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ARTICLE

Epigenetic effects on the embryo as a result of periconceptional environment and assisted reproduction technology

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Following a Master's degree in assisted reproduction technology, Emma Lucas completed her PhD at the University of Nottingham in the laboratories of Professor Lorraine Young and Dr Chris Denning, examining the DNA methylation profiles of embryonic stem cells and differentiated fibroblasts for exploitation in epigenetic reprogramming. She then undertook a post-doctoral position in Professor Tom Fleming's laboratory, investigating the molecular and epigenetic mechanisms through which the mouse preimplantation embryo senses and responds to the maternal dietary environment. She is now a post-doctoral research fellow at the University of Warwick, in Professor Jan Brosens' laboratory, investigating the role of the epigenome of the human endometrium in recurrent pregnancy loss.

Abstract The early embryonic environment has been shown to be remarkably influential on the developing organism, despite the relative brevity of this developmental stage. The cells of the zygote and cleavage-stage embryo hold the potential to form all cell lineages of the embryonic and extra-embryonic tissues, with gradual fate restriction occurring from the time of compaction and blastocyst formation. As such, these cells carry with them the potential to influence the phenotype of all successive cell types as the organism grows, differentiates and ages. The implication is, therefore, that sublethal adverse conditions which alter the developmental trajectory of these cells may have long-term implications for the health and development of the resulting offspring. One confirmed mechanism for the translation of environmental cues to phenotypic outcome is epigenetic modification of the genome to modulate chromatin packaging and gene expression in a cell- and lineage-specific manner. The influence of the periconceptional milieu on the epigenetic profile of the developing embryo has become a popular research focus in the quest to understand the effects of environment, nutrition and assisted reproduction technology on human development and health.

KEYWORDS: assisted reproduction technology, DNA methylation, epigenetics, maternal environment, preimplantation

Introduction

As worldwide dietary and lifestyle habits have changed in the past century, interest has turned to the effects of both nutrient quality and quantity and of activity levels not only on the health of the individual but also on reproductive success and on the health of each subsequent generation. Equally, as the population of human children born after intervention with assisted reproduction technologies increases, it has become possible to learn more about their development through childhood, puberty and into adulthood. The increased understanding of the relationship between periconceptional environment and the long-term health and disease risk of offspring, derived from both

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human and animal studies, raises the issues of how to identify causative changes during early development and what recommendations can be made to reduce the risk of adverse effects. This review aims to examine current understanding of the sensitivity of the periconceptional period of development to environmental factors as well as how epigenetic modification of the genome might contribute to the translation of environmental cues into phenotypic outcomes.

Due to the limitations of studying human pregnancy and ethical implications of research intervention, as well as the limited availability of human embryos for research, mouse and other mammalian models (e.g. sheep, cow, rat, rabbit) have been used widely for developmental and epigenetic profiling studies. This review will focus largely on information gained from animal studies, although data from human studies will be highlighted wherever appropriate

Phenotypic similarities exist between human assisted reproduction children and offspring in animal models of both assisted reproduction treatment and suboptimal maternal diet during the periconceptional period, including the occurrence of cardiovascular changes, altered metabolic activity and body fat characteristics, behavioural changes, advanced bone development and altered immune system responsiveness (reviewed by Fleming et al., 2011; Sinclair et al., 2007). Thus, comparison of animal and human data is relevant and offers the opportunity to understand this sensitive period of development more clearly. These animal models of assisted reproduction treatment and maternal dietary effects are also associated with epigenetic changes in the embryo as well as in fetal and adult offspring and have provided the stimulus, as well as the tools, to investigate the epigenetic status of the human embryo. For example, DNA methylation changes are observed in fetal liver of sheep exposed to periconceptional methyl donor-deficient diet (Sinclair et al., 2007) and maternal dietary effects on epigenetic and health status can be ameliorated in rat offspring by folic acid supplementation (Lillycrop et al., 2005; Burdge et al., 2009).

Epigenetics and preimplantation development

Epigenetic modifications refer to the association of specific alterations to the DNA and its packaging within chromatin which do not alter the DNA sequence itself but which control the expression of associated genes. These include DNA methylation at cytosine bases, predominantly those located in a CpG context, which is a particularly stable and heritable mark (reviewed by Jones, 2012) as well as the modification of a number of histone tail residues, which alters the electrophysical relationships between the histone tail and local DNA as well as recruiting chromatin modifying proteins to the modified region (reviewed by Richly et al., 2010; Lennartsson and Ekwall, 2009). Studies of environmental effects on the human embryonic genome have focused predominantly on DNA methylation, due to a greater understanding of this modification and its more discrete role compared with the complex network of histone modifications. However, some histone modification data are available in animal models and will be discussed.

Whole-genome sequencing of DNA methylation in mouse oocytes and spermatozoa has confirmed that these cells exhibit highly different levels and localization of DNA methylation (Kobayashi et al., 2012). Following fertilization, rapid DNA demethylation occurs in the sperm-derived DNA prior to combination of the parental genomes at syngamy ready for first cell division (Ma et al., 2012; Smith et al., 2012). In the mouse embryo, DNA methylation is progressively lost from the maternally derived DNA until the blastocyst stage. At this point in development, the first major lineage specification event takes place resulting in an inner cell mass, which will give rise to the embryo and yolk sac, and in trophectoderm, which will form the placenta. Selective remethylation occurs in the inner cell mass and continues as lineage specification takes place (Smith et al., 2012, reviewed by Hackett and Surani, 2013). Studies up to the blastocyst stage suggest this process is largely conserved in human, albeit with paternal demethylation occurring to a lesser extent (Santos et al., 2010; Ma et al., 2012). Studies of mouse somatic cell nuclear transfer embryos suggest the process of DNA methylation remodelling targets specific genomic regions, likely through oocyte-derived factors, to ensure the appropriate methylation patterns are established for continuing development (Chan et al., 2012). Species variability in the DNA demethylation process has been observed (Beaujean et al., 2004a,b), most likely reflecting differences in the timing of embryonic genome activation and implantation between species. However, global and sequence-specific analyses confirm some conservation of preimplantation DNA methylation remodelling across species, including in the human embryo (Fulka et al., 2004; Beaujean et al., 2004a), as well as a failure of this process in abnormal somatic cell nuclear transfer embryos (Chan et al., 2012)

Studies in the mouse embryo demonstrate that histone tail modifications also undergo a remodelling process during the preimplantation period (Huang et al., 2007). Markers of mature heterochromatin, such as localization of heterochromatin protein 1 (HP1α) to highly compact chromatin regions and trimethylation of lysine 20 on histone 4 (H4K20me3), are removed in parallel to DNA methylation loss and/or are largely absent during preimplantation development, reappearing during mid-gestation in association with later lineage specification (Wongtawan et al., 2011).

A cascade of transcriptional activation in the human embryo, initiating at the 2-cell stage of development with significant activation from the 4- and 8-cell stages, confirms the importance of appropriate epigenetic remodelling during preimplantation development (Vassena et al., 2011; **Figure 1**). A failure to initiate transcription of specific genes may have downstream effects on the activation of specific expression pathways essential for successful development. In the human blastocyst, dynamic regulation of epigenetic genes such as DNA methyltransferases, chromatin modifiers and histone deacetylases has been reported during trophectoderm specification (Assou et al., 2012). This suggests that, as in animal models, the human trophectoderm lineage may be highly susceptible to epigenetic misregulation (Rugg-Gunn, 2012). However, the only human embryos available to research are from subfertile couples that have been produced and cultured *in vitro* — a considerable caveat to interpretation.

Certain sequences have been identified as being protected from DNA methylation remodelling, including

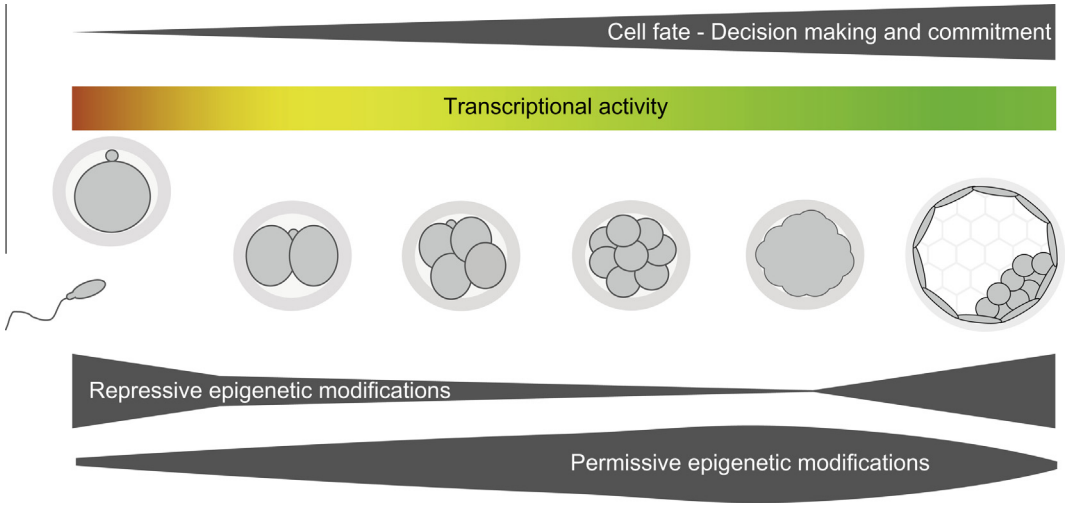

Figure 1 During preimplantation development, the highly specialized oocyte and spermatozoon undergo extensive chromatin remodelling in order to achieve the appropriate nuclear packaging for the initiation of embryonic gene expression. DNA methylation and repressive histone modifications are removed during cleavage development and gradually replaced as the embryonic lineages begin to differentiate at the blastocyst stage. Active histone modifications coincide with the initiation of embryonic transcription. The dynamic nature of this very early developmental window makes it susceptible to perturbation, the consequences of which may have a significant impact on downstream development.

repetitive transposon-derived sequences and imprinted genes (reviewed by Hackett and Surani, 2013), although absolute protection from remodelling is unlikely (Smallwood et al., 2011). Imprinted genes are of particular interest for two reasons. The first is that imprinted genes are frequently associated with control of growth and perturbation of their expression can therefore have significant downstream consequences. The second reason is that the expression level of imprinted genes is controlled by methylation of one allele in a parent-specific manner. The dosage-control mechanism imparted by this unique status makes them informative sequences to analyse in terms of the epigenetic variability induced by environmental changes. Although imprinted genes have been studied extensively in the context of developmental programming and epigenetic susceptibility to the periconceptional environment, a recent study suggests that these genes are neither more susceptible to perturbation due to their unique epigenetic status nor are they protected (Radford et al., 2012). Further, a recent systematic review has reported that in-vitro culture and maturation of oocytes does not increase the risk of imprinting disorders in several animal models, although data on human oocyte maturation is limited (Anckaert et al., 2012). These reports are consistent with variability in the methylation status of imprinted genes (Huntriss et al., 2013) and in their responsiveness to environment, reported both in studies of embryonic development (Puumala et al., 2012) and also in embryonic stem cell lines subjected to in-vitro mimics of altered maternal environment (Kim et al., 2007). The importance of imprinted genes in the developmental control of growth and placentation likely explains the high incidence of their alteration in those manipulations resulting in changes to offspring growth and development (Radford et al., 2012).

Epigenetic effects of assisted reproduction technologies

Emerging evidence suggests that the protocols and culture media used in human IVF can influence embryonic cell numbers and the birthweights of children from singleton pregnancies (Vergouw et al., 2012; Nelissen et al., 2012; Dumoulin et al., 2010), although comparisons to naturally conceived children are often lacking. Importantly, although birthweight is convenient to measure, it is not an infallible marker of adverse periconceptional environment and should not be used as the sole predictive measure of periconceptional environment on later health (Schulz, 2010). This highlights the need for animal studies to identify those markers which could be used to predict the long-term health of offspring exposed to suboptimal environments during the periconceptional period

Assisted reproduction protocols

Transcriptional analysis of human oocytes suggests that those exposed to in-vitro maturation (IVM) procedures retain an immature gene expression profile despite displaying morphological maturity (Wells and Patrizio, 2008) although IVM has been reported not to influence birthweight versus comparable cycles without IVM (Fadini et al., 2012). In mouse, IVM reportedly down-regulates the expression and protein concentrations of epigenetic modifiers GCN5 and HDAC1, associated with histone acetylation levels, in the oocyte and 2-cell embryo (Wang et al., 2010). No change in acetylation of their common target, histone H3, was observed and expression levels recovered by the later cleavage stages; however, it remains to be seen whether other proteins were compensating for the loss of these enzymes

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and also how the IVM process influenced the expression of the transcripts (Wang et al., 2010).

Globally, the extent of DNA demethylation in the paternal pronucleus of rat zygotes was reduced after IVF or intracytoplasmic sperm injection (ICSI) compared with in-vivo embryos (Yoshizawa et al., 2010), although the influence of in-vitro culture *per se* is unclear. Conflicting reports suggest that there either is no effect of culture on in-vivo fertilized rat embryos (Yoshizawa et al., 2010) or that culture itself limits the demethylation process (Zaitseva et al., 2007). Procedural variations cannot be excluded in these differing outcomes, but analysis of specific loci is lacking in both studies precluding further interpretation. In mouse embryos, incomplete DNA demethylation status by the 2-cell stage of development was influenced by in-vitro culture protocol as well as by the use of superovulation (Shi and Haaf, 2002). In mouse models, brief culture alone has been shown to be sufficient to induce phenotypic change in offspring following blastocyst collection and embryo transfer (Watkins et al., 2007) including raised systolic blood pressure and altered metabolic parameters.

Another study in mouse drawing comparison between IVF- and ICSI-produced blastocysts reported that the influence of ICSI on the blastocyst transcriptome was considerably larger than the effect of suboptimal culture conditions (to be discussed), with almost 25-fold more genes altered by ICSI according to microarray analysis (Giritharan et al., 2010). Interestingly, the same study found reduced trophoectoderm and inner cell mass cell numbers in ICSI embryos, despite morphological stage-matching, suggesting that growth may also be altered in these embryos (Giritharan et al., 2010). Although it is tempting to speculate that these differences may be due to epigenetic change, a recent report in human showed no difference in global DNA methylation levels and chromatin organization between IVF- and ICSI-produced embryos, although abnormal profiles in both groups were consistent with developmental failure (Santos et al., 2010). Furthermore, studies in human embryos and gametes suggest that the *H19* differentially methylated region (DMR) is not susceptible to culture-induced DNA methylation changes, since control blastocysts exhibited the expected paternal methylation as did 21 of 28 abnormal blastocysts examined (Ibala-Romdhane et al., 2011). In addition, although increased variability in DNA methylation has been reported in IVF twins at imprinted DMR (*H19*/*IGF2* and KvDMR1) compared with naturally conceived twins, the differences were not significant (Li et al., 2011b). The same authors report that IVF–embryo transfer in mice does not result in significant DNA methylation changes at imprinted loci, nor is there an increased risk of altered methylation in F2 and F3 generations (Li et al., 2011a). Less well examined is the possible role of changes to histone modifications. In mouse, IVF embryos have been found to exhibit lower levels of transcriptional activation-associated trimethylation at lysine 4 of histone H3 (H3K4me3) when compared with in-vivo fertilized embryos or IVF embryos treated with the deacetylase inhibitor Trichostatin A (Wu et al., 2012), which results in an associated increase in H3K9me3. Although implantation and development to birth and adulthood were not examined in this study, a failure to activate genes required for early post-implantation development may lead to alteration in the differentiative capacity of the embryonic cells which feasibly could impact upon the formation of the placenta or on the development of early lineages during gastrulation of the embryo; either scenario having the potential for longer-term impact on the offspring.

Cryopreservation strategies have also been shown to influence birthweight (Vergouw et al., 2012; Nelissen et al., 2012) and embryonic gene expression (Shaw et al., 2012), although interlaboratory variability in freezing and thawing protocols will make it difficult to identify specific factors involved in such outcomes.

Embryo culture media

A large-scale comparison of 13 human embryo culture protocols in a comprehensive mouse system revealed extremes of developmental success including litter size, cell number and gene expression variability depending on the media used (Schwarzer et al., 2012). Although the results cannot be extrapolated directly to potential effects in human embryo culture, this study addressed a considerable issue of human treatment – the inability to optimize conditions on the model in which they are intended for use. Large-scale animal studies are the only route through which such testing is available but the limitations are considerable. Alterations in cell number observed between culture protocols were attributed to an active process (i.e. early lineage decisions) rather than passive (i.e. cell death in a specific lineage) based on apoptotic labelling experiments (Schwarzer et al., 2012) which supports the hypothesis that the early environment indeed can influence the trajectory of the embryo via differentiation processes. These findings provide some explanation for the birthweight and pregnancy rate differences reported by Dumoulin and colleagues (2010) signifying that embryonic/extra-embryonic lineage decisions initiated during the culture period or indeed in response to the in-vivo environment may have a significant impact on development in human and confirming existing reports in animal models (Watkins et al., 2007, 2008; Makinen et al., 2012). Another study comparing a smaller panel of five commercially available human embryo culture media in a mouse model focused specifically on imprinted gene methylation, finding that all the media examined resulted in a loss of methylation versus in-vivo-derived embryos (between 7% and 50% loss depending on the media and locus) (Market-Velker et al., 2010). The loss of methylation was not dependent on the parental origin of the methylated allele as both paternally methylated (*H19*) and maternally methylated (*Snrpn* and *Peg3*) regions were affected (Market-Velker et al., 2010). Loss of methylation did not translate directly to an increase in expression of the imprinted allele in the same blastocysts and without an investigation into the post-implantation development of these embryos, it is difficult to extrapolate from methylation change to a physiological outcome for each individual media system. However, methylation change at these loci is associated with control of fetal growth in animal models and with imprinting disorders in human children (Butler, 2009; Gabory et al., 2010), therefore the importance of these findings should not be overlooked. A further finding of the paper was that the addition of superovulation to the protocol increased the loss of imprinting effect seen at the *H19*

DMR (Market-Velker et al., 2010), consistent with reports from human studies of increased incidence of the imprinting disorder BWS following complex assisted reproduction protocols (Chang et al., 2005). Indeed, superovulation in mouse has been shown to affect DNA methylation remodelling post fertilization (Shi and Haaf, 2002) and alter the expression of developmentally important genes by the morula stage (Linke et al., 2012). Interestingly, a further study found that altered methylation at imprinted loci in villus samples was associated with errors in the paternal spermatozoa, rather than with assisted reproduction treatment itself, and although these samples had often undergone ICSI, the procedure itself was not the origin of the imprinting error (Kobayashi et al., 2009).

A comparison of the effects of two in-vitro culture media systems on the placental transcriptome in mice found that both systems, M16 (a single complex mouse embryo culture media) and G1/G2 (a two-step simplex media system), resulted in a significant alteration in the gene expression profile of the mid-gestation placenta in comparison to in-vivo-produced controls (Fauque et al., 2010). Predominant changes were seen in X chromosome transcripts and imprinted genes, both being largely induced, while across the whole genome, affected transcripts were repressed under both culture conditions (Fauque et al., 2010). Placental transport efficiency has been demonstrated to depend on appropriate expression of imprinted genes (reviewed by Angiolini et al., 2006); thus, epigenetic and gene expression changes within this tissue or its originating trophoblast lineage will clearly influence maternal–fetal transport and thus the growth and health of the developing fetus.

Alterations in histone modifications during preimplantation mouse development are sufficient to result in altered gene expression in adult animals and are reportedly transmitted to the following generation. Preimplantation culture with fetal calf serum to provide suboptimal conditions altered the tail phenotype of *Axin1*^{Fu} mice, resulting in a significant increase in the tail kink phenotype observed in these mice (Fernandez-Gonzalez et al., 2010). This was observed in association with reduced trimethylation at H3K9 and increased acetylation at the same residue, consistent with the phenotype observed after culture with trichostatin A. Culture alone, with or without fetal calf serum, also altered the dimethylation of H3K4 (Fernandez-Gonzalez et al., 2010).

Preimplantation environment and post-implantation epigenetic change

Epigenetic and gene expression changes initiated in response to the preimplantation environment may be important not only at the time of initiation but may have implications for downstream development. The success of implantation and placentation is one medium-term outcome which may be affected, although this period of development is almost impossible to access in humans except in the case of pregnancy loss or termination.

Initial analyses have revealed epigenetic changes in chorionic villus samples from spontaneously aborted pregnancies versus induced abortions at a small number of loci, including *H19* hypermethylation. However, no difference between spontaneously aborted samples from normal conception or IVF/ICSI pregnancies was observed (Zheng et al., 2012). Similarly, Zechner et al. (2010) observed no difference in chorionic villus methylation between IVF and ICSI pregnancies after spontaneous loss, nor an increased rate of epimutation in assisted reproduction pregnancies overall versus natural conception. The difficulty in interpreting these data are that they do not inform on methylation status of fetal tissues, although clearly there are significant issues precluding such analysis. Placental tissue is widely reported to be hypomethylated in relation to fetal tissue and may exhibit more natural variability (reviewed by Yuen and Robinson, 2011; Rugg-Gunn, 2012) Furthermore, whether epigenetic abnormalities are causative or simply a result of upstream alterations in the developmental programme is not known and may be answered only by animal model studies.

Comparison of villus trophoblast and decidual samples from normal patients and early pregnancy loss patients revealed a reduced level of the maintenance DNA methyltransferase *DNMT1* expression in early pregnancy loss trophoblast, although decidual levels did not differ (Yin et al., 2012). This suggests that abnormal methylation characteristics in the embryonic tissues may be related to early pregnancy loss at a global level, despite the findings of the locus-focused reports. However, altered methylation levels in spontaneously aborted tissue have been reported to be independent of conception method (Zheng et al., 2011). In a sheep model, reduced placental expression and activity of *DNMT1* is associated with early losses of in-vitro-produced embryos (Ptak et al., 2012). Furthermore reduced mRNA expression levels of *DNMT1* cofactors, *UHRF1* and *PCNA* were also observed (Ptak et al., 2012). *DNMT1* concentrations were comparable to control concentrations in those pregnancies surviving past 24 weeks gestation, suggesting that intact methylation pathways are correlated with developmental competence in in-vitro-produced offspring (Ptak et al., 2012). Interestingly, mouse embryos exposed to the DNA methyltransferase 1 inhibitor procainamide showed a reduction in their ability *in vitro* to adhere to and invade an Ishikawa cell monolayer (Yin et al., 2012). Furthermore, administration of procainamide to the mouse uterus at the time of embryo implantation resulted in reduced implantation success, reduced embryonic DNA methylation and increased fetal abnormalities (Yin et al., 2012).

Together, these findings confirm that adverse epigenetic change in developing trophoectoderm may feasibly result in downstream pregnancy loss or altered placental efficiency. Further investigation will be necessary to link directly the preimplantation environment and post-implantation developmental changes to determination of adult phenotype, health and disease risk.

Influence of the maternal dietary environment

Although there is much data emerging now from human assisted reproduction cohorts, a considerable contribution to the understanding of periconceptional sensitivity has been made by the study of maternal dietary effects on the developing embryo and resulting offspring (Fleming et al., 2011). Clearly, the diversity of human diets and the

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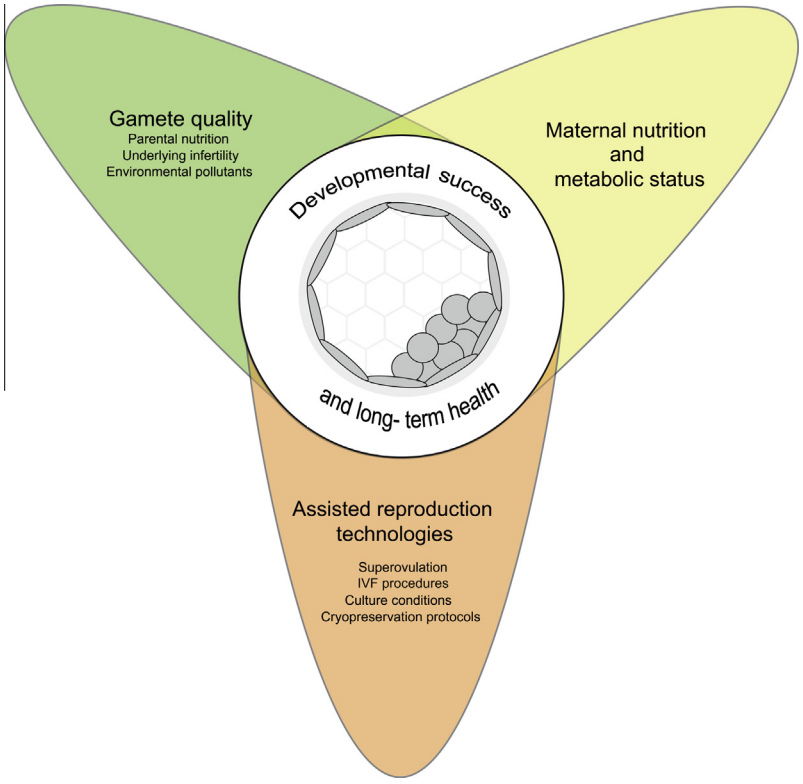

Figure 2 Successful embryo development (and indeed development to adulthood) is influenced by numerous intrinsic and environmental factors. The combination of gamete quality, influenced by parental environment and genetics, and conception method will have immediate consequences on preimplantation development. The influence of the environment *in utero* may then modify the developmental trajectory in response to nutrient cues and other environmental effects. Interaction between these and other factors will ultimately determine developmental success.

limitations of intervention during pregnancy mean that much of the data originates from animal studies.

Periconceptional macro-nutrient content and maternal physiology

Macro-nutrient studies of maternal nutritional influence (protein, fat, carbohydrate content) have been characterized broadly and reviewed thoroughly elsewhere (Sandovici et al., 2012; Fleming et al., 2012). Maternal metabolic status has been shown to influence the histone modification profile of mid-gestation fetuses in mouse, with specific alterations attributable to different metabolic profiles such as maternal diabetes or obesity (Bermejo-Alvarez et al., 2012). Such changes leading to altered gene expression of specific developmentally important genes, such as those involved in neural tube closure, have clear implications for developmental success, but in the case of small widespread changes in gene expression, the outcomes may simply result in phenotypic variation rather than poor health or disease.

Diet-induced obesity in mice has been shown to result in reduced fertility via effects on ovarian function and cyclicity and gene expression changes are observed in obtained blastocysts despite apparently normal development (Bermejo-Alvarez et al., 2012). High fat feeding from conception to mid-gestation has been shown to result in altered DNA methylation of mouse placenta in a sex-specific manner, with female placentae becoming globally hypomethylated (Gallou-Kabani et al., 2010). However, sexual dimorphism in placental methylation even in control pregnancies highlights the complexity of interpreting such data (Gallou-Kabani et al., 2010; Gabory et al., 2012). Differential sensitivity of the sexes to maternal diet or the in-vitro periconceptional environment will make the predictive power of extrapolating to human development even more difficult.

Periconceptional dietary micro-nutrient content and epigenetic change

A pilot study in human subjects reported that preconception maternal dietary supplementation with micronutrients including folate, zinc and B vitamins resulted in altered DNA methylation at imprinted loci (Cooper et al., 2012). Cord blood DNA samples showed a significant sex-specific reduction in DNA methylation at the *IGF2R* DMR in female offspring and at the *GTL2* DMR2 in male offspring, although these differences were not evident in DNA from the same infants at 9 months old (Cooper et al., 2012). In addition, methylation differences became evident in female infants at 9 months of age at the *GNASAS* and *PEG1* DMR suggesting longer-term influences of maternal diet on the offspring epigenetic profile (Cooper et al., 2012). However, in the case of both cord and peripheral blood samples, gene expression

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analysis was not reported so it is not possible as yet to determine a functional effect of these methylation changes.

A prospective study focusing specifically on maternal folate status in human pregnancy and offspring methylation found no association between periconceptional or first-trimester supplementation and offspring imprinted gene (*PEG3*, *IGF2*, *SNRPN*) or *LINE-1* methylation in umbilical cord blood (Haggarty et al., 2012); however, supplementation in the second trimester of pregnancy was associated significantly with increased methylation of the *IGF2* DMR and decreased methylation of *PEG3* and *LINE-1* sequences (Haggarty et al., 2012). In another report, maternal folic acid supplementation alone during the periconceptional period has been linked to the methylation status of *IGF2* DMR in whole-blood genomic DNA from 17-month-old children, with increased methylation levels associated to supplementation and with an inverse correlation between methylation status and birthweight (Steegers-Theunissen et al., 2009). Although blood sampling is a relatively easy source of DNA for analysis, it is unclear how readily methylation and expression data in blood can be translated to more global effects. The relatively short lifespan of blood cells suggests that the haematopoietic precursors would need to be affected in order to see long-term effects in blood samples. Clearly this is more difficult to examine. Although the link between folate availability and haematopoiesis is an important one, the role of folate in the methyl cycle and one-carbon metabolism will contribute to methylation status in many tissues. For example, periconceptional deficiency of methionine and B vitamins, including folate, in sheep resulted in alteration of around 4% of loci examined by a global screening method, RLGS, in offspring liver (Sinclair et al., 2007). Of these 88% were either hypomethylated or completely unmethylated (Sinclair et al., 2007) suggesting a consistent directional response to changes in maternal nutrient availability during the periconceptional period.

Conclusions

Early embryonic development is a period with remarkable influence on the later health of the developing organism. The role of the preimplantation environment in the success of early development cannot be underestimated (**Figure 2**). However, while animal studies have shown clear interactions between the periconceptional environment, be it *in vivo* or *in vitro*, and both short- and long-term developmental measures in the offspring, conflicting reports arise from human studies. This is attributable in large part to the presence of multiple confounding issues (genetic, lifestyle and clinical) and the difficulty in studying early human development. However, as discussed in this article, it appears that in some cases the animal findings may translate directly. The merit of animal work lies in the ability to control the elements that confound human research and identify causal relationships between the embryonic environment and the ongoing developmental success and health of the offspring. The difficulty lies in the application of such findings to population advice both without exaggeration and with realism. With increasing use of assisted reproduction treatment and rising maternal ages, as well as the trend towards obesity and associated health issues in Western societies there is an increasing need to understand the environmental effects on gamete and embryo development and the potential for these effects to be transferred to subsequent generations.

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