport and implications for fetal growth. *Nutr. Res. Rev.* **15**, 211–230.

Bertram C., Khan O., Ohri S., Phillips D. I., Matthews S. G. and Hanson M. A. 2008 Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. *J. Physiol.* **586**, 2217–2229.

Bian C. and Yu X. 2014 PGC7 suppresses TET3 for protecting DNA methylation. *Nucleic Acids Res.* **42**, 2893–2905.

Binder N. K., Beard S. A., Kaitu'u-Lino T. J., Tong S., Hannan N. J. and Gardner D. K. 2015 Paternal obesity in a rodent model affects placental gene expression in a sex-specific manner. *Reproduction* **149**, 435–444.

Bloise E., Feuer S. K. and Rinaudo P. F. 2014 Comparative intrauterine development and placental function of ART concepti: implications for human reproductive medicine and animal breeding. *Hum. Reprod. Update* **20**, 822–839.

Bocheva G. and Boyadjieva N. 2011 Epigenetic regulation of fetal bone development and placental transfer of nutrients: progress for osteoporosis. *Interdiscip. Toxicol.* **4**, 167–172.

Broad K. D. and Keverne E. B. 2011 Placental protection of the fetal brain during short-term food deprivation. *Proc. Natl. Acad. Sci. USA* **108**, 15237–15241.

Buckley M. 2015 Ancient collagen reveals evolutionary history of the endemic South American 'ungulates'. *Proc. Biol. Sci.* **282**, 20142671.

Buermans H. P. and den Dunnen J. T. 2014 Next generation sequencing technology: Advances and applications. *Biochim. Biophys. Acta* **1842**, 1932–1941.

Bunkar N., Pathak N., Lohiya N. K. and Mishra P. K. 2016 Epigenetics: A key paradigm in reproductive health. *Clin. Exp. Reprod. Med.* **43**, 59–81.

Burton G. J. and Fowden A. L. 2015 The placenta: a multifaceted, transient organ. *Philos. Trans. R. Soc. London, B Biol. Sci.* **370**, 20140066.

Carone B. R., Fauquier L., Habib N., Shea J. M., Hart C. E., Li R. *et al.* 2010 Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* **143**, 1084–1096.

Challier J. C., Basu S., Bintein T., Minium J., Hotmire K., Catalano P. M. and Hauguel-de Mouzon S. 2008 Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta* **29**, 274–281.

Chen F., Wang T., Feng C., Lin G., Zhu Y., Wu G. *et al.* 2015 Proteome differences in placenta and endometrium between normal and intrauterine growth restricted pig fetuses. *PLoS One* **10**, e0142396.

Chim S. S., Shing T. K., Hung E. C., Leung T. Y., Lau T. K., Chiu R. W. and Lo Y. M. 2008 Detection and characterization of placental microRNAs in maternal plasma. *Clin. Chem.* **54**, 482–490.

Cho I., Yamanishi S., Cox L., Methe B. A., Zavadil J., Li K. *et al.* 2012 Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* **488**, 621–626.

Choux C., Carmignac V., Bruno C., Sagot P., Vaiman D. and Fauque P. 2015 The placenta: phenotypic and epigenetic modifications induced by Assisted Reproductive Technologies throughout pregnancy. *Clin. Epigenet.* **7**, 87.

Chu W., Fant M. E., Geraghty D. E. and Hunt J. S. 1998 Soluble HLA-G in human placentas: synthesis in trophoblasts and interferon-gamma-activated macrophages but not placental fibroblasts. *Hum. Immunol.* **59**, 435–442.

Coan P. M., Fowden A. L., Constancia M., Ferguson-Smith A. C., Burton G. J. and Sibley C. P. 2008 Disproportional effects of Igf2 knockout on placental morphology and diffusional exchange characteristics in the mouse. *J. Physiol.* **586**, 5023–5032.

Cordova-Palomera A., Fatjo-Vilas M., Gasto C., Navarro V., Krebs M. O. and Fananas L. 2015 Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins. *Transl. Psychiatry* **5**, e557.

Crean A. J., Kopps A. M. and Bonduriansky R. 2014 Revisiting telegony: offspring inherit an acquired characteristic of their mother's previous mate. *Ecol. Lett.* **17**, 1545–1552.

Crews D., Gore A. C., Hsu T. S., Dangleben N. L., Spinetta M., Schallert T. *et al.* 2007 Transgenerational epigenetic imprints on mate preference. *Proc. Natl. Acad. Sci. USA* **104**, 5942–5946.

Cross J. C., Baczyk D., Dobric N., Hemberger M., Hughes M., Simmons D. G. *et al.* 2003 Genes, development and evolution of the placenta. *Placenta* **24**, 123–130.

Davis E. P. and Sandman C. A. 2010 The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev.* **81**, 131–148.

Dawe G. S., Tan X. W. and Xiao Z. C. 2007 Cell migration from baby to mother. *Cell Adh. Migr.* **1**, 19–27.

Dempster E. L., Pidsley R., Schalkwyk L. C., Owens S., Georgiades A., Kane F. *et al.* 2011 Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. *Hum. Mol. Genet.* **20**, 4786–4796.

Diaz P., Powell T. L. and Jansson T. 2014 The role of placental nutrient sensing in maternal-fetal resource allocation. *Biol. Reprod.* **91**, 82.

Diego M. A., Jones N. A., Field T., Hernandez-Reif M., Schanberg S., Kuhn C. and Gonzalez-Garcia A. 2006 Maternal psychological distress, prenatal cortisol, and fetal weight. *Psychosom. Med.* **68**, 747–753.

Diejomaoh M. F., Bello Z., Al Jassar W., Jirous J., Karunakaran K. and Mohammed A. T. 2015 Consecutive successful pregnancies subsequent to intravenous immunoglobulin therapy in a patient with recurrent spontaneous miscarriage. *Int. Med. Case Rep. J.* **8**, 337–344.

Dioum E. M., Chen R., Alexander M. S., Zhang Q., Hogg R. T., Gerard R. D. and Garcia J. A. 2009 Regulation of hypoxia-inducible factor 2alpha signaling by the stress-responsive deacetylase sirtuin 1. *Science* **324**, 1289–1293.

Dominguez-Salas P., Moore S. E., Baker M. S., Bergen A. W., Cox S. E., Dyer R. A. *et al.* 2014 Maternal nutrition at conception modulates DNA methylation of human metastable epialleles. *Nat. Commun.* **5**, 3746.

Dupressoir A., Lavialle C. and Heidmann T. 2012 From ancestral infectious retroviruses to bona fide cellular genes: role of the captured syncytins in placentation. *Placenta* **33**, 663–671.

Edwards C. A. and Ferguson-Smith A. C. 2007 Mechanisms regulating imprinted genes in clusters. *Curr. Opin. Cell Biol.* **19**, 281–289.

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

El Hajj N., Schneider E., Lehnen H. and Haaf T. 2014 Epigenetics and life-long consequences of an adverse nutritional and diabetic intrauterine environment. *Reproduction* **148**, R111–R120.

Fainaru O., Shseyov D., Hantisteanu S. and Groner Y. 2005 Accelerated chemokine receptor 7-mediated dendritic cell migration in Runx3 knockout mice and the spontaneous development of asthma-like disease. *Proc. Natl. Acad. Sci. USA* **102**, 10598–10603.

Farkas S. A., Bottiger A. K., Isaksson H. S., Finnell R. H., Ren A. and Nilsson T. K. 2013 Epigenetic alterations in folate transport genes in placental tissue from fetuses with neural tube defects and in leukocytes from subjects with hyperhomocysteinemia. *Epigenetics* **8**, 303–316.

Feng S., Jacobsen S. E. and Reik W. 2010 Epigenetic reprogramming in plant and animal development. *Science* **330**, 622–627.

Fernandez-Twinn D. S., Constancia M. and Ozanne S. E. 2015 Intergenerational epigenetic inheritance in models of developmental programming of adult disease. *Semin. Cell Dev. Biol.* **43**, 85–95.

Filkowski J. N., Ilnytskyy Y., Tamminga J., Koturbash I., Golubov A., Bagnyukova T. *et al.* 2010 Hypomethylation and genome instability in the germline of exposed parents and their progeny is associated with altered miRNA expression. *Carcinogenesis* **31**, 1110–1115.

Fowden A. L., Coan P. M., Angiolini E., Burton G. J. and Constancia M. 2011 Imprinted genes and the epigenetic regulation of placental phenotype. *Prog. Biophys. Mol. Biol.* **106**, 281–288.

Gabory A., Attig L. and Junien C. 2011 Epigenetic mechanisms involved in developmental nutritional programming. *World J. Diabetes* **2**, 164–175.

Gabory A., Ferry L., Fajardy I., Jouneau L., Gothie J. D., Vige A. *et al.* 2012 Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS One* **7**, e47986.

Gadi V. K. 2009 Fetal microchimerism and cancer. *Cancer Lett.* **276**, 8–13.

Gadi V. K. and Nelson J. L. 2007 Fetal microchimerism in women with breast cancer. *Cancer Res.* **67**, 9035–9038.

Gheorghe C. P., Goyal R., Mittal A. and Longo L. D. 2010 Gene expression in the placenta: maternal stress and epigenetic responses. *Int. J. Dev. Biol.* **54**, 507–523.

Giussani D. A. 2016 The fetal brain sparing response to hypoxia: physiological mechanisms. *J. Physiol.* **594**, 1215–1230.

Gluckman P. D., Lillycrop K. A., Vickers M. H., Pleasants A. B., Phillips E. S., Beedle A. S. *et al.* 2007 Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc. Natl. Acad. Sci. USA* **104**, 12796–12800.

Godfrey K. M. 2002 The role of the placenta in fetal programming-a review. *Placenta* **23**, suppl A, S20–S27.

Gordon L., Joo J. E., Powell J. E., Ollikainen M., Novakovic B., Li X. *et al.* 2012 Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. *Genome Res.* **22**, 1395–1406.

Gronowitz S., Westerlund C., Hogberg T., Ramsby S., Hall H. and Lindberg U. H. 1987 Synthetic and receptor binding studies on bicyclic annelated systems related to cyproheptadine. *Acta Pharm. Suec.* **24**, 1–14.

Guleria I. and Sayegh M. H. 2007 Maternal acceptance of the fetus: true human tolerance. *J. Immunol.* **178**, 3345–3351.

Guttmacher A. E., Maddox Y. T. and Spong C. Y. 2014 The human placenta project: placental structure, development, and function in real time. *Placenta* **35**, 303–304.

Hales C. N. and Barker D. J. 1992 Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* **35**, 595–601.

Han K. H., Kim M. K., Kim H. S., Chung H. H. and Song Y. S. 2013 Protective effect of progesterone during pregnancy against ovarian cancer. *J. Cancer Prev.* **18**, 113–122.

Han L., Witmer P. D., Casey E., Valle D. and Sukumar S. 2007 DNA methylation regulates MicroRNA expression. *Cancer Biol. Ther.* **6**, 1284–1288.

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

Heijmans B. T., Tobi E. W., Stein A. D., Putter H., Blauw G. J., Susser E. S. *et al.* 2008 Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. USA* **105**, 17046–17049.

Holmes R., Montemagno R., Jones J., Preece M., Rodeck C. and Soothill P. 1997 Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth. *Early Hum. Dev.* **49**, 7–17.

Holness M. J. and Sugden M. C. 2006 Epigenetic regulation of metabolism in children born small for gestational age. *Curr. Opin. Clin. Nutr. Metab. Care* **9**, 482–488.

Hu M., Richard J. E., Maliqueo M., Kokosar M., Fornes R., Benrick A. *et al.* 2015 Maternal testosterone exposure increases anxiety-like behavior and impacts the limbic system in the offspring. *Proc. Natl. Acad. Sci. USA* **112**, 14348–14353.

Iwaki T., Yamamoto K., Matsuura T., Sugimura M., Kobayashi T. and Kanayama N. 2004 Alteration of integrins under hypoxic stress in early placenta and choriocarcinoma cell line BeWo. *Gynecol. Obstet. Invest.* **57**, 196–203.

Iwasa Y. 1998 The conflict theory of genomic imprinting: how much can be explained? *Curr. Top. Dev. Biol.* **40**, 255–293.

Jaenisch R. 1997 DNA methylation and imprinting: why bother? *Trends Genet.* **13**, 323–329.

Jahan S., Zinnat R., Hasan Z., Ahmed C. M., Habib S. H., Saha S. and Ali L. 2009 An investigation into genetic contribution to the relationship between insulin resistance and birth weight. *Int. J. Diabetes Dev. Ctries* **29**, 12–18.

Jansson N., Nilsfelt A., Gellerstedt M., Wennergren M., Rossander-Hulthen L., Powell T. L. and Jansson T. 2008 Maternal hormones linking maternal body mass index and dietary intake to birth weight. *Am. J. Clin. Nutr.* **87**, 1743–1749.

Jansson N., Rosario F. J., Gaccioli F., Lager S., Jones H. N., Roos S. *et al.* 2013 Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *J. Clin. Endocrinol. Metab.* **98**, 105–113.

Jansson T. and Powell T. L. 2007 Role of the placenta in fetal programming: underlying mechanisms and potential interventional approaches. *Clin. Sci. (Lond)* **113**, 1–13.

Jansson T., Wennergren M. and Illsley N. P. 1993 Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. *J. Clin. Endocrinol. Metab.* **77**, 1554–1562.

Katari S., Turan N., Bibikova M., Erinle O., Chalian R., Foster M. *et al.* 2009 DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum. Mol. Genet.* **18**, 3769–3778.

Kawakatsu M., Urata Y., Goto S., Ono Y. and Li T. S. 2013 Placental extract protects bone marrow-derived stem/progenitor cells against radiation injury through anti-inflammatory activity. *J. Radiat. Res.* **54**, 268–276.

Keverne E. B. 2014 Significance of epigenetics for understanding brain development, brain evolution and behaviour. *Neuroscience* **264**, 207–217.

Khosla S., Dean W., Brown D., Reik W. and Feil R. 2001 Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol. Reprod.* **64**, 918–926.

Kinoshita T., Ikeda Y. and Ishikawa R. 2008 Genomic imprinting: a balance between antagonistic roles of parental chromosomes. *Semin. Cell Dev. Biol.* **19**, 574–579.

Kobayashi H., Hiura H., John R. M., Sato A., Otsu E., Kobayashi N. *et al.* 2009 DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. *Eur. J. Hum. Genet.* **17**, 1582–1591.

Krebs C., Longo L. D. and Leiser R. 1997 Term ovine placental vasculature: comparison of sea level and high altitude conditions by corrosion cast and histomorphometry. *Placenta* **18**, 43–51.

Krisher R. L. 2004 The effect of oocyte quality on development. *J. Anim. Sci.* **82**, e-suppl. E14–E23.

Kuramochi-Miyagawa S., Watanabe T., Gotoh K., Totoki Y., Toyoda A., Ikawa M. *et al.* 2008 DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Dev.* **22**, 908–917.

Lambertini L. 2014 Genomic imprinting: sensing the environment and driving the fetal growth. *Curr. Opin. Pediatr.* **26**, 237–242.

Leddy M. A., Power M. L. and Schulkin J. 2008 The impact of maternal obesity on maternal and fetal health. *Rev. Obstet. Gynecol.* **1**, 170–178.

Li E., Bestor T. H. and Jaenisch R. 1992 Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69**, 915–926.

Li X., Ito M., Zhou F., Youngson N., Zuo X., Leder P. and Ferguson-Smith A. C. 2008 A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. *Dev. Cell* **15**, 547–557.

Lof M., Olausson H., Bostrom K., Janerot-Sjoberg B., Sohlstrom A. and Forsum E. 2005 Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. *Am. J. Clin. Nutr.* **81**, 678–685.

Maccani J. Z. and Maccani M. A. 2015 Altered placental DNA methylation patterns associated with maternal smoking: current perspectives. *Adv. Genomics Genet.* **2015**, 205–214.

Maccani M. A. and Marsit C. J. 2009 Epigenetics in the placenta. *Am. J. Reprod. Immunol.* **62**, 78–89.

Mainigi M. A., Olalere D., Burd I., Sapienza C., Bartolomei M. and Coutifaris C. 2014 Peri-implantation hormonal milieu: elucidating mechanisms of abnormal placentation and fetal growth. *Biol. Reprod.* **90**, 26.

Malki K., Koritskaya E., Harris F., Bryson K., Herbster M. and Tosto M. G. 2016 Epigenetic differences in monozygotic twins discordant for major depressive disorder. *Transl. Psychiatry* **6**, e839.

Manikkam M., Guerrero-Bosagna C., Tracey R., Haque M. M. and Skinner M. K. 2012 Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. *PLoS One* **7**, e31901.

Mann M. R., Lee S. S., Doherty A. S., Verona R. I., Nolen L. D., Schultz R. M. and Bartolomei M. S. 2004 Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* **131**, 3727–3735.

Mayer W., Smith A., Fundele R. and Haaf T. 2000 Spatial separation of parental genomes in preimplantation mouse embryos. *J. Cell Biol.* **148**, 629–634.

Ment L. R. and Vohr B. R. 2008 Preterm birth and the developing brain. *Lancet Neurol.* **7**, 378–379.

Miech R. P. 2010 The role of fetal microchimerism in autoimmune disease. *Int. J. Clin. Exp. Med.* **3**, 164–168.

Mill J., Tang T., Kaminsky Z., Khare T., Yazdanpanah S., Bouchard L. *et al.* 2008 Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am. J. Hum. Genet.* **82**, 696–711.

Moore L. D., Le T. and Fan G. 2013 DNA methylation and its basic function. *Neuropsychopharmacology* **38**, 23–38.

Mosca M., Curcio M., Lapi S., Valentini G., D'Angelo S., Rizzo G. and Bombardieri S. 2003 Correlations of Y chromosome microchimerism with disease activity in patients with SLE: analysis of preliminary data. *Ann. Rheum. Dis.* **62**, 651–654.

Nagamani M., McDonough P. G., Ellegood J. O. and Mahesh V. B. 1978 Maternal and amniotic fluid 17 alpha-hydroxyprogesterone levels during pregnancy: diagnosis of congenital adrenal hyperplasia in utero. *Am. J. Obstet. Gynecol.* **130**, 791–794.

Nelissen E. C., van Montfoort A. P., Dumoulin J. C. and Evers J. L. 2011 Epigenetics and the placenta. *Hum. Reprod. Update* **17**, 397–417.

O'Leary M. A., Bloch J. I., Flynn J. J., Gaudin T. J., Giallombardo A., Giannini N. P. *et al.* 2013 The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* **339**, 662–667.

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

Olsson D., Ekstrom M. and Forsberg B. 2012 Temporal variation in air pollution concentrations and preterm birth-a population based epidemiological study. *Int. J. Environ. Res. Public Health* **9**, 272–285.

Oswald J., Engemann S., Lane N., Mayer W., Olek A., Fundele R. *et al.* 2000 Active demethylation of the paternal genome in the mouse zygote. *Curr. Biol.* **10**, 475–478.

Ozanne S. E., Jensen C. B., Tingey K. J., Storgaard H., Madsbad S. and Vaag A. A. 2005 Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. *Diabetologia* **48**, 547–552.

Padmanabhan N., Jia D., Geary-Joo C., Wu X., Ferguson-Smith A. C., Fung E. *et al.* 2013 Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell* **155**, 81–93.

Padoan A., Rigano S., Ferrazzi E., Beaty B. L., Battaglia F. C. and Galan H. L. 2004 Differences in fat and lean mass proportions in normal and growth-restricted fetuses. *Am. J. Obstet. Gynecol.* **191**, 1459–1464.

Palmeira P., Quinello C., Silveira-Lessa A. L., Zago C. A. and Carneiro-Sampaio M. 2012 IgG placental transfer in healthy and pathological pregnancies. *Clin. Dev. Immunol.* **2012**, 985646.

Parham P. 2004 NK cells and trophoblasts: partners in pregnancy. *J. Exp. Med.* **200**, 951–955.

Pasini D., Bracken A. P., Jensen M. R., Lazzerini Denchi E. and Helin K. 2004 Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* **23**, 4061–4071.

Penninga L. and Longo L. D. 1998 Ovine placentome morphology: effect of high altitude, long-term hypoxia. *Placenta* **19**, 187–193.

Pentsuk N. and van der Laan J. W. 2009 An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. *Birth Defects Res. B. Dev. Reprod. Toxicol.* **86**, 328–344.

Perez-Torrero E., Torrero C. and Salas M. 2001 Effects of perinatal undernourishment on neuronal development of the facial motor nucleus in the rat. *Brain Res.* **905**, 54–62.

Petit C., Chevrier C., Durand G., Monfort C., Rouget F., Garlantezec R. and Cordier S. 2010 Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: a prospective cohort study in Brittany, France. *Environ. Health* **9**, 71.

Plessinger M. A. and Woods Jr J. R. 1993 Maternal, placental, and fetal pathophysiology of cocaine exposure during pregnancy. *Clin. Obstet. Gynecol.* **36**, 267–278.

Poutahidis T., Varian B. J., Levkovich T., Lakritz J. R., Mirabal S., Kwok C. *et al.* 2015 Dietary microbes modulate transgenerational cancer risk. *Cancer Res.* **75**, 1197–1204.

Puschendorf M., Terranova R., Boutsma E., Mao X., Isono K., Brykczynska U. *et al.* 2008 PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat. Genet.* **40**, 411–420.

Reik W. and Lewis A. 2005 Co-evolution of X-chromosome inactivation and imprinting in mammals. *Nat. Rev. Genet.* **6**, 403–410.

Roh C. R., Budhraja V., Kim H. S., Nelson D. M. and Sadovsky Y. 2005 Microarray-based identification of differentially expressed genes in hypoxic term human trophoblasts and in placental villi of pregnancies with growth restricted fetuses. *Placenta* **26**, 319–328.

Roifman M., Choufani S., Turinsky A. L., Drewlo S., Keating S., Brudno M. *et al.* 2016 Genome-wide placental DNA methylation analysis of severely growth-discordant monochorionic twins reveals novel epigenetic targets for intrauterine growth restriction. *Clin. Epigenet.* **8**, 70.

Romano-Keeler J. and Weitkamp J. H. 2015 Maternal influences on fetal microbial colonization and immune development. *Pediatr. Res.* **77**, 189–195.

Rossant J. and Cross J. C. 2001 Placental development: lessons from mouse mutants. *Nat. Rev. Genet.* **2**, 538–548.

Roth M., Neuner F. and Elbert T. 2014 Transgenerational consequences of PTSD: risk factors for the mental health of children whose mothers have been exposed to the Rwandan genocide. *Int. J. Ment. Health Syst.* **8**, 12.

Ruebner M., Strissel P. L., Ekici A. B., Stiegler E., Dammer U., Goecke T. W. *et al.* 2013 Reduced syncytin-1 expression levels in placental syndromes correlates with epigenetic hypermethylation of the ERVW-1 promoter region. *PLoS One* **8**, e56145.

Russell S. L., Gold M. J., Hartmann M., Willing B. P., Thorson L., Wlodarska M. *et al.* 2012 Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep.* **13**, 440–447.

Rutherford J. N. 2009 Fetal signaling through placental structure and endocrine function: illustrations and implications from a nonhuman primate model. *Am. J. Hum. Biol.* **21**, 745–753.

Sachdeva P., Patel B. G. and Patel B. K. 2009 Drug use in pregnancy; a point to ponder! *Indian J. Pharm. Sci.* **71**, 1–7.

Santos F., Hendrich B., Reik W. and Dean W. 2002 Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev. Biol.* **241**, 172–182.

Satokari R., Gronroos T., Laitinen K., Salminen S. and Isolauri E. 2009 Bifidobacterium and Lactobacillus DNA in the human placenta. *Lett. Appl. Microbiol.* **48**, 8–12.

Schneider E., El Hajj N., Richter S., Roche-Santiago J., Nanda I., Schempp W. *et al.* 2014 Widespread differences in cortex DNA methylation of the "language gene" CNTNAP2 between humans and chimpanzees. *Epigenetics* **9**, 533–545.

Schroeder D. I., Blair J. D., Lott P., Yu H. O., Hong D., Crary F. *et al.* 2013 The human placenta methylome. *Proc. Natl. Acad. Sci. USA* **110**, 6037–6042.

Serman L., Vlahovic M., Sijan M., Bulic-Jakus F., Serman A., Sincic N. *et al.* 2007 The impact of 5-azacytidine on placental weight, glycoprotein pattern and proliferating cell nuclear antigen expression in rat placenta. *Placenta* **28**, 803–811.

Siervo G. E., Vieira H. R., Ogo F. M., Fernandez C. D., Goncalves G. D., Mesquita S. F. *et al.* 2015 Spermatic and testicular damages in rats exposed to ethanol: influence of lipid peroxidation but not testosterone. *Toxicology* **330**, 1–8.

Smallwood S. A. and Kelsey G. 2012 De novo DNA methylation: a germ cell perspective. *Trends Genet.* **28**, 33–42.

Sood R., Zehnder J. L., Druzin M. L. and Brown P. O. 2006 Gene expression patterns in human placenta. *Proc. Natl. Acad. Sci. USA* **103**, 5478–5483.

Spungin B., Avolio J., Arden S. and Satir P. 1987 Dynein arm attachment probed with a non-hydrolyzable ATP analog. Structural evidence for patterns of activity. *J. Mol. Biol.* **197**, 671–677.

Stevens A. M. 2016 Maternal microchimerism in health and disease. *Best Pract. Res. Clin. Obstet. Gynaecol.* **31**, 121–130.

Stouder C. and Paoloni-Giacobino A. 2010 Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. *Reproduction* **139**, 373–379.

Stout M. J., Conlon B., Landeau M., Lee I., Bower C., Zhao Q. *et al.* 2013 Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. *Am. J. Obstet. Gynecol.* **208**, 226 e1–e7.

Suzuki T., Asami M. and Perry A. C. 2014 Asymmetric parental genome engineering by Cas9 during mouse meiotic exit. *Sci. Rep.* **4**, 7621.

Tachibana M., Sugimoto K., Nozaki M., Ueda J., Ohta T., Ohki M. *et al.* 2002 G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev.* **16**, 1779–1791.

Tachibana M., Ueda J., Fukuda M., Takeda N., Ohta T., Iwanari H. *et al.* 2005 Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9. *Genes Dev.* **19**, 815–826.

Tan X. W., Liao H., Sun L., Okabe M., Xiao Z. C. and Dawe G. S. 2005 Fetal microchimerism in the maternal mouse brain: a novel population of fetal progenitor or stem cells able to cross the blood-brain barrier? *Stem Cells* **23**, 1443–1452.

Tunster S. J., Jensen A. B. and John R. M. 2013 Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* **145**, R117–R137.

Turan N., Katari S., Gerson L. F., Chalian R., Foster M. W., Gaughan J. P. *et al.* 2010 Inter- and intra-individual variation in allele-specific DNA methylation and gene expression in children conceived using assisted reproductive technology. *PLoS Genet.* **6**, e1001033.

van Montfoort A. P., Hanssen L. L., de Sutter P., Viville S., Geraedts J. P. and de Boer P. 2012 Assisted reproduction treatment and epigenetic inheritance. *Hum. Reprod. Update* **18**, 171–197.

Varner M. W. and Esplin M. S. 2005 Current understanding of genetic factors in preterm birth. *BJOG* **112,** suppl 1, 28–31.

Waller-Wise R. 2011 Umbilical cord blood: information for childbirth educators. *J. Perinat. Educ.* **20**, 54–60.

Waterland R. A., Kellermayer R., Laritsky E., Rayco-Solon P., Harris R. A., Travisano M. *et al.* 2010 Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. *PLoS Genet.* **6**, e1001252.

Watkins A. J., Lucas E. S., Marfy-Smith S., Bates N., Kimber S. J. and Fleming T. P. 2015 Maternal nutrition modifies trophoblast giant cell phenotype and fetal growth in mice. *Reproduction* **149**, 563–575.

Wildman D. E., Chen C., Erez O., Grossman L. I., Goodman M. and Romero R. 2006 Evolution of the mammalian placenta revealed by phylogenetic analysis. *Proc. Natl. Acad. Sci. USA* **103**, 3203–3208.

Yan Y., Wang C., Zhou W., Shi Y., Guo P., Liu Y. *et al.* 2016 Elevation of circulating miR-210-3p in high-altitude hypoxic environment. *Front. Physiol.* **7**, 84.

Yanase T., Imai T., Simpson E. R. and Waterman M. R. 1992 Molecular basis of 17alpha-hydroxylase/17,20-lyase deficiency. *J. Steroid Biochem. Mol. Biol.* **43**, 973–979.

Yang K. 1997 Placental 11 beta-hydroxysteroid dehydrogenase: barrier to maternal glucocorticoids. *Rev. Reprod.* **2**, 129–132.

Yuen R. K., Penaherrera M. S., von Dadelszen P., McFadden D. E. and Robinson W. P. 2010 DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. *Eur. J. Hum. Genet.* **18**, 1006–1012.

Yusuf K. and Kliman H. J. 2008 The fetus, not the mother, elicits maternal immunologic rejection: lessons from discordant dizygotic twin placentas. *J. Perinat. Med.* **36**, 291–296.

Zamudio S., Baumann M. U. and Illsley N. P. 2006 Effects of chronic hypoxia in vivo on the expression of human placental glucose transporters. *Placenta* **27**, 49–55.

Zhang J., Landy H. J., Branch D. W., Burkman R., Haberman S., Gregory K. D. *et al.* 2010 Contemporary patterns of spontaneous labor with normal neonatal outcomes. *Obstet. Gynecol.* **116**, 1281–1287.

Zheng J., Xiao X., Zhang Q. and Yu M. 2014 DNA methylation: the pivotal interaction between early-life nutrition and glucose metabolism in later life. *Br. J. Nutr.* **112**, 1850–1857.

Corresponding editor: INDRAJIT NANDA