Human Reproduction Update, Vol.17, No.3 pp. 397–417, 2011
Advanced Access publication on October 19, 2010 doi:10.1093/humupd/dmq052

human reproduction update

Epigenetics and the placenta

Ewka C.M. Nelissen*, Aafke P.A. van Montfoort, John C.M. Dumoulin, and Johannes L.H. Evers

Department of Obstetrics and Gynaecology, Research Institute Growth & Development (GROW), Center for Reproductive Medicine, Maastricht University Medical Centre, MUMC+, PO Box 5800, 6202 AZ Maastricht, The Netherlands

*Correspondence address. Tel: +31-43-3876764; Fax: +31-43-3874765; E-mail: e.nelissen@mumc.nl

Submitted on May 21, 2010; resubmitted on September 7, 2010; accepted on September 22, 2010

TABLE OF CONTENTS

BACKGROUND: The placenta is of utmost importance for intrauterine fetal development and growth. Deregulation of placentation can lead to adverse outcomes for both mother and fetus, e.g. gestational trophoblastic disease (GTD), pre-eclampsia and fetal growth retardation. A significant factor in placental development and function is epigenetic regulation.

METHODS: This review summarizes the current knowledge in the field of epigenetics in relation to placental development and function. Relevant studies were identified by searching PubMed, Medline and reference sections of all relevant studies and reviews.

RESULTS: Epigenetic regulation of the placenta evolves during preimplantation development and further gestation. Epigenetic marks, like DNA methylation, histone modifications and non-coding RNAs, affect gene expression patterns. These expression patterns, including the important parent-of-origin-dependent gene expression resulting from genomic imprinting, play a pivotal role in proper fetal and placental development. Disturbed placental epigenetics has been demonstrated in cases of intrauterine growth retardation and small for gestational age, and also appears to be involved in the pathogenesis of pre-eclampsia and GTD. Several environmental effects have been investigated so far, e.g. ethanol, oxygen tension as well as the effect of several aspects of assisted reproduction technologies on placental epigenetics.

CONCLUSIONS: Studies in both animals and humans have made it increasingly clear that proper epigenetic regulation of both imprinted and non-imprinted genes is important in placental development. Its disturbance, which can be caused by various environmental factors, can lead to abnormal placental development and function with possible consequences for maternal morbidity, fetal development and disease susceptibility in later life.

Key words: epigenetics / placenta / genomic imprinting / environmental effects

Introduction

From its earliest stages till the end of pregnancy, the placenta is of paramount importance for the intrauterine development and growth of the fetus. It is responsible for the establishment of a tight contact between mother and conceptus, enabling the exchange of gas, nutrients and waste products. The placenta protects the fetus from maternal immune system attacks and secretes pregnancy-associated hormones and growth factors (Rossant and Cross, 2001).

Deregulation of placentation can be detrimental to both mother and child; derailed growth can lead to invasive, sometimes malignant trophoblast disease in the mother (Hui *et al.*, 2005), whereas hypoplastic development is associated with maternal hypertension (pre-eclampsia; Cross, 2003; Fisher, 2004) and fetal growth retardation (Chaddha *et al.*, 2004; Gluckman *et al.*, 2004; Monk *et al.*, 2004). Intrauterine growth retardation and low birthweight (LBW) are major negative health predictors for newborns and children, and are associated with chronic diseases expressed later in life, e.g. cardiovascular disease, hypertension and type 2 diabetes (Barker *et al.*, 1993; Curhan *et al.*, 1996a, b; Boyko, 2000; Eriksson *et al.*, 2003; Ross and Beall, 2008). A significant factor in placental development and function is epigenetic regulation. Imprinted genes for instance, which are epigenetically regulated, are abundantly expressed in the placenta and usually lacking in non-placental organisms (Reik and Walter, 2001).

In the present review, we discuss the role of epigenetics in placental development and outline the progress in both animal and human research in recent years. Our intention is to offer the clinician insight into the consequences of disturbed placental epigenetics and to focus on environmental effects which may cause these disturbances.

Methods

To generate this review, a thorough literature search was repeatedly made in PubMed and Medline, with a limitation for articles written in the English language. Search terms used were placenta, epigenetic, DNA methylation, histone modification, non-coding RNA, imprinting, environment. In addition, reference sections of all relevant studies or reviews were manually searched for more information.

Epigenetics

Although the cells in a human body contain the same DNA sequence, the function and phenotype differ (Reik, 2007). This implies that, apart from genetic programming, the phenotype is regulated by another phenomenon. This is called epi-(Greek for upon, above)-genetics. Epigenetics is defined as the study of changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. The usage of the word in scientific discourse is more narrow, referring to changes in gene function which are heritable (over rounds of cell division and sometimes transgenerationally; Russo *et al.*, 1996). During development, differentiated cells accumulate epigenetic modifications (or marks) that differ from those of pluripotent cells, and differentiated cells of different lineages also accumulate different marks (Reik, 2007). Certain epigenetic marks can be removed before a cell divides or within very few cell divisions (short-term flexibility), while other marks can be maintained for many divisions (long-term stability and heritability). Under the definition which strictly requires heritability, alterations that last less than one cell cycle do not qualify as epigenetic. Bird recently discussed the restrictiveness of this heritable view and redefined epigenetics as 'the structural adaption of chromosomal regions to register, signal or perpetuate altered activity states' (Bird, 2007). This definition focuses not only on genes but also on chromosomes without the constraint of heritability.

Epigenetic marks, deposited early in development, are able to adapt themselves throughout life in response to intrinsic and environmental stimuli. Accordingly, epigenetic disturbance may lead to different phenotypes and diseases like cancer. Epigenetic regulation controls transcription at three levels, which are also displayed in the placenta: DNA (DNA methylation), protein (histone modifications) and RNA (non-coding RNAs).

DNA methylation

DNA methylation is the best-characterized epigenetic modification. Enzymes called DNA methyltransferases (DNMTs) catalyse the addition of a methyl group to the cytosine ring to form methyl cytosine, using S-adenosylmethionine as a methyl donor (Herman and Baylin, 2003). After DNA replication, DNMT1 is the predominant mammalian DNA methylating enzyme responsible for the restoration of hemi-methylated sites to full methylation, called maintenance methylation. DNMT3A and DNMT3B are mainly involved in the methylation of new sites, called *de novo* methylation (Laird, 2003). DNMT3L is postulated to play a regulatory role in DNA methylation without DNA methyltransferase activity in itself (Okano *et al.*, 1998). In humans and other mammals, the post-replicative DNA modification occurs predominantly on cytosines that precede a guanosine in the DNA sequence (the CpG dinucleotide; Herman and Baylin, 2003). These dinucleotides can be clustered in small stretches of DNA termed CpG islands, which are often associated with promoter regions. Most CpG sites outside of CpG islands are methylated suggesting a role in the global maintenance of the genome, while most CpG islands in gene promoters are unmethylated which allows active gene transcription (Herman and Baylin, 2003; Weber and Schubeler, 2007). The importance of DNA methylation for gene expression, and especially for transcriptional silencing, is apparent in the exceptions to the rule that CpG islands associated with gene promoter regions are unmethylated (Bird, 2002). These exceptions are for instance the fully methylated CpG islands associated with the silenced alleles of some imprinted genes (see below) or on the inactive X chromosome.

DNA methylation in the placenta

In mammalian preimplantation development, DNA methylation of non-imprinted genes is subject to a global reprogramming process after fertilization, with phases of active and passive demethylation followed by *de novo* methylation (Mayer *et al.*, 2000; Horsthemke and Ludwig, 2005). This *de novo* methylation occurs by the blastocyst stage, but is restricted to the inner cell mass (ICM), whereas the trophectoderm (TE) is virtually lacking methylation (Santos *et al.*, 2002). An inequality is thus established between both cell lineages at the time of differentiation into embryonic and extraembryonic lineages (Santos *et al.*, 2002). This epigenetic inequality with higher overall DNA methylation

levels in the embryo compared with the placenta is maintained throughout gestation (Santos *et al.*, 2002). Earlier investigations have also shown that hypomethylation is maintained in all derivates of the extraembryonic lineage (Chapman *et al.*, 1984; Rossant *et al.*, 1986). At term, two regulating domains in the IGF2-H19 region are for instance hypomethylated in placenta, while in neonatal blood these regions are methylated on one of the two alleles (Guo *et al.*, 2008). Despite the global hypomethylation of the trophoblast lineage, DNA methylation is indispensable for normal development of extraembryonic tissues, especially for the invasive behaviour of trophoblast cells. Administration of a single dose of 5′-aza-2′-deoxycytidine (a DNA methylation inhibitor) to pregnant rats at different stages of development, disrupts trophoblast proliferation (Vlahovic *et al.*, 1999; Serman *et al.*, 2007) and in human choriocarcinoma-derived cell lines 5′-aza-2′-deoxycytidine disrupts trophoblast migration (Rahnama *et al.*, 2006). Furthermore, knockout mice studies of DNA methyltransferases *Dnmt1* and *Dnmt3L* have shown that the placentas of these homozygous mice exhibit multiple morphological defects, like chorioallantoic fusion defects and lack of labyrinth formation (Li *et al.*, 1992; Bourc'his *et al.*, 2001; Arima *et al.*, 2006). These effects may be mediated by a loss of imprinting (LOI) (see below) since mutant *Dnmt1* resulted in biallelic expression of certain genes in the regulating domains *Igf2*, *Snrpn* and *Peg3*, while genes in the *Kcnq1* domain were less sensitive to absence of *Dnmt1* (Weaver *et al.*, 2010).

Placentation displays many similarities with tumourigenesis. Trophoblasts proliferate, migrate and invade the uterine wall and its vasculature and escape from immune detection, like cancer cells. Normal human cytotrophoblasts express functional tumour-associated genes, several of which are also essential for the development of certain malignancies (Ferretti *et al.*, 2007; Koslowski *et al.*, 2007). Recent observations showed epigenetic regulation by DNA methylation and histone modification of some tumour suppressor genes like Maspin, RASSF1A and APC in human placentas (Dokras *et al.*, 2006; Chiu *et al.*, 2007; Wong *et al.*, 2008). In addition, Novakovic *et al.* (2008) investigated the extent of tumour-associated methylation in first-trimester cytotrophoblasts and term placenta, and demonstrated a methylation-induced reduction in expression in a small subset of genes as part of normal human placentation.

Besides a role of DNA methylation in placental morphology, it also affects placental physiology. For instance, the tightly controlled regulation of plasma concentrations of biologically active vitamin D is epigenetically uncoupled in pregnancy (Novakovic *et al.*, 2009). Biologically active vitamin D regulates calcium homeostasis, immunomodulation, cellular differentiation and apoptosis (DeLuca, 2004; Dusso *et al.*, 2005; Anderson *et al.*, 2006). In addition, vitamin D deficiency has been linked to placental insufficiency, e.g. pre-eclampsia (Bodnar *et al.*, 2007; Seely, 2007). Promoter methylation down-regulates the promoter activity of vitamin D hydroxylase (CYP24A1) and abolishes vitamin D-mediated feedback activation (Novakovic *et al.*, 2009). This leads to significantly elevated levels of active vitamin D at the fetomaternal interface, suggesting a role in pregnancy progression (Novakovic *et al.*, 2009).

Histone modification

The nucleosome is, together with the genomic DNA, an important constituent of chromatin. The nucleosome is a protein complex, consisting of two copies of each of the four core histones (H2A, H2B, H3 and H4), around which the DNA is wrapped. The N-terminal histone tail protrudes from the chromatin. Epigenetic modifications at the N-terminal tail of nucleosomal histones involve methylation, acetylation, phosphorylation and ubiquitinylation of selected amino acids, that can impose either transcriptionally repressive or transcriptionally permissive chromatin structures (Turner, 2002; Kimura *et al.*, 2004). These modifications are accomplished by a range of enzymes including histone methyltransferases (HMTs), acetyltransferases (HATs), kinases and ubiquitylases, while histone demethylases, deacetylases (HDACs), phosphatases and deubiquitylases are able to remove the mark from the histone tail. Proteins can recognize and bind to these specific modifications and exert an effect on gene activity. Polycomb Group (PcG) proteins for instance, which mutually form complexes, are known for their transcriptional repression (Niessen *et al.*, 2009). Furthermore, histone acetylation usually marks active genes as does di- or trimethylation of lysine residue four of histone H3 (H3K4me2, K4me3), whereas H3K9me2/3 and H3K27me3 constitute repressive marks (Peterson and Laniel, 2004; Sims and Reinberg, 2006). Repressive histone modifications seem to confer short-term, flexible silencing which is important for developmental plasticity, whereas DNA methylation is believed to be a more stable, long-term silencing mechanism (Boyer *et al.*, 2006; Lee *et al.*, 2006; Reik, 2007).

Histone modifications in the placenta

Like DNA methylation, histone methylation also plays a role in cell differentiation at the blastocyst stage. H3 arginine methylation predisposes blastomeres to contribute to the pluripotent cells of the ICM, which appears to require higher global levels of H3 arginine methylation than the TE/trophoblast lineage (Torres-Padilla *et al.*, 2007). This epigenetic disparity, with higher histone modification levels in the embryonic lineage compared with the trophoblast lineage, is seen more globally. Besides in many mouse studies, this has also been described in other mammalian species: bovine, sheep and rabbit (Manes and Menzel, 1981; Santos *et al.*, 2003; Beaujean *et al.*, 2004). Nevertheless, as with DNA methylation, again these lower modification levels in the trophoblast lineage are indispensable for normal extraembryonic development. H3K27 methylation for instance, is mediated by several protein aggregates, like PcG repressive complex (PRC) 2, containing PcG members and HDAC (Hemberger, 2007). Mutants of PcG members are characterized by failure of amnion and chorion formation (O'Carroll *et al.*, 2001; Pasini *et al.*, 2004) or failure of invasive trophoblast giant cell differentiation (Wang *et al.*, 2001, 2002). The above described lineage-specific DNA methylation and histone modification levels can influence cell fate determination (Reik *et al.*, 2003b; Torres-Padilla, 2008) and emphasize the difference in regulatory mechanisms between embryonic and placental tissues.

Selective activation of placental-specific genes requires specific roles for histone modifying enzymes. The expression of the human transcription factor GCMa for instance, necessary for regulating syncytin which mediates proper trophoblastic fusion, depends on regulation by HDAC and histone acetylytransferases (HAT) (Chuang *et al.*, 2006). Another example of histone modification-mediated placental-specific gene regulation concerns five genes of the human growth

hormone (*hGH*) cluster. These are highly conserved in structure, yet are expressed selectively in the placenta or pituitary. Placental growth hormone gradually replaces pituitary growth hormone and these placental secretions appear to have important implications for physiological adjustment to gestation and especially in the control of maternal IGF1 levels (Alsat *et al.*, 1998). The transcriptional activation of the placental-specific and pituitary-specific hGH genes is differentially regulated with different roles for HAT and HMT co-activator complexes in each of these tissues (Kimura *et al.*, 2004).

Non-coding RNAs

In the past years, it has been discovered that the majority of the mammalian genome is transcribed and that these transcripts mainly consist of non-coding (nc) RNAs (Okazaki *et al.*, 2002; Carninci *et al.*, 2005; Katayama *et al.*, 2005; Engstrom *et al.*, 2006). These ncRNAs can be classified according to their function or length. When ncRNAs act in *cis*, they are able to regulate the expression of one or more genes on the same chromosome. On the other hand when ncRNAs act in *trans*, they are able to regulate the expression of one or more genes on different chromosomes or regulate mature RNAs in the cytoplasm (Koerner *et al.*, 2009). *Cis*-acting functions have been associated with macro ncRNAs and *trans*-acting functions with short ncRNAs. Macro ncRNAs can be a few hundred to several hundred thousand nucleotides (nt) long. Examples of short ncRNAs are short interfering (si) RNAs (21 nt), micro (mi) RNAs (~22 nt), piwi-interacting RNAs (26–31 nt) and short nucleolar (sno) RNAs (60–300 nt) (Koerner *et al.*, 2009). Well-studied mammalian macro ncRNAs are *Xist* (inactive X-specific transcript) and *Tsix* (X-specific transcript) which are involved in X chromosome inactivation in female mammals.

ncRNAs in the placenta

In the placenta, repression of multiple genes in the *Igf2r* and *Kcnq1* clusters on the paternal chromosome depends on the macro ncRNAs *Airn* and *Kcnq1ot1* by an unknown mechanism (Sleutels *et al.*, 2002; Mancini-Dinardo *et al.*, 2006; Shin *et al.*, 2008). There are transcription-based silencing models that do not require the ncRNA product itself, but only its transcription which interferes for instance with *cis*-regulatory activator elements or the activation of *cis*-regulatory silencing elements. On the other hand in ncRNA-based silencing models, ncRNAs coat the region containing silenced genes and recruit silencing factors (Pauler *et al.*, 2007). Recently, two studies found evidence for the ncRNA-based silencing model concerning the macro ncRNAs *Airn* and *Kcnq1ot1*. They indicated that *Airn* and *Kcnq1ot1* are themselves directly involved in the repression of placental genes (Pandey *et al.*, 2008; Terranova *et al.*, 2008). For the involvement of ncRNAs in placental gene clusters with parent-of-origin-expression, see section on genomic imprinting.

Additionally, many epigenetic modifiers interact with one another. For example, the acquisition of histone modifications and DNA methylation is interdependent. They interplay with each other and also with regulatory proteins and non-coding RNAs (Lehnertz *et al.*, 2003; Esteve *et al.*, 2006; Vire *et al.*, 2006; Januchowski *et al.*, 2007; Delcuve *et al.*, 2009). In the placenta, for instance the ncRNA *Kcnq1ot1* localizes to chromatin and recruits repressive histone marks to the whole imprinted cluster (Pandey *et al.*, 2008). Furthermore, imprinted macro ncRNAs can serve as host transcripts for *trans*-acting short ncRNAs, which suggests a functional link between the two (Koerner *et al.*, 2009).

Genomic imprinting

Following experiments with maternal and paternal pronuclei, it was discovered that both parental genomes are required in the embryo for viable development (Barton *et al.*, 1984; McGrath and Solter, 1984; Surani *et al.*, 1984). Imprinted genes are defined by the functional non-equivalence of the maternal and paternal copy resulting in monoallelic expression in a parent-of-origin-dependent manner. During the process of imprinting, the male and female germ line confer a sex-specific mark (imprint) on certain chromosomal regions (Reik and Walter, 2001). Only one allele of the imprinted genes, the maternal or paternal, can be active and expressed. Note that the imprints themselves can be associated with activity or inactivity (Ferguson-Smith *et al.*, 2006). This active or inactive allele is epigenetically marked by the earlier described histone modifications, DNA methylation or both (Reik *et al.*, 2001). Imprinted genes are not randomly distributed in the genome, but tend to occur predominantly in clusters. Each cluster is controlled by an imprinting control region (ICR) which usually contains a stably maintained or developmentally changing Differentially Methylated Region (DMR; Wood and Oakey, 2006; Edwards and Ferguson-Smith, 2007). The existence of these ICRs suggests that the primary control of imprinting is not at a single gene level, but at chromosomal level (Buiting *et al.*, 1995). Furthermore, imprinting (parent-of-origin-specific gene transcription) is tissue-specific, species-dependent and developmentally regulated (Morison Reeve, 1998; Fowden *et al.*, 2006; Monk *et al.*, 2006). Besides assuming imprinting as a simple pattern resulting in either paternal or maternal expression, more complex and diverse patterns of effect have been found (Wolf *et al.*, 2008). The quantitative effect of an imprinted allele not only depends on its parent-of-origin, but also on the allele it is paired with at a locus (Wolf *et al.*, 2008).

In addition to allele-specific methylation and histone modifications, imprinting is regulated by non-coding RNAs (Spahn and Barlow, 2003). The macro ncRNAs *Airn*, *Kcnq1ot1* and *Nespas* are involved in genomic imprinting control (Koerner *et al.*, 2009). Each imprinted gene cluster, which contains an ICR, expresses at least one macro ncRNA gene (Edwards and Ferguson-Smith, 2007; Royo and Cavaille, 2008). An unmethylated ICR is required for imprinted macro ncRNA expression and the macro ncRNA is generally expressed from the other chromosome than most imprinted mRNA genes (Koerner *et al.*, 2009). For instance, the imprinted Igf2r cluster contains three maternally expressed genes [*Igf2r*, *Slc22a2* (solute carrier family 22,a2) and *Slc22a3*]. The paternal unmethylated ICR expresses a macro ncRNA named *Airn*, which silences paternal *Igf2r*, *Slc22a2* and *Slc22a3* expression (Royo and Cavaille, 2008). The mechanisms by which these ncRNAs are responsible for the induced repression in imprinted loci are still not well characterized.

After fertilization, a genome-wide DNA demethylation and remethylation takes place in the embryo (Mayer *et al.*, 2000; Horsthemke and Ludwig, 2005). However, imprinted genes escape this epigenetic reprogramming (Mayer *et al.*, 2000). They are protected from demethylation because it is important that the parental imprints are preserved in the developing embryo (Tremblay *et al.*, 1995). Later in fetal development, when the germ cells initially enter

the gonad, genomic imprints are erased. They are newly established during later stages of gametogenesis (paternal imprints in spermatozoa and maternal imprints in oocytes) and maintained during post-zygotic development (Lucifero *et al.*, 2004a; Swales and Spears, 2005). Both Dnmt3a and Dnmt3L are at least required for methylation of most imprinted loci in germ cells and are therefore implicated in maternal and paternal imprinting (Hata *et al.*, 2002; Kaneda *et al.*, 2004). In contrast, Miri and Varmuza recently proposed the 'Mother Knows Best' model of genomic imprinting. Evidence is accumulating from both wild-type and manipulated mammalian embryos indicating that genomic imprinting is a maternal effect regulated by oocyte proteins, and acting in part during the long first cell cycle that precedes cleavage divisions (Miri and Varmuza, 2009). In this proposed model, the oocyte contains remodelling components which are necessary for paternal genome epigenetic establishment just after fertilization (Miri and Varmuza, 2009). In the placenta, maintenance of imprinting has been recently inferred to depend more on repressive histone methylation and ncRNAs than on DNA methylation (Wagschal and Feil, 2006; Wagschal *et al.*, 2008). In fact, placenta-specific imprinting, in contrast to the embryo, along mouse distal chromosome 7 is largely independent of DNA methylation in its maintenance (Tanaka *et al.*, 1999; Lewis *et al.*, 2004; Umlauf *et al.*, 2004).

Genomic imprinting arose during mammalian evolution (around 150 million years ago) and might be associated with the evolution of intrauterine development that requires formation of a placenta (Reik and Walter, 2001). Therefore, many researchers have speculated that some aspect of placentation benefits from genomic imprinting (Coan *et al.*, 2005; Wagschal and Feil, 2006; Charalambous *et al.*, 2007; Renfree *et al.*, 2008). There are several theories about the purpose of genomic imprinting. One hypothesis is that genomic imprinting is a by-product of a self-defence mechanism against exogenous DNAs or retrotransposons without a meaning in itself (Barlow, 1993; Yoder *et al.*, 1997). Accumulating evidence describes the central role of DNA methylation in repression of retroviruses and retrotransposons, as well as genomic imprinting (Yoder *et al.*, 1997; Okano *et al.*, 1999; Bourc'his *et al.*, 2001; Hata *et al.*, 2002; Bourc'his and Bestor, 2004; Kaneda *et al.*, 2004). Also, the mammalian genome contains many retrotransposon-derived sequences compared with the genome of other higher vertebrates. It has been suggested that this phenomenon has evolved over time but this does not explain why not all imprinted genes are methylated.

Another hypothesis is based on parthenogenetic placentation, or the ovarian time bomb hypothesis (Varmuza and Mann, 1994; Weisstein *et al.*, 2002). Parthenotes (two maternal genomes) have well-developed small embryos which develop until early post-implantation stages, but they have almost non-existent trophoblast. Therefore, it is hypothesized that female mammals are protected from malignant ovarian trophoblastic disease. Viable offspring can be generated from mice oocytes where one of the two maternal haploid genomes has a deletion in the paternally imprinted H19 ICR (Kono *et al.*, 2004). This illustrates that paternal imprinting prevents parthenogenetic development in mice. Therefore, genomic imprinting is a barrier to parthenogenesis, which can protect female mammals from malignant trophoblastic disease. They also demonstrated that appropriate expression of *Igf2* and *H19* genes is extremely important for normal development (Kono *et al.*, 2004). This theory does not explain why genes which are not involved in placental development are still imprinted.

Furthermore, the 'complementation hypothesis' has been proposed. It explains the origin of imprinting arguing that it is essential for mammalian development as a mechanism regulating complementary expression profiles of paternal and maternal genomes because both cannot be expressed from the same chromosomes simultaneously, even when the parental imprints are completely erased (Lee *et al.*, 2002; Kaneko-Ishino *et al.*, 2003). This hypothesis uses the mechanism of complementation under the selective pressure predicted by the genetic conflict hypothesis.

Most popular is the conflict or 'battle of the sexes' theory (Moore and Haig, 1991; Haig, 2004). Following this theory, the father wants maximal growth and good health for his offspring to ensure successful survival against competition allowing the paternal genome to be passed on to successive generations. The mother, however, despite wanting the same outcome, also wants to be able to reproduce again and limit the drain on her resources to maintain her own health. This competition within the embryo and/or placenta between paternal genes attempting to enhance growth and maternal genes trying to limit growth is reflected in either intrauterine growth restriction (IUGR) or overgrowth in imprinting disorder diseases. The conflict hypothesis has been challenged by the lack of phenotype observed in knockout mice of the maternally imprinted *Snrpn* gene (Yang *et al.*, 1998) and the growth inhibitory rather than enhancer effect of the paternally expressed *ZAC* gene (Piras *et al.*, 2000).

Imprinting controlling placental and fetal development

The importance of imprinted genes for the placenta is underlined by the many imprinted genes that are expressed in the placenta (Reik and Walter, 2001; Ferguson-Smith *et al.*, 2006; Tycko, 2006; Hemberger, 2007), the distinct imprinted gene expression profile displayed in the placenta and embryo when compared with adult tissues (Steinhoff *et al.*, 2009) and the fact that non-placental organisms like fish, reptiles and chickens lack imprinting (Yokomine *et al.*, 2001; Reik *et al.*, 2003a; Yokomine *et al.*, 2005).

Why is the placenta such an important site of imprinted gene action? Knockout (loss of expression) and transgenic (over-expression) data of placental imprinted genes have revealed a range of (aberrant) phenotypic patterns. Imprinted genes, such as *Ascl2*, *Phlda2* and *Peg10* (a newly discovered retrotransposon-derived gene) appear to be indispensable for proper placental morphology and function, while others, like *Igf2*, are involved in nutrient supply regulation (Guillemot *et al.*, 1994; Salas *et al.*, 2004; Angiolini *et al.*, 2006; Ono *et al.*, 2006). The latter was found to be a major modulator of placental and fetal growth, as it regulates the development of the diffusional permeability capacity in the placenta (Constancia *et al.*, 2002; Sibley *et al.*, 2004). Recently, it has been demonstrated in the mouse that placental phenotype, like morphology and diffusional exchange characteristics, depends on the degree of *Igf2* gene ablation and the interplay between placental and fetal *Igf2* (Coan *et al.*, 2008). As described before, a number of genes are imprinted specifically in the placenta, but genes imprinted in both the embryo and the placenta can also be essential for placental development (Wagschal and Feil, 2006).

To date, more than 130 imprinted genes in mice and at least 70 in humans have been discovered (http://www.geneimprint.com, www.

mgu.har.mrc.ac.uk, http://igc.otago.ac.nz), of which several are imprinted specifically in the placenta. There is an extensive conservation between mouse and human genes that are imprinted in both the embryo and extraembryonic tissues. Conserved maternal expression was found for the imprinted genes *PHLDA2*, *SLC22A18*, *SLC22A1LS* and *CDKN1C* (Monk *et al.*, 2006). Genes from the *IGF2R* domain showed human placental imprinting in only a minority of the samples (Monk *et al.*, 2006). However, a lack of imprinting was demonstrated in the human *KCNQ1* domain and the human orthologues of the mouse placental-specific imprinted genes *Gatm* and *Dcn*. Although maternal expression was observed in the mouse, human expression appeared largely bi-allelic from first-trimester trophoblast till term (Monk *et al.*, 2006).

Although imprinting in human placentas has been increasingly studied, the parental origin of the allele-specific expression of several human placental imprinted genes is still unknown (Table I). Besides, placental imprinting of these genes is a dynamic process which evolves during human pregnancy (Pozharny *et al.*, 2010). Especially in first-trimester placentas, several genes, in contrast to IGF2, displayed a significantly higher rate of LOI than term placentas, although little difference in gene expression was seen (Pozharny *et al.*, 2010). Among these genes is H19 with, before 10 weeks of pregnancy, bi-allelic expression in 28% of the placental tissues. After 10 weeks, only mono-allelic expression was measured (Yu *et al.*, 2009). These investigations demonstrate that the parent-of-origin effect is not established completely in the first trimester of human pregnancy. Instead, placental imprinting is a dynamic, maturational process. It is postulated that these developmental changes in LOI, without necessarily affecting the gene expression, play an important role in placental maturation and development (Pozharny *et al.*, 2010). Also in term placentas, the mono-allelic expression of imprinted genes is not always very strict. Lambertini *et al.* (2008) for instance analysed the expression of nine imprinted genes and found that in apparently normal term placentas several genes showed bi-allelic expression in some but not all placentas (Lambertini *et al.*, 2008). For *ZAC/PLAGL1*, the expression of the maternally imprinted allele accounted for only 1–3% of the total expression of this gene. On the other hand, the maternally imprinted *IGF2* allele could account for 20–50% of the expression and the maternally imprinted *PEG3* sometimes for even more than 50%. Interestingly, *H19* and *DLK1* never showed LOI (Lambertini *et al.*, 2008). This has also been reported in mice. In a few placentas about 20% LOI was reported for *Snrpn* while, just as in human, *H19* escaped LOI (Fortier *et al.*, 2008). There are also imprinted genes that display mono-allelic expression just in first trimester placental tissues, like CTNNA3 (Oudejans *et al.*, 2004; van Dijk *et al.*, 2004). The parent-of-origin effect of this gene is also trophoblast cell type-dependent: bi-allelic expression in extravillous trophoblast, maternal expression in villous cytotrophoblast and expression is lost following epithelial–mesenchymal transition (van Dijk *et al.*, 2004). This trophoblast cell type-dependent imprinting of *CTNNA3* is identical to *CDKN1C* imprinting with respect to trophoblast cell type (villous) and parental origin of the expressed allele (maternal). Therefore, gene dosage compensation of both *CTNNA3* and *CDKN1C* in the placenta is suggested to share a conserved regulatory mechanism correlating with an early step in trophoblast determination, i.e. differentiation into villous or extravillous trophoblast (van Dijk *et al.*, 2004).

Paternally expressed genes tend to enhance fetal growth while maternally expressed genes tend to suppress it (Fowden *et al.*, 2006). Knockout studies of several paternally or maternally imprinted genes result in IUGR and smaller placental size or overgrowth and hyperplasia of placenta, respectively (Lefebvre *et al.*, 1998; Takahashi *et al.*, 2000; Frank *et al.*, 2002). Furthermore, certain maternal genes are required for proper development of the embryo, whereas extra-embryonic tissues depend on the presence of active paternal genes. An excess of paternally derived chromosomes leads to a complete (no maternal genome) or partial (lower number of maternal chromosomes) mole (Devriendt, 2005). Accordingly, hydatidiform moles are characterized by a reduced or even lack of embryonic development and excessive trophoblastic proliferation.

Disturbed placental epigenetics

Several researchers examined the role of epigenetic disturbance in human placental-related pathologies like small for gestational age (SGA), IUGR, pre-eclampsia and gestational trophoblastic disease (GTD) (Table II).

Many cases of IUGR, in which a fetus is not able to achieve its genetically determined potential size, are idiopathic and utero-placental insufficiency is regarded as an important factor in these cases. SGA infants are smaller than the 10th percentile for the gestational age of pregnancy. It is clinically important to distinguish fetal constitutional smallness from fetal growth failure to ensure appropriate medical care, prevent unnecessary elective Caesarean sections and premature births. An IUGR infant may or may not be SGA but it always implies a pathological process like an underlying utero-placental insufficiency (Bamberg and Kalache, 2004; Maulik, 2006). Therefore, further research is conducted to discover possible biomarkers for IUGR and to elucidate the aetiology of IUGR.

McMinn *et al.* (2006) analysed whether altered expression of imprinted genes is implicated in non-syndromic human IUGR. They conducted a genome-wide survey of mRNA expression in late-gestation placental samples of 14 IUGR placentas compared with 15 non-IUGR placentas. Several imprinted genes were differentially expressed. They found for instance increased expression of the paternally imprinted *PHLDA2* and decreased expression of the maternally imprinted *MEST* and *PLAGL1* and paternally imprinted *MEG3*, *GATM* and *GNAS* in IUGR placentas (McMinn *et al.*, 2006). These results support the hypothesis that placental-specific imprinted genes are deregulated in IUGR.

A recent study systematically investigated the expression levels of 74 'putatively' imprinted genes in human late-gestation placental samples from 10 normal and 7 IUGR pregnancies by using quantitative RT–PCR (qRT–PCR) (Diplas *et al.*, 2009). They found that 52 of the 74 genes were expressed in human placental tissue of which nine genes were significantly differentially expressed between both groups. Five genes were up-regulated (*PHLDA2*, *ILK2*, *NNAT*, *CCDC86*, *PEG10*) and four were down-regulated (*PLAGL1*, *DHCR24*, *ZNF331*, *CDKAL1*) in IUGR placentas, without a consistent 'parent-of-origin' pattern. They investigated LOI and found that five of these deregulated genes were not imprinted in placenta. Assessment of 14 placental imprinted genes revealed no correlation between expression and LOI, in both normal and IUGR placentas (Diplas *et al.*, 2009). Therefore, mechanisms other than LOI may be associated with the deregulated expression in IUGR placentas. Gene expression levels may vary according to the site of sampling. Even in

Table I Human placental imprinted genes.

Gene	Name	Location	References	Placental tissue	Mono-allelic expression	Parental expression in placenta			Parental expression (not placental-specific)[a]
						Paternal	Maternal	Not analysed	
DLK1	Delta-like 1 homolog	14q32	Pozharny *et al.* (2010)	First trimester	Minor subset	x			P
			Lambertini *et al.* (2008), Pozharny *et al.* (2010)	Term	Yes	x			P
IGF2	Insulin-like growth factor 2 (somatomedin A)	11p15.5	Pozharny *et al.* (2010)	First trimester	Subset	x			P
			Apostolidou *et al.* (2007), Lambertini *et al.* (2008)	Term	Subset	x			P
MEST/PEG1 isoform 1	Mesoderm-specific transcript homolog	7q32	Pozharny *et al.* (2010)	First trimester	Subset	x			P
			Apostolidou *et al.* (2007), Lambertini *et al.* (2008)	Term	Subset	x			P
MEST/PEG1 isoform 2	Mesoderm-specific transcript homolog	7q32	McMinn *et al.* (2006)	Third trimester/ term	Large subset	x			P
PEG3	Paternally expressed 3	19q13.4	Pozharny *et al.* (2010)	First trimester	Subset	x			P
			Lambertini *et al.* (2008), Pozharny *et al.* (2010)	Term	Subset	x			P
PEG10	Paternally expressed 10	7q21	Pozharny *et al.* (2010)	First trimester	Subset	x			P
			Lambertini *et al.* (2008)	Term	Subset	x			P
SNRPN	Small nuclear ribonucleoprotein polypeptide N	15q11.2	Pozharny *et al.* (2010)	First trimester	Minor subset	x			P
			Diplas *et al.* (2009), Pozharny *et al.* (2010)	Term	yes	x			P
PLAGL1/ZAC	Pleiomorphic adenoma gene-like 1	6q24–q25	Pozharny *et al.* (2010)	First trimester	Minor subset	x			P
			Lambertini *et al.* (2008), Pozharny *et al.* (2010)	Term	Yes	x			P
CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57)	11p15.5	Monk *et al.* (2006)	Term	Yes		x		M
H19	H19, imprinted maternally expressed transcript (non-protein coding)	11p15.5	Pozharny *et al.* (2010), Yu *et al.* (2009)	First trimester	Subset		x		M
			Lambertini *et al.* (2008), Yu *et al.* (2009)	Term	Yes		x		M

Continued

Table I *Continued*

Gene	Name	Location	References	Placental tissue	Mono-allelic expression	Parental expression in placenta			Parental expression (not placental-specific)[a]
						Paternal	Maternal	Not analysed	
MEG3	Maternally expressed 3 (non-protein coding)	14q32	Pozharny *et al.* (2010)	First trimester	Minor subset		x		M
			Lambertini *et al.* (2008), Pozharny *et al.* (2010)	Term	yes		x		M
PHLDA2	Pleckstrin homology-like domain, family A, member 2	11p15.5	Pozharny *et al.* (2010)	First trimester	Minor subset		x		M
			Apostolidou *et al.* (2007), Monk *et al.* (2006), Diplas *et al.* (2009)	Term	yes		x		M
SLC22A18	Solute carrier family 22, member 18	11p15.5	Pozharny *et al.* (2010)	First trimester	Subset		x		M
			Monk *et al.* (2006), Diplas *et al.* (2009), Pozharny *et al.* (2010)	Term	Subset		x		M
SLC22A18AS/ SLC22A1LS	Solute carrier family 22 (organic cation transporter), member 18 antisense	11p15.5	Monk *et al.* (2006)	Term	Yes		x		M
TP73	Tumour protein p73	1p36.3	Pozharny *et al.* (2010)	First trimester	Subset		x		M
			Lambertini *et al.* (2008), Pozharny *et al.* (2010)	Term	Large subset		x		M
CD44	CD44 molecule (Indian blood group)	11p13	Pozharny *et al.* (2010)	First trimester	Minor subset		Postulated		Postulated: M
			Diplas *et al.* (2009), Pozharny *et al.* (2010)	Term	Yes		Postulated		Postulated: M
CTNNA3	Catenin (cadherin-associated protein), alpha 3	10q22.2	Oudejans *et al.* (2004), Van Dijk *et al.* (2004)	First trimester	Subset		x		Provisional data: M
			Diplas *et al.* (2009)	Term	Yes			x	Provisional data: M
CTNND2	Catenin, delta 2 (neural plakophilin-related arm-repeat protein)	5p15.2	Diplas *et al.* (2009)	Term	Yes			x	No record
CYR61	Cysteine-rich, angiogenic inducer, 61	1p31–p22	Diplas *et al.* (2009)	Term	Yes			x	No record
DLX5	Distal-less homeobox 5	7q22	Diplas *et al.* (2009)	Term	Yes			x	M
EPS15	Epidermal growth factor receptor pathway substrate 15	1p32	Pozharny *et al.* (2010)	First trimester	Minor subset		Postulated		No record
			Diplas *et al.* (2009), Pozharny *et al.* (2010)	Term	Yes		Postulated		No record

GDNF	Glial cell-derived neurotrophic factor	5p13.1–p12	Diplas *et al.* (2009)	Term	Yes		x	No record
HYMAI	Hydatidiform mole associated and imprinted (non-protein coding)	6q24.2	Diplas *et al.* (2009)	Term	Yes		x	P
IL1B	Interleukin 1, beta	2q14	Diplas *et al.* (2009)	Term	Yes		x	No record
INS	Insulin	11p15.5	Diplas *et al.* 2009	Term	Yes		x	P
KCNQ1OT1/LIT1	KCNQ1 overlapping transcript 1 (non-protein coding)	11p15	Monk *et al.* (2006)	First trimester	Minor subset	x		P
			Monk *et al.* (2006)	Term	No			P
			Guo *et al.* (2008)	Third trimester/term	Yes	x		P
MAGEL2	MAGE-like 2	15q11-q12	Diplas *et al.* (2009)	Term	Yes		x	P
MAGI2	Membrane-associated guanylate kinase, WW and PDZ domain containing 2	7pq21	Diplas *et al.* (2009)	Term	Yes		x	Predicted: M
MAPK12	Mitogen-activated protein kinase 12	22q13.33	Diplas *et al.* (2009)	Term	Yes		x	No record
MESTIT1	MEST intronic transcript 1 (non-protein coding)	7q32	Diplas *et al.* (2009)	Term	Yes		x	P
MKRN3	Makorin ring finger protein 3	15q11–q13	Diplas *et al.* (2009)	Term	Yes		x	P
NDN	Necdin homolog	15q11.2–q12	Diplas *et al.* (2009)	Term	Yes		x	P
NGF	Nerve growth factor (beta polypeptide)	1p13.1	Diplas *et al.* (2009)	Term	Yes		x	No record
NNAT	Neuronatin	20q11.2–q12	Diplas *et al.* (2009)	Term	Yes		x	P
PCNA	Proliferating cell nuclear antigen	20pter-p12	Diplas *et al.* (2009)	Term	Yes		x	No record
SDHD	Succinate dehydrogenase complex, subunit D, integral membrane protein	11q23	Diplas *et al.* (2009)	Term	Yes		x	Conflicting data
SLC22A3	Solute carrier family 22 (extraneuronal monoamine transporter), member 3	6q26–q27	Monk *et al.* (2006)	First trimester	Yes		x	M
SGCE	Sarcoglycan, epsilon	7q21–q22	Diplas *et al.* (2009)	Term	Yes		x	P
SNORD64	Small nucleolar RNA, C/D box 64	15q12	Diplas *et al.* (2009)	Term	Yes		x	P
SNORD108	Small nucleolar RNA, C/D box 108	15q11.2	Diplas *et al.* (2009)	Term	Yes		x	P
SNURF	SNRPN upstream reading frame	15q12	Diplas *et al.* (2009)	Term	Yes		x	P
UBE3A	ubiquitin protein ligase E3A	15q11-q13	Diplas *et al.* (2009)	Term	Yes		x	M

Continued

Table I *Continued*

Gene	Name	Location	References	Placental tissue	Mono-allelic expression	Parental expression in placenta			Parental expression (not placental-specific)[a]
						Paternal	Maternal	Not analysed	
WT1	Wilms tumor 1	11p13	Diplas *et al.* (2009)	Term	Yes			x	P
			Jinno *et al.* (1994)	Term	Subset		x		P
Putatively placental imprinted genes									
ATP10A	ATPase, class V, type 10A	15q11.2	Steinhoff *et al.* (2009)	Term	Na			x	M
CD81	CD81 molecule	11p15.5	McMinn *et al.* (2006)	Second/third trimester	Na			x	Not imprinted
DCN	Decorin	12q21.33	McMinn *et al.* (2006)	Second/third trimester	Na			x	Unknown
DIO3	Deiodinase, iodothyronine, type III	14q32	McMinn *et al.* (2006)	Second/third trimester	Na			x	Unknown
GATM	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	15q21.1	McMinn *et al.* (2006)	Second/third trimester	Na			x	Unknown
GNAS	GNAS complex locus	20q13.3	Steinhoff *et al.* (2009), McMinn *et al.* (2006)	Term	Na			x	Isoform-dependent
IGF2R	Insulin-like growth factor 2 receptor	6q26	McMinn *et al.* (2006)	Second/third trimester	Na			x	Not imprinted
			Monk *et al.* (2006)	Term	minor subset		x		Not imprinted
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1	11p15.5	Steinhoff *et al.* (2009)	Term	Na			x	M
MKRN1	Makorin ring finger protein 1	7q34	McMinn *et al.* (2006)	Second/third trimester	Na			x	No record
PPP1CC	Protein phosphatase 1, catalytic subunit, gamma isozyme	12q24.1–q24.2	McMinn *et al.* (2006)	Second/third trimester	Na			x	No record
ZIM2	Zinc finger, imprinted 2	19q13.4	Steinhoff *et al.* (2009), McMinn *et al.* (2006)	Term	Na			x	P

[a]Searched in imprinted gene catalogues (www.geneimprint.com, http://igc.otago.ac.nz). Na, not analysed; P, paternal; M, maternal.

Table II Placental epigenetics in relation to placental-related pathologies.[a]

Pathology	Gene	Expressed allele	Methylation (investigated region)	Gene expression (fold change)	Mono-allelic expression (affected number)	Reference[b,c]
IUGR	CDKN1C	M	Na	↑ (3.3)	Na	McMinn *et al.* (2006)
			Na	=	Na	Diplas *et al.* (2009)
	DLK1	P	Na	=	LOI (2/5)	Diplas *et al.* (2009)
	GATM	M	Na	↓ (0.59)	Na	McMinn *et al.* (2006)
			Na	na	Na	Diplas *et al.* (2009)
	GNAS	M	Na	↓ (?)	Na	McMinn *et al.* (2006)
			Na	Na	Na	Diplas *et al.* (2009)
	H19	M	Na	=	LOI (1/5)	Diplas *et al.* (2009)
	IGF2	P	Na	↓ (0.39)	Na	McMinn *et al.* (2006)
			Na	=	=[d]	Diplas *et al.* (2009)
	MEG3	M	Na	↓ (0.52)	Na	McMinn *et al.* (2006)
			Na	=	=	Diplas *et al.* (2009)
	MEST	P	=	↓ (0.72)	Na	McMinn *et al.* (2006)
			Na	=	=[d]	Diplas *et al.* (2009)
	NNAT	P	Na	=	Na	McMinn *et al.* (2006)
			Na	↑	Na	Diplas *et al.* (2009)
	PEG10	P	Na	=	Na	McMinn *et al.* (2006)
			Na	↑	=	Diplas *et al.* (2009)
	PHLDA2	M	= (KvDMR)	↑ (1.27)	Na	McMinn *et al.* (2006)
			Na	↑	=	Diplas *et al.* (2009)
	PLAGL1	P	Na	↓ (0.62)	Na	McMinn *et al.* (2006)
			Na	↓	LOI (2/13)	Diplas *et al.* (2009)
	SERPINA3	M/P	=	↑		Chelbi *et al.* (2007)
	SERPINB2	M/P	Na	↓		Chelbi *et al.* (2007)
	SERPINB7	M/P	Na	↑		Chelbi *et al.* (2007)
	SNRPN	P	Na	=	LOI (2/8)	Diplas *et al.* (2009)
SGA	CDKN1C	M	= (KvDMR)	Na	=	Guo *et al.* (2008)
	H19	M	LOM (H19DMR, (1/24))	=	LOI (1/24)	Guo *et al.* (2008)
	IGF2	P	LOM (H19DMR, (1/24))	↓ (0.57)	=	Guo *et al.* (2008)
	KCNQ1OT1	P	= (KvDMR)	Na	=	Guo *et al.* (2008)
	PHLDA2	M	= (KvDMR)	Na	=	Guo *et al.* (2008)
Lower birthweight	IGF2	M	Na	=	=	Apostolidou *et al.* (2007)
	MEST	P	Na	=	=[e]	Apostolidou *et al.* (2007)
	PHLDA2	M	Na	↑	=	Apostolidou *et al.* (2007)
Pre-eclampsia	H19	M	Na	=	LOI (6/13)	Yu *et al.* (2009)
	SERPINA1	M/P	Na	↓	Nr	Chelbi *et al.* (2007)

Continued

Table II *Continued*

Pathology	Gene	Expressed allele	Methylation (investigated region)	Gene expression (fold change)	Mono-allelic expression (affected number)	Reference[b,c]
	SERPINA3	M/P	LOM	=	Nr	Chelbi *et al.* (2007)
	SERPINB2	M/P	Na	↓	Nr	Chelbi *et al.* (2007)
	miR-210	M/P	Na	↑ (3.0)	Na	Pineles *et al.* (2007)
			Na	↑ (3.64)	Na	Zhu *et al.* (2009)
	miR-182	M/P	Na	↑ (2.1)	Na	Pineles *et al.* (2007)
GTD	OCT4	M/P	GOM	↓	Nr	Zhang *et al.* (2008)
	p16	M/P	GOM	↓	Nr	Xue *et al.* (2004)
	HIC-1	M/P	GOM	↓	Nr	Xue *et al.* (2004)
	TIMP3	M/P	GOM	Na	Nr	Xue *et al.* (2004)
	GSTP1	M/P	=	Na	Nr	Xue *et al.* (2004)
	DSAPK	M/P	=	Na	Nr	Xue *et al.* (2004)
	E-cadherin	M/P	GOM	↓	Nr	Xue *et al.* (2004)

[a]The effects mentioned in the table are those from the affected group compared with a control group. In almost all studies third trimester/term placentas are investigated. Only Xue *et al.* (2004) and Zhang *et al.* (2008) investigated, respectively, first trimester and first trimester and term placentas.
[b]McMinn *et al.* (2006) describe a microarray studies and Diplas *et al.* (2009) analysed 74 imprinted genes. For both studies only the imprinted genes that were differentially expressed in one of the two studies are described.
[c]Zhu *et al.* describe a microarray analysis of miRNAs. Thirty-four miRNAs are deregulated, but only the miRNA that was found to be affected in the study of Pineles *et al.* (2007) is reported.
[d]LOI was reported in some IUGR placentas, but this was comparable with the LOI reported in the non-IUGR placentas
[e]LOI was reported in 8 of 42 placentas, but this was not correlated to birthweight.
GOM, gain of methylation; GTD, gestational trophoblastic disease; IUGR, intrauterine growth reduction; LOI, loss of imprinting; LOM, loss of methylation; Na, not analysed; Nr, not relevant; SGA, small for gestational age; '=', not affected as compared with control.

uncomplicated pregnancies, intra-placenta variability has been described (Pidoux *et al.*, 2004; Wyatt *et al.*, 2005). This potential bias was also studied by Diplas *et al.* (2009) revealing no significant difference with respect to sampling variations.

Guo *et al.* investigated placentas of 20 control cases and 24 SGA with or without IUGR cases. They postulated that alterations in epigenetic control mechanisms leading to deregulation of growth-related imprinted genes on chromosome 11p15 may affect placental and fetal growth and lead to SGA/IUGR. They found that two imprinting clusters (KvDMR and *H19* DMR) maintained normal differential methylation in most SGA placentas, except for one, which showed loss of methylation at *H19* DMR. In this placenta, also LOI was demonstrated at the *H19* DMR as shown by bi-allelic expression of the *H19* gene. Furthermore, a significant decrease of *IGF2* expression, mostly independent of *H19* regulation, was found in all SGA placentas (Guo *et al.*, 2008).

Other researchers investigated the relation between placental imprinting and birthweight. The imprinted genes *IGF2*, *MEST*, *PHLDA2* and *IGF2R*, all with known roles in fetal growth, were studied in 200 normal term placentas (Apostolidou *et al.*, 2007). Elevated placental expression of *PHLDA2* was associated with lower birthweight. It is, however, not clear whether birthweight in this study was within normal ranges or whether also LBW or very LBW cases were included. Furthermore, these increased maternally expressed *PHLDA2* levels were independent of LOI (Apostolidou *et al.*, 2007). Indeed, several studies found that placental LOI usually seems to be unrelated to gene expression levels (Table II).

Epigenetics is also believed to be involved in the pathogenesis of pre-eclampsia. Several miRNAs are expressed in the human placenta (Barad *et al.*, 2004). Differential expression of specific placental miRNAs, among others miR-210, was found in pre-eclampsia placentas compared with normal placentas (Pineles *et al.*, 2007; Zhu *et al.*, 2009). Chelbi and Vaiman (2008) suggest that an abnormal methylation pattern may be a common mechanism leading to pre-eclampsia. Moreover, the proximal promoter of the non-imprinted *SERPINA3* is significantly hypomethylated at specific CpG positions in pre-eclampsia placentas compared with normal placentas (Chelbi *et al.*, 2007). Lastly, up-regulation of the expression of 13 tumour suppressor and growth regulatory genes has been identified in placentas of patients with severe early onset pre-eclampsia (Heikkila *et al.*, 2005).

There might also be a role for imprinted genes in the pathogenesis of pre-eclampsia. LOI of the imprinted *Cdkn1c* gene in a mouse model resulted in some of the features of pre-eclampsia, including hypertension and proteinuria (Kanayama *et al.*, 2002). Development of pre-eclampsia is associated with decreased invasive capacity of trophoblasts. Since down-regulation of *H19* expression is associated with development of choriocarcinoma and its highly invasive capacity (Walsh *et al.*, 1995; Kim *et al.*, 2003), Yu *et al.* (2009) investigated the hypothesis that alterations in paternal *H19* imprinting contribute to the pathogenesis of pre-eclampsia. In the placentas of patients with pre-eclampsia associated with severe hypertension, indeed LOI of the *H19* gene was found (Yu *et al.*, 2009).

Researchers have examined the role of aberrant DNA methylation in GTD. Trophoblast hyperplasia is a common feature of complete hydatidiform moles with the potential for malignant transformation to choriocarcinoma (Li *et al.*, 2002). Investigation of invasive choriocarcinoma cell lines displayed altered methylation patterns consistent with a role of methylation change in GTD (Novakovic *et al.*, 2008).

The transcription factor *Oct4*, plays a crucial role in maintaining pluripotency of embryonic stem cells and its hypermethylation is associated with the differentiation of TE cell lineage, from which the normal placenta derives. Both methylated and unmethylated *Oct4* alleles were observed in normal placenta, while 33% of hydatidiform moles and two choriocarcinoma cell lines displayed only methylated alleles. Down-regulation of *Oct4* by hypermethylation implies an important role in the pathogenesis of GTD (Zhang *et al.*, 2008). In addition, aberrant tumour suppressor gene methylation status has been associated with GTD, specifically hydatidiform mole and choriocarcinoma (Xue *et al.*, 2004).

Environmental effects

Increasing evidence suggests that the environment during pre- and post-natal development can affect the risk on chronic diseases such as cancer, cardiovascular disease, diabetes, obesity and behavioural disorders like schizophrenia by altering epigenetic programming (Jirtle and Skinner, 2007). A well-known example is the effect of maternal diet on coat colour and obesity in A^{vy}/a mice offspring (Waterland and Jirtle, 2003). Also other environmental factors like tobacco smoke, alcohol, radiation and chemicals have been shown to influence disease susceptibility and can even induce transgenerational phenotypic effects (Singh *et al.*, 2003; Feil, 2006; Jirtle and Skinner, 2007; Baccarelli and Bollati, 2009). One of the best known examples in humans is the effect of diethylstilbestrol, which was given to pregnant women in the sixties, to prevent miscarriages (Li *et al.*, 2003). This chemical, however, leads to uterus malformations in the offspring, caused by epigenetic deregulation (Li *et al.*, 2003; Bromer *et al.*, 2009; Sato *et al.*, 2009).

There are several studies which investigated the relationship between environmental effects and placental epigenetics (Table III). Haycock and Ramsay (2009) used a mouse model to investigate whether disruption of imprinting control at the *H19* ICR may be a mechanism of ethanol-induced growth retardation in the event of ethanol exposure during fetal development. Ethanol exposed midgestation placentas and embryos were severely growth retarded when compared with controls. DNA methylation was unaffected in embryos, although the paternal alleles were significantly less methylated in ethanol exposed placentas. Data showing a relationship between placental weight and ethanol treatment suggested that this was partially dependent on DNA methylation at the CCCTC-binding factor (CTCF) site on the paternal allele in placentas (Haycock and Ramsay, 2009). Unfortunately, gene expression was not analysed.

Unlike most cells, placental cytotrophoblasts proliferate in response to hypoxia (Adelman *et al.*, 1999). An important regulator of the responses of a cell to oxygen tension is the hypoxia-inducible factor 1 (HIF1; Maltepe *et al.*, 2005). For normal trophoblast differentiation, crosstalk between the HIFs and HDACs is crucial, which links HIF function with epigenetic regulatory mechanisms (Maltepe *et al.*, 2005). Donker *et al.* (2007) investigated *in vitro* the effect of varying oxygen levels on miRNAs in term human trophoblasts during differentiation and in hypoxic environment. In hypoxic trophoblasts, the expression of miR-93 was up-regulated, whereas the expression of miR-424 was down-regulated. These results indicate that the miRNA biosynthetic pathway is functional even in a hypoxic environment, and that hypoxia regulates the expression level of miRNAs in these cells (Donker *et al.*, 2007).

Assisted reproduction technologies (ART) are increasingly used worldwide in animals and humans to achieve (higher) pregnancy and life-birth rates in subfertile patients. ART in humans have been associated with LBWs, SGA, preterm deliveries (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; McGovern *et al.*, 2004) and birth defects (Rimm *et al.*, 2004; Hansen *et al.*, 2005; Lie *et al.*, 2005). Furthermore, several groups have raised concerns about a possible association of rare imprinting diseases with ART. Analysis of affected ART children have shown methylation defects at the DMRs of *SNRPN* (Angelman Syndrome), *KCNQ1OT1* (Beckwith-Wiedemann Syndrome) and *PEG1/MEST* (Silver-Russell Syndrome) (Cox *et al.*, 2002; DeBaun *et al.*, 2003; Gicquel *et al.*, 2003; Maher *et al.*, 2003; Halliday *et al.*, 2004; Kagami *et al.*, 2007). In women undergoing ART, the risk of pre-eclampsia, stillbirth and placenta praevia is significantly increased (Jackson *et al.*, 2004). Several aspects of ART, such as ovulation induction and embryo culture, have been investigated as a possible cause of imprinting disturbance.

Induction of ovulation with high doses of gonadotrophin is used to obtain multiple mature oocytes. This treatment may force oocytes to undergo the growth and maturation phases too rapidly or rescue oocytes already selected for atresia, possibly leading to inadequate establishment or maintenance of the parent-of-origin effect (Fortier *et al.*, 2008). The timing of imprint establishment in human adult oogenesis is mostly unknown, but in mice it starts post-natally, during the oocyte growth phase, in a locus-specific manner with some genes achieving their full methylation status only very late in oocyte growth (Bao *et al.*, 2000; Obata and Kono, 2002; Lucifero *et al.*, 2004b). According to earlier mouse studies, ovulation induction results in delayed embryo development, decreased implantation rates and increased post-implantation loss (Fossum *et al.*, 1989; Ertzeid and Storeng, 1992, 2001; Van der Auwera and D'Hooghe, 2001). Also, the effect of ovulation induction on DNA methylation and imprinting was investigated. One study in both mouse and human oocytes showed no difference in imprint establishment following ovulation induction in the maternally imprinted DMRs of *MEST*, *KCNQ1OT1* and *ZAC/PLAGL1* (Sato *et al.*, 2007). Surprisingly, increased DNA methylation was found at the paternally imprinted *H19* DMR in superovulated oocytes, which is normally unmethylated in oocytes (Sato *et al.*, 2007). Ovulation induction also induces aberrant expression of the imprinted H19 gene in mouse blastocysts (Fauque *et al.*, 2007) with a normal methylation pattern at its regulatory sequence. Contradictory, in individual blastocyst stage mouse embryos, ovulation induction perturbed methylation in both maternally and paternally imprinted DMRs (loss of *Snrpn*, *Peg3*, *Kcnq1ot1*; gain of *H19* methylation) (Market-Velker *et al.*, 2010). Aberrant imprinted methylation was also dose-dependent, being more frequent at higher hormone dosages (Market-Velker *et al.*, 2010). These studies altogether suggest that ovulation induction might interfere with imprint establishment as well as with imprint maintenance after fertilization. Whether the possible effect of ovulation induction is still present in placental tissue is less well investigated. Indeed, one study reported bi-allelic expression of *H19* and *Snrpn*, and bi-allelic expression of *H19* in 9.5 dpc placentas after ovulation induction/*in vivo* development and ovulation induction/3.5 dpc embryo transfer, respectively (Fortier *et al.*, 2008). There was normal mono-allelic expression of these

Table III Environmental effects on placental epigenetics.[a]

Condition	Time of exposure	Gene	Species	Expressed allele	Methylation	Gene expression	Mono-allelic expression	Reference[d,e]
Ethanol	At 1.5 and 2.5 dpc	H19	Mouse	M	LOM (H19DMR)	Na	Na	Haycock Ramsay (2009)
Ovulation induction and *in vivo* development or embryo transfer	At 3.5 dpc	H19	Mouse	M	= (H19DMR)	Na	LOI (22/36)	Fortier *et al.* (2008)
		Igf2	Mouse	P	Na	↑ (3.4)	=	Fortier *et al.* (2008)
		Kcnq1ot1	Mouse	P	Na	Na	=	Fortier *et al.* (2008)
		Snrpn	Mouse	P	= (SnrpnDMR)	Na	LOI (10/36)	Fortier *et al.* (2008)
Embryo transfer (with embryo culture)	2-cell embryo collection + culture till blastocyst stage	Ascl2	Mouse	M	LOM (KvDMR)	↑ (12.5)	Na	Rivera *et al.* (2008)
		Cdkn1c	Mouse	M	LOM (KvDMR1)	Na	LOI (1/28)	Rivera *et al.* (2008)
		H19	Mouse	M	LOM (H19DMR)	=	LOI (19/28)	Rivera *et al.* (2008)
		Igf2	Mouse	P	Na	↓ (0.4)	=	Rivera *et al.* (2008)
		Kcnq1	Mouse	M	LOM (KvDMR1)	Na	LOI (6/27)	Rivera *et al.* (2008)
		Kcnq1ot1	Mouse	P	LOM (KvDMR1)	Na	LOI (9/27)	Rivera *et al.* (2008)
		Mkrn3	Mouse	P	Na	Na	LOI (26/28)[b]	Rivera *et al.* (2008)
		Peg3	Mouse	P	Na	Na	LOI (8/28)	Rivera *et al.* (2008)
		Snrpn	Mouse	P	Na	Na	LOI (8/27)	Rivera *et al.* (2008)
		Zim1	Mouse	M	Na	Na	=	Rivera *et al.* (2008)
Culture	2-cell embryo collection + culture till blastocyst stage	Ascl2	Mouse	M	Na	Na	LOI	Mann *et al.* (2004)
		H19	Mouse	M	LOM (H19DMR)	Na	LOI	Mann *et al.* (2004)
		Peg3	Mouse	P	Na	Na	LOI	Mann *et al.* (2004)
		Snrpn	Mouse	P	LOM (SnrpnDMR)	Na	LOI	Mann *et al.* (2004)
		Xist	Mouse	In females M or P	Na	Na	LOI[c]	Mann *et al.* (2004)
ART		COPG2	Human	CD	=	↓	Na	Katari *et al.* (2009)
		DLK1	Human	P	=	Na	Na	Katari *et al.* (2009)
					=	Na	Na	Tierling *et al.* (2010)
		GNAS	Human	ID	=	=	Na	Katari *et al.* (2009)
					= (4 DMRs)	Na	Na	Tierling *et al.* (2010)
		GRB10	Human	ID	LOM	Na	Na	Katari *et al.* (2009)
					=	Na	Na	Tierling *et al.* (2010)
		H19	Human	M	=	Na	Na	Katari *et al.* (2009)

					=	Na	Na	Tierling *et al.* (2010)
		KCNQ1OT1	Human	P	=	Na	Na	Katari *et al.* (2009)
					= (KvDMR1)	Na	Na	Tierling *et al.* (2010)
		MEST	Human	P	LOM (2CpGs), GOM (1CpG)	↑ (2.09)	Na	Katari *et al.* (2009)
					=	Na	Na	Tierling *et al.* (2010)
		NNAT	Human	P	=	↓ (0.55)	Na	Katari *et al.* (2009)
		PEG3	Human	P	LOM (3CpGs), GOM (2CpGs)	=	Na	Katari *et al.* (2009)
		SERPINF1	Human	M/P	LOM	↑ (1.81)	Nr	Katari *et al.* (2009)
		SLC22A2	Human	M	LOM	=	Na	Katari *et al.* (2009)
		SNRPN	Human	P	=	Na	Na	Katari *et al.* (2009)
					=	Na	Na	Tierling *et al.* (2010)
Hypoxia	*In vitro* exposure of term trophoblast	miR-93	Human	M/P	Na	↑	Na	Donker *et al.* (2007)
		miR-424	Human	M/P	Na	↓	Na	Donker *et al.* (2007)

[a]The effects mentioned in the table are those from the exposed group compared with a control group.
[b]Mkrn3 displayed also severe LOI in control samples, but the LOI was more severe in placentas from cultured and transferred embryos.
[c]Xist was inappropriately expressed in male placental tissue after embryo culture.
[d]Katari *et al.* (2009) performed an array-based methylation analysis. Forty genes were different between the *in vivo* and *in vitro* group. Of these only the ones subject to further expression analysis or the ones affected according to the study by Tierling *et al.* (2010) are reported here.
[e]In the study by Tierling *et al.* (2010) chorion and amnion samples are analysed, instead of throphoblast as with the other studies.
CD, conflicting data; GOM, gain of methylation; ID, isoform dependent; IUGR, intrauterine growth reduction; LOI, loss of imprinting; LOM, loss of methylation; Na, not analysed; Nr, not relevant; '=', not affected when compared with control.

genes in the embryo. These results suggest an apparently primary effect of ovulation induction on the expression of imprinted genes in the placenta when compared with the embryo. The expression of *Igf2*, an important placental growth factor, was increased following ovulation induction despite retaining mono-allelic expression (Fortier *et al.*, 2008). Earlier in this review, we discussed that the placenta displays lower overall methylation levels compared with the embryo. Fortier *et al.* (2008) also noted that the overall levels of methylation at the DMRs of both *Snrpn* and *H19* were lower in placentas than in embryos, without an effect on the mono-allelic expression of these genes. This could suggest again that imprint maintenance in the placenta is regulated primarily by histone modifications instead of by DNA methylation.

The role of oxygen tension during embryo culture was studied in both animal and human studies. In preimplantation mouse embryos, culture in 20% oxygen resulted in far greater perturbations in the global pattern of gene regulation than culture in 5% oxygen, when compared with embryos that developed *in vivo* (Rinaudo *et al.*, 2006). In humans, such perturbations might be the cause of the less optimal embryo development (Kovacic and Vlaisavljevic, 2008; Waldenstrom *et al.*, 2009) and lower rates of live birth implantation and live births (Meintjes *et al.*, 2009; Waldenstrom *et al.*, 2009) after culture in an atmospheric (19–21%) oxygen environment. Preimplantation culture of embryos can also influence birthweight in a negative and positive manner through changes in culture condition. Recently, research in humans has indicated that the type of medium used for culturing IVF embryos during the first few days after fertilization significantly affects the birthweight of the resulting human newborns (Dumoulin *et al.*, 2010). Overgrowth abnormalities, collectively referred to as 'large offspring syndrome', are frequently seen in sheep and cattle (Thompson *et al.*, 1995; Sinclair *et al.*, 2000). Preimplantation embryo culture has been shown to affect methylation and expression of imprinted genes in several animal models. In sheep, *in vitro* culture with serum until the blastocyst stage gives rise to fetuses that show aberrant methylation and lack of expression of a well-known imprinted locus *Igf2r* leading to large offspring (Young *et al.*, 2001). The addition of fetal calf serum to M16 medium for mouse embryonic culture, also alters the expression of imprinted genes but contrarily reduces the birthweight (Khosla *et al.*, 2001). Not all imprinted genes were affected (Doherty *et al.*, 2000; Khosla *et al.*, 2001), but especially H19 seems to be vulnerable for culture medium-induced aberrations of methylation and expression (Sasaki *et al.*, 1995; Doherty *et al.*, 2000; Mann *et al.*, 2004). Only a subset of individual cultured mouse blastocysts were affected (Mann *et al.*, 2004). Moreover, proper imprinted expression for the most part was preserved in the embryo, while placental tissues displayed activation of the normally silent allele for *H19*, *Ascl2*, *Snrpn*, *Peg3* and *Xist* (Mann *et al.*, 2004). Embryo transfer, with or without embryo culture, also led to LOI in placental tissue but not in the embryo (Rivera *et al.*, 2008). This indicates that appropriate imprinting is not restored during post-implantation development of the placenta. The findings of Mann *et al.* and Rivera *et al.* parallel the findings of Haycock and Ramsay (ethanol) and Fortier *et al.* (ovulation induction) suggesting that tissues of TE origin are more sensitive to preimplantation epigenetic disturbance than embryonic tissues.

Very recently, the complete placental transcriptome after ART in mice was analysed, revealing a substantial alteration of placental gene expression at 10.5 days post-fertilization (Fauque *et al.*, 2010). About 6% of placental transcripts were altered at the 2-fold threshold, with most genes being down-regulated, after IVF and embryo culture compared with placentas of embryos produced *in vivo*. Conversely, imprinted genes, as well as X-linked genes, were overall more up-regulated than the rest of the transcriptome (Fauque *et al.*, 2010). Interestingly, analysis of some imprinted genes showed that paternally expressed genes were primarily up-regulated. Moreover, genes involved in cellular proliferation and cell cycle pathways were induced, whereas genes involved in angiogenic signalling and the immune system were reduced (Fauque *et al.*, 2010). These findings, although in mice, may explain future placental problems, e.g. pre-eclampsia, as seen after ART in humans (Jackson *et al.*, 2004).

So far, few studies have been conducted to examine the effect of ART on human placental epigenetics. Recently, Gomes *et al.* reported a higher incidence of hypomethylation of the KvDMR1 in clinically normal children conceived by ART. They investigated blood samples and found aberrant hypomethylation in 3 ART children (3/18) and in none of the 30 non-ART newborns (Gomes *et al.*, 2009). Interestingly, each of these three children had a dizygotic sibling with discordant methylation. Also placental tissue was obtained. Unfortunately, no placental samples were collected after birth of the hypomethylated children. Katari *et al.* (2009) performed an array-based methylation analysis in placenta and cord blood of 10 ART and 13 non-ART children. Moderate but significant methylation differences were observed between groups with the ART children displaying lower mean methylation levels at specific CpG sites in placenta and higher mean methylation levels in cord blood (Katari *et al.*, 2009). In contrast, Tierling *et al.* (2010) found no significant methylation difference at 10 DMRs in amnion/chorion tissue and umbilical cord blood of 185 phenotypically normal children, comprising 77 children conceived by ICSI, 35 by IVF and 73 spontaneously. However, the latter studies both found that MEST was hypomethylated in placental cells compared with umbilical cord blood.

Conclusion and future prospects

In recent years, there has been an explosion of knowledge in the field of epigenetics. Epigenetics is gradually recognized as having an important role in placental development and functioning. Proper epigenetic regulation of imprinted genes in particular, but also of non-imprinted genes is crucial in the placenta. This epigenetic regulation evolves during preimplantation development and further gestation. Environmental effects can disturb placental epigenetics and herewith placental development and function, with possible downstream consequences for maternal morbidity, fetal development and disease susceptibility in later life (summarized in Fig. 1). Especially since several animal studies have suggested that placental tissues are more sensitive to preimplantation epigenetic disturbance than embryonic tissues and ART are increasingly used worldwide, this emphasizes the need for further investigations in human placentas. Placental tissue sampled after placental-related pathologies and from pregnancies resulting from ART, can be used to screen for epigenetic disturbances, instead of being discarded as a meaningless by-product. Early screening using this tissue may lead to earlier intervention or better surveillance of children who may be at risk later in life. With epigenetic marks being reversible, epigenetic therapy may eventually provide promising

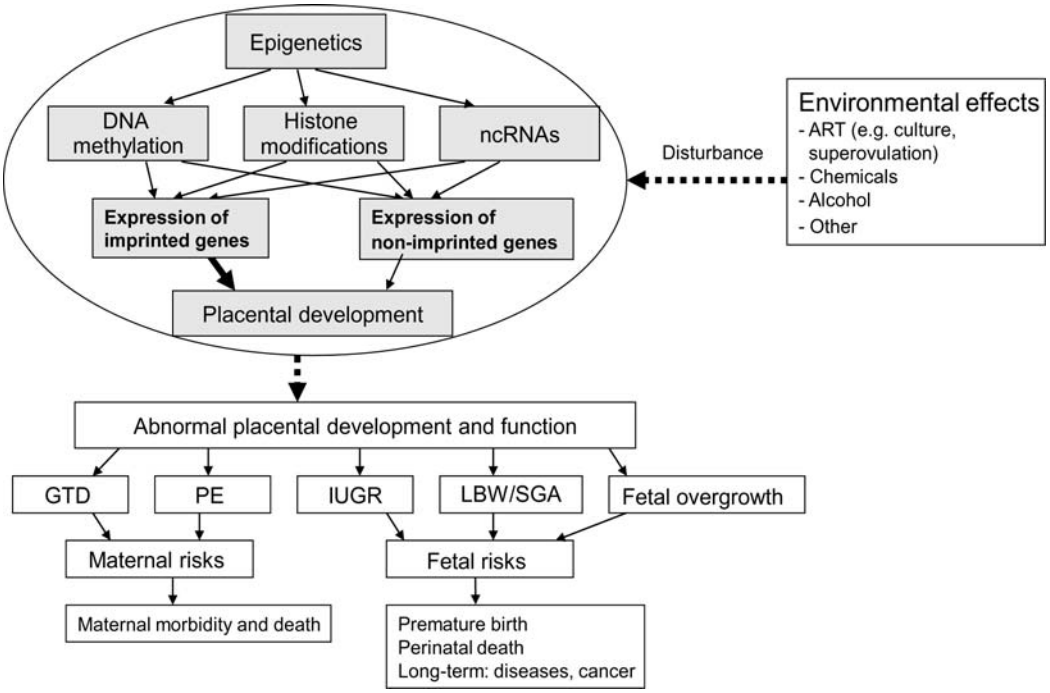

Figure 1 The role of epigenetics in placental development and the possible consequences of its disturbance which can be caused by environmental effects. Arrows indicate relationships between the different steps. Bold arrow indicates the importance of imprinted genes for placental development. Dashed arrows indicate the effect of environment on placental disturbance. ncRNAs, non-coding RNAs; ART, assisted reproduction technologies; GTD, gestational trophoblastic disease; PE, pre-eclampsia; IUGR, intrauterine growth restriction; LBW, low birthweight; SGA, small for gestational age.

new challenges. This also implies the need for more fundamental research to investigate the involvement of histone modifications and ncRNAs in placental gene regulation.

Acknowledgements

We thank Professor J.P.M. Geraedts for his insightful comment on our manuscript.

References

Adelman DM, Maltepe E, Simon MC. Multilineage embryonic hematopoiesis requires hypoxic ARNT activity. *Genes Dev* 1999;**13**:2478–2483.

Alsat E, Guibourdenche J, Couturier A, Evain-Brion D. Physiological role of human placental growth hormone. *Mol Cell Endocrinol* 1998;**140**:121–127.

Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. *Cancer Chemother Pharmacol* 2006; **57**:234–240.

Angiolini E, Fowden A, Coan P, Sandovici I, Smith P, Dean W, Burton G, Tycko B, Reik W, Sibley C *et al.* Regulation of placental efficiency for nutrient transport by imprinted genes. *Placenta* 2006;**27**:S98–102.

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

Arima T, Hata K, Tanaka S, Kusumi M, Li E, Kato K, Shiota K, Sasaki H, Wake N. Loss of the maternal imprint in Dnmt3Lmat-/- mice leads to a differentiation defect in the extraembryonic tissue. *Dev Biol* 2006;**297**:361–373.

Baccarelli A, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr* 2009;**21**:243–251.

Bamberg C, Kalache KD. Prenatal diagnosis of fetal growth restriction. *Semin Fetal Neonatal Med* 2004;**9**:387–394.

Bao S, Obata Y, Carroll J, Domeki I, Kono T. Epigenetic modifications necessary for normal development are established during oocyte growth in mice. *Biol Reprod* 2000;**62**:616–621.

Barad O, Meiri E, Avniel A, Aharonov R, Barzilai A, Bentwich I, Einav U, Gilad S, Hurban P, Karov Y *et al.* MicroRNA expression detected by oligonucleotide microarrays: system establishment and expression profiling in human tissues. *Genome Res* 2004;**14**:2486–2494.

Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 1993;**36**:62–67.

Barlow DP. Methylation and imprinting: from host defense to gene regulation? *Science* 1993;**260**:309–310.

Barton SC, Surani MA, Norris ML. Role of paternal and maternal genomes in mouse development. *Nature* 1984;**311**:374–376.

Beaujean N, Hartshorne G, Cavilla J, Taylor J, Gardner J, Wilmut I, Meehan R, Young L. Non-conservation of mammalian preimplantation methylation dynamics. *Curr Biol* 2004;**14**:R266–267.

Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002; **16**:6–21.

Bird A. Perceptions of epigenetics. *Nature* 2007;**447**:396–398.

Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab* 2007;**92**:3517–3522.

Bourc'his D, Bestor TH. Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* 2004;**431**:96–99.

Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH. Dnmt3L and the establishment of maternal genomic imprints. *Science* 2001;**294**:2536–2539.

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK *et al.* Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 2006; **441**:349–353.

Boyko EJ. Proportion of type 2 diabetes cases resulting from impaired fetal growth. *Diabetes Care* 2000;**23**:1260–1264.

Bromer JG, Wu J, Zhou Y, Taylor HS. Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. *Endocrinology* 2009;**150**:3376–3382.

Buiting K, Saitoh S, Gross S, Dittrich B, Schwartz S, Nicholls RD, Horsthemke B. Inherited microdeletions in the Angelman and Prader–Willi syndromes define an imprinting centre on human chromosome 15. *Nat Genet* 1995;**9**:395–400.

Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C *et al.* The transcriptional landscape of the mammalian genome. *Science* 2005;**309**:1559–1563.

Chaddha V, Viero S, Huppertz B, Kingdom J. Developmental biology of the placenta and the origins of placental insufficiency. *Semin Fetal Neonatal Med* 2004;**9**:357–369.

Chapman V, Forrester L, Sanford J, Hastie N, Rossant J. Cell lineage-specific undermethylation of mouse repetitive DNA. *Nature* 1984;**307**:284–286.

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

Chelbi ST, Vaiman D. Genetic and epigenetic factors contribute to the onset of preeclampsia. *Mol Cell Endocrinol* 2008;**282**:120–129.

Chelbi ST, Mondon F, Jammes H, Buffat C, Mignot TM, Tost J, Busato F, Gut I, Rebourcet R, Laissue P *et al.* Expressional and epigenetic alterations of placental serine protease inhibitors: SERPINA3 is a potential marker of preeclampsia. *Hypertension* 2007;**49**:76–83.

Chiu RW, Chim SS, Wong IH, Wong CS, Lee WS, To KF, Tong JH, Yuen RK, Shum AS, Chan JK *et al.* Hypermethylation of RASSF1A in human and rhesus placentas. *Am J Pathol* 2007;**170**:941–950.

Chuang HC, Chang CW, Chang GD, Yao TP, Chen H. Histone deacetylase 3 binds to and regulates the GCMa transcription factor. *Nucleic Acids Res* 2006;**34**:1459–1469.

Coan PM, Burton GJ, Ferguson-Smith AC. Imprinted genes in the placenta—a review. *Placenta* 2005;**26**:S10–20.

Coan PM, Fowden AL, Constancia M, Ferguson-Smith AC, Burton GJ, Sibley CP. Disproportional effects of Igf2 knockout on placental morphology and diffusional exchange characteristics in the mouse. *J Physiol* 2008;**586**:5023–5032.

Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, Stewart F, Kelsey G, Fowden A, Sibley C *et al.* Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* 2002;**417**:945–948.

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

Cross JC. The genetics of pre-eclampsia: a feto-placental or maternal problem? *Clin Genet* 2003;**64**:96–103.

Curhan GC, Chertow GM, Willett WC, Spiegelman D, Colditz GA, Manson JE, Speizer FE, Stampfer MJ. Birth weight and adult hypertension and obesity in women. *Circulation* 1996a;**94**:1310–1315.

Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation* 1996b;**94**:3246–3250.

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

Delcuve GP, Rastegar M, Davie JR. Epigenetic control. *J Cell Physiol* 2009;**219**:243–250.

DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004;**80**:1689S–1696S.

Devriendt K. Hydatidiform mole and triploidy: the role of genomic imprinting in placental development. *Hum Reprod Update* 2005;**11**:137–142.

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

Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000;**62**:1526–1535.

Dokras A, Coffin J, Field L, Frakes A, Lee H, Madan A, Nelson T, Ryu GY, Yoon JG, Madan A. Epigenetic regulation of maspin expression in the human placenta. *Mol Hum Reprod* 2006;**12**:611–617.

Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. *Mol Hum Reprod* 2007;**13**:273–279.

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

Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005;**289**:F8–28.

Edwards CA, Ferguson-Smith AC. Mechanisms regulating imprinted genes in clusters. *Curr Opin Cell Biol* 2007;**19**:281–289.

Engstrom PG, Suzuki H, Ninomiya N, Akalin A, Sessa L, Lavorgna G, Brozzi A, Luzi L, Tan SL, Yang L *et al.* Complex Loci in human and mouse genomes. *PLoS Genet* 2006;**2**:e47.

Eriksson JG, Forsen TJ, Osmond C, Barker DJ. Pathways of infant and childhood growth that lead to type 2 diabetes. *Diabetes Care* 2003;**26**:3006–3010.

Ertzeid G, Storeng R. Adverse effects of gonadotrophin treatment on pre- and postimplantation development in mice. *J Reprod Fertil* 1992;**96**:649–655.

Ertzeid G, Storeng R. The impact of ovarian stimulation on implantation and fetal development in mice. *Hum Reprod* 2001;**16**:221–225.

Esteve PO, Chin HG, Smallwood A, Feehery GR, Gangisetty O, Karpf AR, Carey MF, Pradhan S. Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Genes Dev* 2006;**20**:3089–3103.

Fauque P, Jouannet P, Lesaffre C, Ripoche MA, Dandolo L, Vaiman D, Jammes H. Assisted Reproductive Technology affects developmental kinetics, H19 Imprinting Control Region methylation and H19 gene expression in individual mouse embryos. *BMC Dev Biol* 2007;**7**:116.

Fauque P, Mondon F, Letourneur F, Ripoche MA, Journot L, Barbaux S, Dandolo L, Patrat C, Wolf JP, Jouannet P *et al.* In vitro fertilization and embryo culture strongly impact the placental transcriptome in the mouse model. *PLoS One* 2010;**5**:e9218.

Feil R. Environmental and nutritional effects on the epigenetic regulation of genes. *Mutat Res* 2006;**600**:46–57.

Ferguson-Smith AC, Moore T, Detmar J, Lewis A, Hemberger M, Jammes H, Kelsey G, Roberts CT, Jones H, Constancia M. Epigenetics and imprinting of the trophoblast—a workshop report. *Placenta* 2006;**27**:S122–126.

Ferretti C, Bruni L, Dangles-Marie V, Pecking AP, Bellet D. Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts. *Hum Reprod Update* 2007;**13**:121–141.

Fisher SJ. The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. *Reprod Biol Endocrinol* 2004;**2**:53.

Fortier AL, Lopes FL, Darricarrere N, Martel J, Trasler JM. Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. *Hum Mol Genet* 2008;**17**:1653–1665.

Fossum GT, Davidson A, Paulson RJ. Ovarian hyperstimulation inhibits embryo implantation in the mouse. *J In Vitro Fert Embryo Transf* 1989;**6**:7–10.

Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. *Horm Res* 2006;**65**:50–58.

Frank D, Fortino W, Clark L, Musalo R, Wang W, Saxena A, Li CM, Reik W, Ludwig T, Tycko B. Placental overgrowth in mice lacking the imprinted gene Ipl. *Proc Natl Acad Sci USA* 2002;**99**:7490–7495.

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

Gluckman PD, Hanson MA. Maternal constraint of fetal growth and its consequences. *Semin Fetal Neonatal Med* 2004;**9**:419–425.

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

Guillemot F, Nagy A, Auerbach A, Rossant J, Joyner AL. Essential role of Mash-2 in extraembryonic development. *Nature* 1994;**371**:333–336.

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

Haig D. Genomic imprinting and kinship: how good is the evidence? *Annu Rev Genet* 2004;**38**:553–585.

Halliday J, Oke K, Breheny S, Algar E, Amor DJ. Beckwith–Wiedemann syndrome and IVF: a case–control study. *Am J Hum Genet* 2004;**75**:526–528.

Hansen M, Bower C, Milne E, de Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects—a systematic review. *Hum Reprod* 2005;**20**:328–338.

Hata K, Okano M, Lei H, Li E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* 2002;**129**:1983–1993.

Haycock PC, Ramsay M. Exposure of mouse embryos to ethanol during preimplantation development: effect on DNA methylation in the h19 imprinting control region. *Biol Reprod* 2009;**81**:618–627.

Heikkila A, Tuomisto T, Hakkinen SK, Keski-Nisula L, Heinonen S, Yla-Herttuala S. Tumor suppressor and growth regulatory genes are overexpressed in severe early-onset preeclampsia—an array study on case-specific human preeclamptic placental tissue. *Acta Obstet Gynecol Scand* 2005;**84**:679–689.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *Br Med J* 2004;**328**:261.

Hemberger M. Epigenetic landscape required for placental development. *Cell Mol Life Sci* 2007;**64**:2422–2436.

Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003;**349**:2042–2054.

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

Hui P, Martel M, Parkash V. Gestational trophoblastic diseases: recent advances in histopathologic diagnosis and related genetic aspects. *Adv Anat Pathol* 2005;**12**:116–125.

Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;**103**:551–563.

Januchowski R, Dabrowski M, Ofori H, Jagodzinski PP. Trichostatin A down-regulate DNA methyltransferase 1 in Jurkat T cells. *Cancer Lett* 2007;**246**:313–317.

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

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

Kanayama N, Takahashi K, Matsuura T, Sugimura M, Kobayashi T, Moniwa N, Tomita M, Nakayama K. Deficiency in p57Kip2 expression induces preeclampsia-like symptoms in mice. *Mol Hum Reprod* 2002;**8**:1129–1135.

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* 2004;**429**:900–903.

Kaneko-Ishino T, Kohda T, Ishino F. The regulation and biological significance of genomic imprinting in mammals. *J Biochem* 2003;**133**:699–711.

Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, Gaughan JP, Coutifaris C, Sapienza C. DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum Mol Genet* 2009;**18**:3769–3778.

Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, Nishida H, Yap CC, Suzuki M, Kawai J *et al.* Antisense transcription in the mammalian transcriptome. *Science* 2005;**309**:1564–1566.

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

Kim SJ, Park SE, Lee C, Lee SY, Kim IH, An HJ, Oh YK. Altered imprinting, promoter usage, and expression of insulin-like growth factor-II gene in gestational trophoblastic diseases. *Gynecol Oncol* 2003;**88**:411–418.

Kimura AP, Liebhaber SA, Cooke NE. Epigenetic modifications at the human growth hormone locus predict distinct roles for histone acetylation and methylation in placental gene activation. *Mol Endocrinol* 2004;**18**:1018–1032.

Koerner MV, Pauler FM, Huang R, Barlow DP. The function of non-coding RNAs in genomic imprinting. *Development* 2009;**136**:1771–1783.

Kono T, Obata Y, Wu Q, Niwa K, Ono Y, Yamamoto Y, Park ES, Seo JS, Ogawa H. Birth of parthenogenetic mice that can develop to adulthood. *Nature* 2004;**428**:860–864.

Koslowski M, Sahin U, Mitnacht-Kraus R, Seitz G, Huber C, Tureci O. A placenta-specific gene ectopically activated in many human cancers is essentially involved in malignant cell processes. *Cancer Res* 2007;**67**:9528–9534.

Kovacic B, Vlaisavljevic V. Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts in vitro: a prospective study on sibling oocytes. *Reprod Biomed Online* 2008;**17**:229–236.

Laird PW. The power and the promise of DNA methylation markers. *Nat Rev Cancer* 2003;**3**:253–266.

Lambertini L, Diplas AI, Lee MJ, Sperling R, Chen J, Wetmur J. A sensitive functional assay reveals frequent loss of genomic imprinting in human placenta. *Epigenetics* 2008;**3**:261–269.

Lee J, Inoue K, Ono R, Ogonuki N, Kohda T, Kaneko-Ishino T, Ogura A, Ishino F. Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. *Development* 2002;**129**:1807–1817.

Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K *et al.* Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 2006;**125**:301–313.

Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA. Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. *Nat Genet* 1998;**20**:163–169.

Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, Peters AH. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr Biol* 2003;**13**:1192–1200.

Lewis A, Mitsuya K, Umlauf D, Smith P, Dean W, Walter J, Higgins M, Feil R, Reik W. Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. *Nat Genet* 2004;**36**:1291–1295.

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

Li HW, Tsao SW, Cheung AN. Current understandings of the molecular genetics of gestational trophoblastic diseases. *Placenta* 2002;**23**:20–31.

Li S, Hursting SD, Davis BJ, McLachlan JA, Barrett JC. Environmental exposure, DNA methylation, and gene regulation: lessons from diethylstilbesterol-induced cancers. *Ann N Y Acad Sci* 2003;**983**:161–169.

Lie RT, Lyngstadaas A, Orstavik KH, Bakketeig LS, Jacobsen G, Tanbo T. Birth defects in children conceived by ICSI compared with children conceived by other IVF-methods; a meta-analysis. *Int J Epidemiol* 2005;**34**:696–701.

Lucifero D, Chaillet JR, Trasler JM. Potential significance of genomic imprinting defects for reproduction and assisted reproductive technology. *Hum Reprod Update* 2004a;**10**:3–18.

Lucifero D, Mann MR, Bartolomei MS, Trasler JM. Gene-specific timing and epigenetic memory in oocyte imprinting. *Hum Mol Genet* 2004b;**13**:839–849.

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

Maltepe E, Krampitz GW, Okazaki KM, Red-Horse K, Mak W, Simon MC, Fisher SJ. Hypoxia-inducible factor-dependent histone deacetylase activity determines stem cell fate in the placenta. *Development* 2005;**132**:3393–3403.

Mancini-Dinardo D, Steele SJ, Levorse JM, Ingram RS, Tilghman SM. Elongation of the Kcnq1ot1 transcript is required for genomic imprinting of neighboring genes. *Genes Dev* 2006;**20**:1268–1282.

Manes C, Menzel P. Demethylation of CpG sites in DNA of early rabbit trophoblast. *Nature* 1981;**293**:589–590.

Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM, Bartolomei MS. Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* 2004;**131**:3727–3735.

Market-Velker BA, Zhang L, Magri LS, Bonvissuto AC, Mann MR. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Hum Mol Genet* 2010;**19**:36–51.

Maulik D. Fetal growth compromise: definitions, standards, and classification. *Clin Obstet Gynecol* 2006;**49**:214–218.

Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. *Nature* 2000;**403**:501–502.

McGovern PG, Llorens AJ, Skurnick JH, Weiss G, Goldsmith LT. Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. *Fertil Steril* 2004;**82**:1514–1520.

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

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

Meintjes M, Chantilis SJ, Douglas JD, Rodriguez AJ, Guerami AR, Bookout DM, Barnett BD, Madden JD. A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. *Hum Reprod* 2009;**24**:300–307.

Miri K, Varmuza S. Imprinting and extraembryonic tissues-mom takes control. *Int Rev Cell Mol Biol* 2009;**276**:215–262.

Monk D, Moore GE. Intrauterine growth restriction—genetic causes and consequences. *Semin Fetal Neonatal Med* 2004;**9**:371–378.

Monk D, Arnaud P, Apostolidou S, Hills FA, Kelsey G, Stanier P, Feil R, Moore GE. Limited evolutionary conservation of imprinting in the human placenta. *Proc Natl Acad Sci USA* 2006;**103**:6623–6628.

Moore T, Haig D. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet* 1991;**7**:45–49.

Morison IM, Reeve AE. A catalogue of imprinted genes and parent-of-origin effects in humans and animals. *Hum Mol Genet* 1998;**7**:1599–1609.

Niessen HE, Demmers JA, Voncken JW. Talking to chromatin: post-translational modulation of polycomb group function. *Epigenetics Chromatin* 2009;**2**:10.

Novakovic B, Rakyan V, Ng HK, Manuelpillai U, Dewi C, Wong NC, Morley R, Down T, Beck S, Craig JM *et al.* Specific tumour-associated methylation in normal human term placenta and first-trimester cytotrophoblasts. *Mol Hum Reprod* 2008;**14**:547–554.

Novakovic B, Sibson M, Ng HK, Manuelpillai U, Rakyan V, Down T, Beck S, Fournier T, Evain-Brion D, Dimitriadis E *et al.* Placenta-specific methylation of the vitamin D 24-hydroxylase gene: implications for feedback autoregulation of active vitamin D levels at the fetomaternal interface. *J Biol Chem* 2009;**284**:14838–14848.

O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T. The polycomb-group gene Ezh2 is required for early mouse development. *Mol Cell Biol* 2001;**21**:4330–4336.

Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. *J Biol Chem* 2002;**277**:5285–5289.

Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet* 1998;**19**:219–220.

Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999;**99**:247–257.

Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, Nikaido I, Osato N, Saito R, Suzuki H *et al.* Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* 2002;**420**:563–573.

Ono R, Nakamura K, Inoue K, Naruse M, Usami T, Wakisaka-Saito N, Hino T, Suzuki-Migishima R, Ogonuki N, Miki H *et al.* Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. *Nat Genet* 2006;**38**:101–106.

Oudejans CB, Mulders J, Lachmeijer AM, van Dijk M, Konst AA, Westerman BA, van Wijk IJ, Leegwater PA, Kato HD, Matsuda T *et al.* The parent-of-origin effect of 10q22 in pre-eclamptic females coincides with two regions clustered for genes with down-regulated expression in androgenetic placentas. *Mol Hum Reprod* 2004;**10**:589–598.

Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-Dinardo D, Kanduri C. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Mol Cell* 2008;**32**:232–246.

Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E, Helin K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *Embo J* 2004;**23**:4061–4071.

Pauler FM, Koerner MV, Barlow DP. Silencing by imprinted noncoding RNAs: is transcription the answer? *Trends Genet* 2007;**23**:284–292.

Peterson CL, Laniel MA. Histones and histone modifications. *Curr Biol* 2004;**14**:R546–551.

Pidoux G, Gerbaud P, Laurendeau I, Guibourdenche J, Bertin G, Vidaud M, Evain-Brion D, Frendo JL. Large variability of trophoblast gene expression within and between human normal term placentae. *Placenta* 2004;**25**:469–473.

Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, Draghici S, Espinoza J, Kusanovic JP, Mittal P *et al.* Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. *Am J Obstet Gynecol* 2007;**196**:e261–266.

Piras G, El Kharroubi A, Kozlov S, Escalante-Alcalde D, Hernandez L, Copeland NG, Gilbert DJ, Jenkins NA, Stewart CL. Zac1 (Lot1), a potential tumor suppressor gene, and the gene for epsilon-sarcoglycan are maternally imprinted genes: identification by a subtractive screen of novel uniparental fibroblast lines. *Mol Cell Biol* 2000;**20**:3308–3315.

Pozharny Y, Lambertini L, Ma Y, Ferrara L, Litton CG, Diplas A, Jacobs AR, Chen J, Stone JL, Wetmur J *et al.* Genomic loss of imprinting in first-trimester human placenta. *Am J Obstet Gynecol* 2010;**202**:e391–398.

Rahnama F, Shafiei F, Gluckman PD, Mitchell MD, Lobie PE. Epigenetic regulation of human trophoblastic cell migration and invasion. *Endocrinology* 2006;**147**:5275–5283.

Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 2007;**447**:425–432.

Reik W, Walter J. Genomic imprinting: parental influence on the genome. *Nat Rev Genet* 2001;**2**:21–32.

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

Reik W, Constancia M, Fowden A, Anderson N, Dean W, Ferguson-Smith A, Tycko B, Sibley C. Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *J Physiol* 2003a;**547**:35–44.

Reik W, Santos F, Mitsuya K, Morgan H, Dean W. Epigenetic asymmetry in the mammalian zygote and early embryo: relationship to lineage commitment? *Philos Trans R Soc Lond B Biol Sci* 2003b;**358**:1403–1409; discussion 1409.

Renfree MB, Ager EI, Shaw G, Pask AJ. Genomic imprinting in marsupial placentation. *Reproduction* 2008;**136**:523–531.

Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet* 2004;**21**:437–443.

Rinaudo PF, Giritharan G, Talbi S, Dobson AT, Schultz RM. Effects of oxygen tension on gene expression in preimplantation mouse embryos. *Fertil Steril* 2006;**86**:1252–1265.

Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM, Bartolomei MS. Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. *Hum Mol Genet* 2008;**17**:1–14.

Ross MG, Beall MH. Adult sequelae of intrauterine growth restriction. *Semin Perinatol* 2008;**32**:213–218.

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

Rossant J, Sanford JP, Chapman VM, Andrews GK. Undermethylation of structural gene sequences in extraembryonic lineages of the mouse. *Dev Biol* 1986;**117**:567–573.

Royo H, Cavaille J. Non-coding RNAs in imprinted gene clusters. *Biol Cell* 2008;**100**:149–166.

Russo VEA, Martienssen RA, Riggs AD. *Epigenetic Mechanisms of Gene Regulation*. Woodbury: Cold Spring Harbor Laboratory Press, 1996.

Salas M, John R, Saxena A, Barton S, Frank D, Fitzpatrick G, Higgins MJ, Tycko B. Placental growth retardation due to loss of imprinting of Phlda2. *Mech Dev* 2004;**121**:1199–1210.

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

Santos F, Zakhartchenko V, Stojkovic M, Peters A, Jenuwein T, Wolf E, Reik W, Dean W. Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. *Curr Biol* 2003;**13**:1116–1121.

Sasaki H, Ferguson-Smith AC, Shum AS, Barton SC, Surani MA. Temporal and spatial regulation of H19 imprinting in normal and uniparental mouse embryos. *Development* 1995;**121**:4195–4202.

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

Sato K, Fukata H, Kogo Y, Ohgane J, Shiota K, Mori C. Neonatal exposure to diethylstilbestrol alters expression of DNA methyltransferases and methylation of genomic DNA in the mouse uterus. *Endocr J* 2009;**56**:131–139.

Seely EW. Calciotropic hormones in preeclampsia: a renewal of interest. *J Clin Endocrinol Metab* 2007;**92**:3402–3403.

Serman L, Vlahovic M, Sijan M, Bulic-Jakus F, Serman A, Sincic N, Matijevic R, Juric-Lekic G, Katusic A. The impact of 5-azacytidine on placental weight,

glycoprotein pattern and proliferating cell nuclear antigen expression in rat placenta. *Placenta* 2007;**28**:803–811.

Shin JY, Fitzpatrick GV, Higgins MJ. Two distinct mechanisms of silencing by the KvDMR1 imprinting control region. *Embo J* 2008;**27**:168–178.

Sibley CP, Coan PM, Ferguson-Smith AC, Dean W, Hughes J, Smith P, Reik W, Burton GJ, Fowden AL, Constancia M. Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *Proc Natl Acad Sci USA* 2004;**101**:8204–8208.

Sims RJ 3rd, Reinberg D. Histone H3 Lys 4 methylation: caught in a bind. *Genes Dev* 2006;**20**:2779–2786.

Sinclair KD, Young LE, Wilmut I, McEvoy TG. In-utero overgrowth in ruminants following embryo culture: lessons from mice and a warning to men. *Hum Reprod* 2000;**15**:68–86.

Singh SM, Murphy B, O'Reilly RL. Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. *Clin Genet* 2003;**64**:451–460.

Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* 2002;**415**:810–813.

Spahn L, Barlow DP. An ICE pattern crystallizes. *Nat Genet* 2003;**35**:11–12.

Steinhoff C, Paulsen M, Kielbasa S, Walter J, Vingron M. Expression profile and transcription factor binding site exploration of imprinted genes in human and mouse. *BMC Genomics* 2009;**10**:144.

Surani MA, Barton SC, Norris ML. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature* 1984;**308**:548–550.

Swales AK, Spears N. Genomic imprinting and reproduction. *Reproduction* 2005; **130**:389–399.

Takahashi K, Kobayashi T, Kanayama N. p57(Kip2) regulates the proper development of labyrinthine and spongiotrophoblasts. *Mol Hum Reprod* 2000;**6**:1019–1025.

Tanaka M, Puchyr M, Gertsenstein M, Harpal K, Jaenisch R, Rossant J, Nagy A. Parental origin-specific expression of Mash2 is established at the time of implantation with its imprinting mechanism highly resistant to genome-wide demethylation. *Mech Dev* 1999;**87**:129–142.

Terranova R, Yokobayashi S, Stadler MB, Otte AP, van Lohuizen M, Orkin SH, Peters AH. Polycomb group proteins Ezh2 and Rnf2 direct genomic contraction and imprinted repression in early mouse embryos. *Dev Cell* 2008;**15**:668–679.

Thompson JG, Gardner DK, Pugh PA, McMillan WH, Tervit HR. Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. *Biol Reprod* 1995;**53**:1385–1391.

Tierling S, Souren NY, Gries J, Lo Porto C, Groth M, Lutsik P, Neitzel H, Utz-Billing I, Gillessen-Kaesbach G, Kentenich H *et al.* Assisted reproductive technologies do not enhance the variability of DNA methylation imprints in human. *J Med Genet* 2010;**47**:371–76.

Torres-Padilla ME. Cell identity in the preimplantation mammalian embryo: an epigenetic perspective from the mouse. *Hum Reprod* 2008;**23**:1246–1252.

Torres-Padilla ME, Parfitt DE, Kouzarides T, Zernicka-Goetz M. Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature* 2007; **445**:214–218.

Tremblay KD, Saam JR, Ingram RS, Tilghman SM, Bartolomei MS. A paternal-specific methylation imprint marks the alleles of the mouse H19 gene. *Nat Genet* 1995; **9**:407–413.

Turner BM. Cellular memory and the histone code. *Cell* 2002;**111**:285–291.

Tycko B. Imprinted genes in placental growth and obstetric disorders. *Cytogenet Genome Res* 2006;**113**:271–278.

Umlauf D, Goto Y, Cao R, Cerqueira F, Wagschal A, Zhang Y, Feil R. Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. *Nat Genet* 2004; **36**:1296–1300.

Van der Auwera I, D'Hooghe T. Superovulation of female mice delays embryonic and fetal development. *Hum Reprod* 2001;**16**:1237–1243.

van Dijk M, Mulders J, Konst A, Janssens B, van Roy F, Blankenstein M, Oudejans C. Differential downregulation of alphaT-catenin expression in placenta: trophoblast cell type-dependent imprinting of the CTNNA3 gene. *Gene Expr Patterns* 2004; **5**:61–65.

Varmuza S, Mann M. Genomic imprinting—defusing the ovarian time bomb. *Trends Genet* 1994;**10**:118–123.

Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM *et al.* The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 2006;**439**:871–874.

Vlahovic M, Bulic-Jakus F, Juric-Lekic G, Fucic A, Maric S, Serman D. Changes in the placenta and in the rat embryo caused by the demethylating agent 5-azacytidine. *Int J Dev Biol* 1999;**43**:843–846.

Wagschal A, Feil R. Genomic imprinting in the placenta. *Cytogenet Genome Res* 2006; **113**:90–98.

Wagschal A, Sutherland HG, Woodfine K, Henckel A, Chebli K, Schulz R, Oakey RJ, Bickmore WA, Feil R. G9a histone methyltransferase contributes to imprinting in the mouse placenta. *Mol Cell Biol* 2008;**28**:1104–1113.

Waldenstrom U, Engstrom AB, Hellberg D, Nilsson S. Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. *Fertil Steril* 2009;**91**:2461–2465.

Walsh C, Miller SJ, Flam F, Fisher RA, Ohlsson R. Paternally derived H19 is differentially expressed in malignant and nonmalignant trophoblast. *Cancer Res* 1995;**55**:1111–1116.

Wang J, Mager J, Chen Y, Schneider E, Cross JC, Nagy A, Magnuson T. Imprinted X inactivation maintained by a mouse Polycomb group gene. *Nat Genet* 2001; **28**:371–375.

Wang J, Mager J, Schnedier E, Magnuson T. The mouse PcG gene eed is required for Hox gene repression and extraembryonic development. *Mamm Genome* 2002; **13**:493–503.

Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003;**23**:5293–5300.

Weaver JR, Sarkisian G, Krapp C, Mager J, Mann MR, Bartolomei MS. Domain-Specific Response of Imprinted Genes to Reduced DNMT1. *Mol Cell Biol* 2010;**30**:3916–3928.

Weber M, Schubeler D. Genomic patterns of DNA methylation: targets and function of an epigenetic mark. *Curr Opin Cell Biol* 2007;**19**:273–280.

Weisstein AE, Feldman MW, Spencer HG. Evolutionary genetic models of the ovarian time bomb hypothesis for the evolution of genomic imprinting. *Genetics* 2002;**162**:425–439.

Wolf JB, Cheverud JM, Roseman C, Hager R. Genome-wide analysis reveals a complex pattern of genomic imprinting in mice. *PLoS Genet* 2008;**4**:e1000091.

Wong NC, Novakovic B, Weinrich B, Dewi C, Andronikos R, Sibson M, Macrae F, Morley R, Pertile MD, Craig JM *et al.* Methylation of the adenomatous polyposis coli (APC) gene in human placenta and hypermethylation in choriocarcinoma cells. *Cancer Lett* 2008;**268**:56–62.

Wood AJ, Oakey RJ. Genomic imprinting in mammals: emerging themes and established theories. *PLoS Genet* 2006;**2**:e147.

Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky Y. The correlation between sampling site and gene expression in the term human placenta. *Placenta* 2005;**26**:372–379.

Xue WC, Chan KY, Feng HC, Chiu PM, Ngan HY, Tsao SW, Cheung AN. Promoter hypermethylation of multiple genes in hydatidiform mole and choriocarcinoma. *J Mol Diagn* 2004;**6**:326–334.

Yang T, Adamson TE, Resnick JL, Leff S, Wevrick R, Francke U, Jenkins NA, Copeland NG, Brannan CI. A mouse model for Prader–Willi syndrome imprinting-centre mutations. *Nat Genet* 1998;**19**:25–31.

Yoder JA, Walsh CP, Bestor TH. Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet* 1997;**13**:335–340.

Yokomine T, Kuroiwa A, Tanaka K, Tsudzuki M, Matsuda Y, Sasaki H. Sequence polymorphisms, allelic expression status and chromosome locations of the chicken IGF2 and MPR1 genes. *Cytogenet Cell Genet* 2001;**93**:109–113.

Yokomine T, Shirohzu H, Purbowasito W, Toyoda A, Iwama H, Ikeo K, Hori T, Mizuno S, Tsudzuki M, Matsuda Y *et al.* Structural and functional analysis of a 0.5-Mb chicken region orthologous to the imprinted mammalian Ascl2/ Mash2-Igf2-H19 region. *Genome Res* 2005;**15**:154–165.

Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001; **27**:153–154.

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

Zhang HJ, Siu MK, Wong ES, Wong KY, Li AS, Chan KY, Ngan HY, Cheung AN. Oct4 is epigenetically regulated by methylation in normal placenta and gestational trophoblastic disease. *Placenta* 2008;**29**:549–554.

Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. *Am J Obstet Gynecol* 2009;**200**:e661–667.