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Minireview

The Embryo and Its Future[1]

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ABSTRACT

The preimplantation mammalian embryo from different species appears sensitive to the environment in which it develops, either in vitro or in vivo, for example, in response to culture conditions or maternal diet. This sensitivity may lead to long-term alterations in the characteristics of fetal and/or postnatal growth and phenotype, which have implications for clinical health and biotechnological applications. We review the breadth of environmental influences that may affect early embryos and their responses to such conditions along epigenetic, metabolic, cellular, and physiological directions. In addition, we evaluate how embryo environmental responses may influence developmental potential and phenotype during later gestation. We conclude that a complex of different mechanisms may operate to associate early embryo environment with future health.

conceptus, early development, embryo, epigenetics, female reproductive tract, fetal and postnatal development, gene expression regulation, metabolism, pregnancy

INTRODUCTION

Several studies using animal models have shown that preimplantation embryos are sensitive to environmental conditions that can affect future growth and developmental potential, both pre- and postnatally. These studies initiated with the observation that cultured mouse embryos, after transfer, resulted in reduced fetal growth compared with that of in vivo counterparts [1, 2]. Further studies on the mouse have confirmed this effect and identified possible culture conditions, particularly inclusion of serum, which may act deleteriously [3–6; see also reviews 7, 8]. For example, embryos cultured in medium containing serum had a reduced viability and, after transfer, gave rise to fetuses at Day 14 that were 20% smaller than those derived from embryos cultured in the absence of serum or developing in vivo before transfer [6].

In addition to these rodent examples, early embryos from ruminants also display environmental sensitivity, which may, after transfer, lead to the condition known as large offspring syndrome (LOS). LOS has been identified in both sheep and cows and is characterized by aberrant fetal and placental development, increased fetal myogenesis, dystocia, dysfunctional perinatal pulmonary activity, organomegaly, and increased mortality in early postnatal life [9, 10]. Those animals that survive maintain features of abnormal organ sizing during later life [11]. LOS occurrence is also associated particularly with the use of sera and complex media [12, 13]. In addition, cloned embryos derived from somatic nuclear transfer in both ruminants and mice experience similar abnormalities in fetal growth, reduced viability, and increased perinatal death [8, 14]. The human embryo must also be considered as potentially at risk to environmental conditions that may have long-term consequences [15–19]. Several, but not all, outcome studies show an increase in preterm delivery, low birth weight, and perinatal mortality in singleton pregnancies following assisted reproduction technologies (ART) compared with natural conception [18, 20–24]. However, the breadth of clinical and technical parameters that may complicate the interpretation of human studies needs to be borne in mind [18]. Nevertheless, an association between low birth weight and ART is a cause for concern because susceptibility to adult-onset diseases such as coronary heart disease (CHD), stroke, hypertension, type II diabetes, and osteoporosis, while having adult life-style risk factors, correlate independently and more strongly with early life factors such as low weight and small size and thinness at birth [reviewed in 25, 26].

Abnormalities in developmental potential arising from embryonic environment are not limited to in vitro culture and may arise from the specific conditions experienced in vivo. Maternal diet can impact both on preimplantation phenotype and long-term development. High-protein diets in sheep during the periconceptional period have been associated with reduced developmental viability and increased fetal and birth weights as observed in LOS [27, 28]. A low-protein diet fed to rats exclusively during the preimplantation period before return to control diet for the remainder of gestation is associated with several changes in postnatal phenotype even though offspring were fed a normal diet. These effects included low birth weight, subsequent overcompensatory adolescent growth, onset of adult hypertension, and alterations in relative organ sizing in a gender-specific manner [29]. Periconceptional maternal undernutrition in sheep has also been shown to affect later fetal development and physiology [30, 31] and can also

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influence the response to undernutrition in late gestation through its influence on fetal growth factor expression [32]. Poor nutrition around the time of conception has further been reported to influence fetal growth and birth weight in the human [33]. In a related context, maternal micronutrient intake periconceptionally, particularly of folic acid and B_{12} vitamin, which act as methyl donors important in DNA and protein modification, has long-lasting effects contributing to reduced incidence of neural tube defects later in development [34].

Collectively, these in vitro and in vivo studies lend support to the notion that an embryo's environment is critical for its future. The concept is a general one, evident across species, although species-specific differences arising in fetal growth rate are apparent. In the human, the relationship between smallness at birth and susceptibility to adult-onset disease largely derives from maternal undernutrition during pregnancy and intrauterine growth restriction. In global terms, therefore, the contribution that embryo environment in vivo may make to later fetal growth is an important issue to evaluate and the parallel analysis of in vivo and in vitro models is required to understand mechanisms. Moreover, the phenotypic consequences of early embryonic environment are potentially broad. Apart from growth, cardiovascular and metabolic conditions, low birth weight in humans may also be a significant risk factor for development of different neurological disorders, including psychopathologies associated with stress, such as depression and suicide [35] and the condition of autism [36]. An important and well-controlled study has recently demonstrated that mouse embryo culture conditions resulted in an altered behavioral activity in offspring following embryo transfer [37].

What mechanisms relate preimplantation environment with long-term developmental potential? Is there a single underlying cause or are multiple interactive processes responsible? The links between early environment and programming of fetal or postnatal growth and phenotype are being actively pursued by several laboratories using different animal models. This review will evaluate our current understanding of underlying mechanisms (Fig. 1).

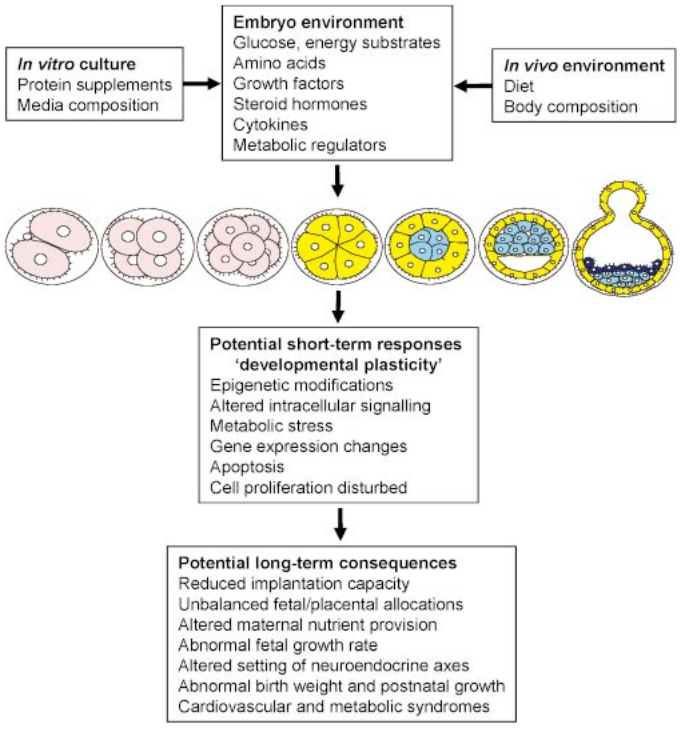

FIG. 1. Schematic representing the potential interactions between the environment of the embryo, either in vitro or in vivo, the embryo's short-term responses, and their long-term consequences. Different stages and lineages of embryo development are shown in different colors representing undifferentiated cells (pink), trophectoderm (yellow), ICM (pale blue), and primitive endoderm (deep blue).

AN EPIGENETIC BASIS FOR EMBRYO-DERIVED PROGRAMMING

There is growing and convincing evidence from analysis of different species that epigenetic events in the early embryo contribute to altered developmental potential [7, 38]. Imprinted genes, normally showing allele-specific expression due to epigenetic modification at regulatory CpG islands (differentially methylated regions, DMRs) mediated by the pattern of DNA methylation [38], appear particularly sensitive in this respect. Modification to histone proteins, including acetylation and methylation, occur dynamically and in concert with DNA methylation [39]. The regulatory roles of imprinted genes encoding insulin-like growth factor 2 and its receptor (Igf2, Igf2R) in fetal growth [40, 41] and of Igf2, Mash-2, Ip1, Peg-3, and other genes in placental growth and nutrient supply [42–45] make these potentially sensitive targets for embryo epigenetic modification [38, 46].

Gametogenesis and early development are critical periods for the erasure, acquisition, and maintenance of genomic imprints. Although genome-wide demethylation of nonimprinted genes occurs consecutively on paternal and then maternal alleles by active and passive processes, respectively, during mouse preimplantation development, the gametic methylation pattern on imprinted genes needs to be preserved for future allele-specific expression [38]. Preservation of imprinted gene methylation status during preimplantation development appears critically dependent on the oocyte DNA methyl transferase variant, DNMT1o [47, 48]. Thus, exclusion of DNMT1o from the nucleus during early cleavage may be responsible for global demethylation of the nonimprinted maternally derived genome [47].

The pattern of methylation of imprinted genes has been shown to alter in a gene-specific manner in response to embryo culture conditions, indicating effects of the environment on the maintenance of their methylation profile. Thus, mouse embryos cultured in Whitten medium show deregulation of the allele-specific methylation and expression profile of the H19 gene (regulator of IGF2), while embryos cultured in KSOM medium containing amino acids maintained the normal paternal methylated imprint at the regulatory domain and a maternal allele-specific expression profile; corresponding culture effects on the Snrpn-imprinted gene were not observed [49]. There is growing evidence that culture-mediated epigenetic changes are heritable during cell cycling. Thus, Igf2 and H19 expression are reduced and H19 DMR hypermethylated in mouse fetuses that show reduced growth rate following embryo culture in medium containing serum compared with medium lacking serum [6]. Conversely, Igf2R expression, but not that of Igf2, is significantly reduced in fetal sheep exhibiting LOS, thereby enhancing the bioavailability of Igf2 and likely contributing to the syndrome [50]. Bovine fetuses derived from in vitro production also show higher expression of Igf2 in liver than do in vivo controls [51]. Mouse extraembryonic tissues may also show disturbed H19 methylation following embryo culture [52]. Moreover, male rat

embryos derived from mothers fed a low-protein diet during preimplantation development exhibit reduced expression of H19 in the extraembryonic blastocyst trophectoderm lineage [53]. Cloned mouse and bovine embryos also show abnormal epigenetic organization with incomplete reprogramming of imprinted gene methylation pattern in the donated somatic cell nucleus [14]. Indeed, mice generated completely from embryonic stem (ES) cells show aberrant fetal growth and increased perinatal mortality associated with abnormal expression and methylation of Igf2 and Igf2R gene clusters in the original cultured ES cells, which is maintained during later development [54]. Abnormal epigenetics and expression of imprinted genes are also associated with the human aberrant fetal growth and neurological syndromes such as Beckwith-Weidemann, Prader-Willi, and Angelman [19, 38].

EMBRYO METABOLISM AND FUTURE HEALTH

The causal mechanisms relating embryo environmental sensitivity with abnormal fetal and postnatal development are unlikely to reside exclusively at the epigenetic level. We need to consider how embryos interact with their normal in vivo environment; how this interaction may alter under suboptimal in vitro or in vivo conditions; and what type of responses may occur in embryos to compensate, a measure of their developmental plasticity, essential for survival. Disturbance in developmental and/or nutrient cues across the maternal-embryonic interface may have long-lasting effects given the evolutionarily favorable concept that the setting of fetal growth trajectory may derive from a perception of maternal nutrient supply gained at an early stage of development. We also need to evaluate the contribution of egg quality to this assessment of plasticity and potential, an issue covered elsewhere in recent reviews [55–57]. In this context, diets high in energy or protein applied in ruminants prior to conception reduce oocyte fertility and developmental potential due to exposure to elevated urea levels [58].

The ionic and nutrient composition of the oviduct and uterus experienced by cleavage-stage embryos has been reviewed recently [8, 59]. There is some evidence that energy substrate levels may vary between human oviduct and uterine fluids [60] and that nutrient levels are relatively low compared with those routinely present within culture media [61, 62]. However, data are scarce with regard to metabolite composition experienced by embryos in vivo at different stages of cleavage. In contrast, we know from extensive in vitro studies with human and different animal models that metabolic activity and substrate preferences appear to change between early and late cleavage with elevated glucose and oxygen consumption evident as embryos approach cavitation [63, 64; reviewed in 62]. However, nutrient consumption data vary dependent on in vitro culture composition and conditions, and the extent of exchange between the embryo and its environment is further complicated by the extent of endogenous stores [8, 62].

In vivo, other factors regulating nutrient exchange will derive from the maternal hormonal status responsive to dietary intake. Thus, progesterone level during the first 3 days of development in sheep has been shown to enhance subsequent fetal growth [65, 66]. Recently, ghrelin, the growth hormone secretogogue receptor (GHSR) ligand, implicated in modulating feeding behavior and energy metabolism, has been identified in mouse uterine fluid and endometrium as well as in morula and blastocyst stages [67]. Embryos also express GHSR, and ghrelin is inhibitory to blastocyst development in vitro; uterine levels of ghrelin increase during fasting, suggesting it may modulate embryo metabolic demands in line with maternal nutrient availability [67]. Although such environmental conditions will influence nutrient exchange kinetics, from the perspective of the embryo, it has been proposed that low metabolite consumption rates (quiet metabolism) more closely equate with developmental competence and viability than do high consumption rates (active metabolism), which are characteristic of metabolic stress [62, 68]. Embryo metabolic parameters may therefore be a significant contributor to the programming of future growth and physiological activity.

Hyperglycemia and Metabolic Stress

Hyperglycaemic conditions illustrate the association between embryo metabolism, stress, and future potential. Although glucose consumption rate does increase in late cleavage, oxidative phosphorylation and not glycolysis is the primary source of energy production in blastocysts across the species due to the corresponding elevation in oxygen consumption [62, 64]. In fact, elevated glucose metabolism, particularly via glycolysis, can be viewed as a stress response in embryos [15, 68], and mouse blastocysts showing high glycolytic rates have a lower capacity for implantation [69]. Sheep embryos collected from ewes fed a high-protein diet during the periconceptional period exhibit a higher metabolic rate and consume more glucose, associated with reduced developmental potential, than do embryos from control diets [27].

While elevated glycolysis is an indicator of metabolic stress within embryos, the extent of glucose uptake itself has been shown to be a positive indicator of fetal developmental potential after transfer [70]. However, in rodent diabetic models where a hyperglycemic embryo environment with significantly elevated glucose levels are utilized, embryo potential is reduced. Here, high external glucose acts to deplete glucose transporter expression at both mRNA and protein levels [71], leading to glucose starvation and activation of apoptotic pathways, chromatin degradation, and nuclear fragmentation [72], which culminate in reduced blastocyst cell numbers [73]. Similar responses to high glucose have been identified in bovine embryos [74]. These detrimental effects in embryos can also derive from perturbation in oocyte maturation in response to high glucose levels [75]. Following embryo transfer, embryos derived from a diabetic environment show increased resorptions and pregnancy loss, illustrating the link between embryo stress response and future developmental capacity. Similar reduction in longer term potential has been shown to occur in response to inhibition in glucose transporter activity during mouse cleavage [76]. A deficient inner cell mass (ICM) cell number has also been identified as a potential causative component of fetal growth retardation and large placenta, which are characteristic of the inherited BB/E diabetic rat model [73].

The effect of high glucose concentration on the embryo in diabetic models may also disturb later morphogenesis due to a breakdown in normal inductive interactions between blastocyst ICM and trophectoderm lineages by downregulation of ICM Fgf-4 expression required for maintaining trophectoderm proliferative activity [77]. Further support for the importance of embryo glucose/insulin balance has been demonstrated following short-term exposure of preimplantation mouse embryos to insulin, shown to stimulate ICM proliferation [78], which caused a long-term

increase in fetal growth rate after transfer [79]. Hyperglycemia is also implicated in the rat low-protein diet models. This diet fed to dams exclusively during the preimplantation period caused transient, mild maternal hyperglycemia associated with blastocyst cell number depletion and abnormal programming of postnatal growth [29]. When the diet is applied 2 wk before mating and during postimplantation development, a transient upregulation of glycolysis is evident in isolated, intact rat conceptuses during 9.5–10.5 days of development coincident with organogenesis [80].

Reactive Oxygen Species

A further stress identified in embryos in response to culture conditions is an increase in hydrogen peroxide production and attendant risk from reactive oxygen species (ROS) [81, 82]. Oxidative stress in the bovine embryo has been shown to lead to DNA damage [83]. ROS exposure will enhance the demand for antioxidant enzymes to maintain homeostatic control, which may further compromise developmental potential [84]. ROS damage in bovine embryos is suppressed by inclusion of vitamin E in culture medium, which stimulated development both before and after embryo transfer [85].

The implications of impaired metabolic activity within embryos for fetal and postnatal development are potentially serious, but direct consequences are yet to be explored mechanistically. Elevated glucose levels can lead to suppression of insulin and glucokinase expression, decreased mitochondrial function, increased ROS formation, and accelerated apoptosis, as well as activation of common stress-activated signaling pathways, which could readily influence proliferative, metabolic, and neuroendocrinal axes during later development [reviewed in 86, 87]. For example, one major intracellular target of hyperglycemia is the transcription factor nuclear factor-$_k$B (NF-$_k$B), which in turn can regulate the expression of diverse growth factors, cytokines, and adhesion molecules, all of which have the potential to modulate the phenotypic response to early embryo environment [87].

Amino Acids

Another and perhaps better example of adverse embryo programming working through metabolic pathways concerns amino acids and their turnover by the embryo. This is not surprising given the multitude of roles ascribed to amino acids in early development; in addition to protein biosynthesis, they also stimulate activation of the embryonic genome, blastocyst formation and hatching, and contribute to energy production, osmoregulation, pH control, cell homeostasis, and, perhaps significantly, to signal transduction cascades [88]. The embryo is equipped with an array of sodium-dependent and -independent transporter systems to regulate amino acid flux and availability [88]. Clear differences in embryo amino acid intracellular content have been demonstrated between in vivo and in vitro developing mouse embryos, indicative of environmental influence on exchange rates [89]. Indeed, amino acid turnover has been shown to vary between individual human embryos assayed noninvasively during early cleavage. Significantly, the pattern of exchange correlated with capacity to form a blastocyst, with those embryos showing lower turnover exhibiting greater viability [90], supporting the concept of a quiet metabolism being linked with future potential [62, 68]. Culture of mouse embryos with amino acids has also been reported to enhance fetal development after transfer [91]. In addition, culture of sheep embryos with amino acids leads to significantly improved fetal development than in medium containing serum [12]. The correct balance of amino acids is important because amino acids, particularly glutamine, may spontaneously break down in culture to produce ammonium ions, which can be deleterious to development in the short- and long-term. Addition of ammonium to mouse culture produced a concentration-dependent reduction in blastocyst and ICM cell numbers, increased apoptosis, and altered the pattern of H19 gene expression; this treatment, after transfer, also reduced the implantation rate, impaired fetal development at Day 15, and caused a higher rate of exencephaly [92, 93].

The importance of amino acid environment during early development is further implicated from in vivo studies. Maternal low-protein diet administered to rats during the preimplantation period induced a transient reduction in maternal serum essential amino acid concentrations, associated with reductions in blastocyst ICM and trophectoderm (TE) cell numbers and leading to abnormal programming of growth and hypertension postnatally [29]. Our current studies reveal, using the mouse model, that maternal protein undernutrition alters the profile of amino acids present within the uterine fluid during the period of morula and blastocyst occupancy, prior to implantation [94]. These studies further indicate that specific amino acids, notably the branch-chain group of leucine, isoleucine, and valine, are significantly depleted in both serum and uterine fluid in response to low-protein diet [29, 94]. Significant uptake of these amino acids has been shown to occur during blastocyst formation and expansion in vitro, suggestive of a critical developmental role [95]. Leucine in particular has a key signaling function in early development as activator of the mammalian target of rapamycin (mTOR), a serine-threonine kinase pathway that phosphorylates regulatory targets involved in protein translation and biosynthesis [96, 97]. The mTOR signaling has been associated with several growth and patterning events and phenotypic changes during development and differentiation [98, 99]. Significantly, in the early embryo, mTOR activity has been shown to be required for inducing an invasive and migratory behavior in trophoblast cells during the implantation period [100] and, through this activity dependent on amino acid transport, could coordinate downstream signaling pathways within the embryo [101].

In another mechanistic direction, changes in amino acid environment to embryos may lead to abnormal epigenetic effects. Maternal low-protein diet fed to rodents during the periconceptional period led to elevation in maternal serum homocysteine levels [102], which may cause folate deficiency and interfere with methyl group donation required for DNA methylation [103]. The importance of maternal dietary methyl supplementation on epigenetic regulation and the extent of DNA methylation has been demonstrated in the *Agouti* mouse model [104, 105].

FERTILIZATION EVENTS AND DEVELOPMENTAL POTENTIAL

A further mechanism that may associate egg and embryo environment with future developmental potential is the number or pattern of calcium oscillations that take place upon oocyte meiotic maturation and activation and that control cortical granule exocytosis and cell-cycle resumption at fertilization [106]. Altering the calcium oscillation number experimentally has also been shown to affect the

relative number of ICM and TE cells in the blastocyst [107], the rate of apoptosis [108], and the extent of fetal development of parthenogenetic embryos [107, 109]. Whether these longer term effects are mediated through epigenetic pathways influencing gene expression or cell cycling remains to be determined.

FROM METABOLISM TO GENE EXPRESSION AND HOMEOSTASIS

The disturbance in embryo metabolism, proliferative capacity, signaling competence, and maternal developmental cues associated with a suboptimal environment, either in vitro or in vivo, will activate a condition of stress, which in turn will promote further responses within embryos to maintain homeostatic balance [15, 16]. Environmental influence on embryo gene expression is a sensitive measure of stress; increased expression of hsp70.1 and the growth-arrest gene CHOP-10 occurs in response to diverse stresses in rodent and bovine embryos [110, 111]. Expression of growth-regulating genes is also susceptible to the environment. Mouse embryos developing in culture express lower levels of the growth factors Igf-1 and -2 than do embryos in vivo [112, 113], while culture in KSOM medium with amino acids results in gene expression of Igf-1 and -2 and their receptors at a higher level that in embryos cultured in Whitten medium and more similar to in vivo embryos [114].

A range of metabolic and differentiation-related genes has been shown to alter their expression profile in response to culture conditions in bovine embryos [115, 116]. For example, genes critical in trophectoderm differentiation and intercellular junction formation are expressed at different levels dependent on in vivo or in vitro derivation and, for the latter, the type of medium used [117]. In the case of the human, in vitro-cultured embryos show significant variation in expression of genes involved in apoptotic [118, 119] and differentiation [120, 121] pathways and show wide variation in apoptotic rate and blastocyst cell numbers [122], indicative of variable developmental potential.

EMBRYO PROLIFERATION AND FUTURE POTENTIAL

Environmental factors commonly influence embryo proliferation (or apoptosis) and blastocyst ICM and trophectoderm cell numbers. This has been demonstrated in various species, including the human, following in vitro culture in response to growth factors, amino acids, glucose, and many other factors [15, 123, 124]. Similarly, in vivo embryo development in mice in response to maternal age [125], periconceptional protein undernutrition [29], or periconceptional zinc deficiency [126] can coincide with reduced blastocyst cell numbers.

What is the significance of depleted blastocyst cell numbers in the propagation of embryo environmental response into future development? At one level, reduced proliferation can be viewed merely as a symptom or marker of metabolic stress as discussed above. However, the stem cell pool for fetal and extraembryonic lineages is limited and, if depleted, may influence the pattern of later development. Past experimental studies using mostly the mouse have demonstrated some resilience and regulative capacity within embryos to reductions in cell numbers mediated by different manipulative procedures such as cell ablation, embryo bisection, or by mitomycin treatment to deplete the ICM [127–132]. These studies have revealed that, during postimplantation development, from about Day 12 or later, proliferative control mechanisms may compensate for early depletion of cells and result in reasonable rates of fetal survival. Nevertheless, artificial reduction in embryo cell numbers may significantly decrease the proportion of ICM derivatives [128], reduce the rate of primitive endoderm formation and the size of egg cylinder stage conceptuses up to Day 8 after transfer, delay the timing of gastrulation, retard morphogenesis, and significantly increase the rate of pregnancy loss [127, 129–132]. However, during postnatal development, mice derived from half embryos grow at similar rates to controls [133]. It is also worth remembering that the early mouse embryo is equipped with self-regulating mechanisms to maintain ICM and TE cells and their ratio within relative narrow limits, indicating their importance to future development. Thus, while considerable variation may occur between embryos in allocation of cells to the ICM lineage during the 16-cell morula period, the second and final allocation at the 32-cell transition is inversely related to the first [134]. This may compensate for inter-embryo variation and appears to be mediated by a combination of cell interactions, mitotic spindle orientation, and cell shape changes [134–136].

There is good evidence that blastocyst proliferative potential and relative lineage sizes influence future growth and viability. Quantitative measurements of the human ICM are highly indicative of blastocyst implantation potential with larger ICM size increasing potential [137]. Moreover, a significant relationship between mouse blastocyst and ICM cell numbers, stimulated by amino acid availability during in vitro culture, and fetal viability after transfer has been reported [91]. Reduced blastocyst cell numbers have also been implicated in the poorer developmental potential of in vitro-cultured bovine embryos compared with in vivo counterparts [138] that may lead to LOS. Similarly, reduced trophectoderm and ICM cell numbers in rat blastocysts following periconceptional maternal low-protein diet is associated with postnatal abnormal growth and hypertension [29]. In this latter model, if low-protein diet is extended to Day 10 of development, the size of the conceptus and visceral yolk sac (VYS) are reduced compared with controls [29]. The VYS is derived from the ICM and performs a critical role in fetal nutritive support through endocytosis of maternal proteins, their lysosomal degradation, and release as amino acids for biosynthetic activity within the fetus [139]. Recently, we have investigated the effect of maternal low-protein diet on mouse VYS endocytic activity and found that maternal undernutrition reduces the rate of receptor-mediated protein (BSA) uptake and amino acid release per unit weight of yolk sac protein (unpublished results). This suggests that depleted ICM cell numbers can associate with altered physiological activity of derivative lineages, propagating, and indeed amplifying the effect of nutrient deprivation.

EMBRYO ENVIRONMENT AND MECHANISMS OF FETAL PROGRAMMING

Embryos respond to their environment in vitro or in vivo in diverse ways that can influence their future growth and health. As discussed above, developmental plasticity, evident in epigenetic, metabolic, and proliferative states can lead to changes in fetal development through changes in imprinted gene expression, nutrient, and stress-related signaling pathways or cell cycling and apoptotic rates. Last, we consider the interaction between the embryo-centric

phenotype changes and the maternal environment during subsequent gestation.

The search for basic mechanisms underlying the intrauterine programming of fetal and postnatal growth, cardiovascular, and metabolic physiology and ultimately the enhanced risk of adult-onset disease has indicated a complex network of interactions with a central role for maternal-fetal neuroendocrine signaling [reviewed in 140–142]. Maternal undernutrition during gestation alters maternal steroid hormone levels, including elevation of glucocorticoids (GC; corticosterone, cortisol), the stress hormones, which can profoundly influence the physiological condition of the conceptus [143]. Fetal protection against maternal GC is enzymatically regulated by placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts GC to an inactive form [144]. Placental 11β-HSD2 expression and activity are reduced by maternal undernutrition, resulting in increased GC exposure to the fetus [145–147]. This exposure can alter the setting of the fetal hypothalamus-pituitary-adrenal (HPA) axis, leading to elevated fetal GC activity which, through the nuclear GC receptors, can modify the expression of many downstream genes controlling growth and metabolism, including cardiovascular and renal physiology [140–142]. Vascular function and setting of resting blood pressure can be further modified via the renin-angiotensin system and kidney nephrogenesis in response to HPA modulation [141, 148].

How important are these neuroendocrinal pathways in initiating fetal and postnatal alteration in growth, metabolic, and cardiovascular phenotype following environmental manipulation during preimplantation development? The effect of restricted periconceptional nutrition (70% control feed allowance) before mating and for 7 days after mating, prior to control feed provision for the duration of gestation, has been investigated on the development of the fetal HPA axis in sheep [30, 31]. Periconceptional undernutrition resulted in higher fetal plasma concentrations of pituitary adrenocorticotropic hormone (ACTH) between 110 and 145 days of gestation, a significantly greater cortisol response to corticotropin-releasing hormone and an increase in fetal blood pressure in twin fetuses in late gestation [30, 31]. A change in the setting of the fetal HPA axis following periconceptional undernutrition may derive from reduced trophectoderm development and a change in the secretion of placental hormones such as prostaglandin E_2, which is thought to control ACTH secretion in late gestation [30, 149].

The consequences of early embryo environment on future development may also be mediated through disturbance in the balance of placental-fetal growth and function derived from i) nonequivalent epigenetic changes to imprinted genes expressed in fetal or placental pathways and/or ii) inappropriate allocation of cells during early lineage specification [15, 44, 46]. The influence of placental function and placental/fetal exchange on fetal programming has been reviewed recently [150]. Many imprinted genes contribute to placental function and nutrient exchange [38, 46] and may therefore amplify early epigenetic effects in embryos caused by environmental conditions with physiological impairment to growth due to reduced nutrient supply. Thus, deletion of the mouse Igf2 isoform Igf2P0, which is expressed exclusively in the labyrinthine trophoblast of the placenta, results in reduced placental growth and transport capacity and subsequently retardation in fetal growth [44]. Abnormal allantoic development and defective placentation resulting from in vitro culture of ruminant embryos and leading to fetal pathology has been proposed as a mechanistic basis for LOS [16, 151, 152].

CONCLUSIONS

The embryo represents a developmental window during which susceptibility to environmental conditions is prevalent. The breadth of interacting responses within embryos to these conditions illustrates the complexity of the search for underlying mechanisms of developmental plasticity contributing to long-term changes in phenotype. From the main effects observed to date, we can surmise a multifactorial process whereby short-term epigenetic, metabolic, and proliferative conditions, possibly coupled with an altered maternal physiology, impose homeostatic changes in gene expression and setting of the neuroendocrine axis during later gestation. While the use of different animal models has contributed to our understanding of embryo sensitivity and response, we cannot assume equivalent sensitivity in the human. Nevertheless, caution in the handling and manipulation of human embryos and minimizing the duration of in vitro culture would likely reduce the potential of adverse consequences. The subject of developmental programming is still mainly descriptive in nature, and more use of genomic and proteomic tools will help identify critical gene and signaling pathways involved. The environmental sensitivity of egg polarity and establishment of developmental spatial axes [153] should also be investigated. There is a need for further experimental research with animal models, particularly employing intervention designs in vitro, to understand the temporal relationships of developmental plasticity.

REFERENCES

1. Biggers JD, Moore BD, Whittingham DG. Development of mouse embryos in vivo after cultivation from two-cell ova to blastocysts in vitro. Nature 1965; 206:734–735.
2. Bowman P, McLaren A. Viability and growth of mouse embryos after in vitro culture and fusion. J Embryol Exp Morphol 1970; 23: 693–704.
3. Caro CM, Trounson A. The effect of protein on preimplantation mouse embryo development in vitro. J In Vitro Fertil Embryo Transf 1984; 1:183–187.
4. Arny M, Nachtigall L, Quagliarello J. The effect of preimplantation culture conditions on murine embryo implantation and fetal development. Fertil Steril 1987; 48:861–865.
5. Van der Auwera I, Pijnenborg R, Koninckx PR. The influence of in-vitro culture versus stimulated and untreated oviductal environment on mouse embryo development and implantation. Hum Reprod 1999; 14:2570–2574.
6. 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.
7. Khosla S, Dean W, Reik W, Feil R. Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. Hum Reprod Update 2001; 7:419–427.
8. Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. Hum Reprod Update 2003; 9:557–582.
9. Walker SK, Hartwich KM, Robinson JS. Long-term effects on offspring of exposure of oocytes and embryos to chemical and physical agents. Hum Reprod Update 2000; 6:564–577.
10. 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; Suppl 5:68–86.
11. McEvoy TG, Sinclair KD, Broadbent PJ, Goodhand KL, Robinson JJ. Post-natal growth and development of Simmental calves derived from in vivo or in vitro embryos. Reprod Fertil Dev 1998; 10:459–464.
12. 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.

13. Sinclair KD, McEvoy TG, Maxfield EK, Maltin CA, Young LE, Wilmut I, Broadbent PJ, Robinson JJ. Aberrant fetal growth and development after in vitro culture of sheep zygotes. J Reprod Fertil 1999; 116:177–186.
14. Yanagimachi R. Cloning: experience from the mouse and other animals. Mol Cell Endocrinol 2002; 187:241–248.
15. Leese HJ, Donnay I, Thompson JG. Human assisted conception: a cautionary tale. Lessons from domestic animals. Hum Reprod 1998; 13(suppl 4):184–202.
16. Thompson JG, Kind KL, Roberts CT, Robertson SA, Robinson JS. Epigenetic risks related to assisted reproductive technologies: short- and long-term consequences for the health of children conceived through assisted reproduction technology: more reason for caution? Hum Reprod 2002; 17:2783–2786.
17. Winston RM, Hardy K. Are we ignoring potential dangers of in vitro fertilization and related treatments? Nat Cell Biol 2002; 4(suppl): s14–18.
18. Olivennes F, Fanchin R, Ledee N, Righini C, Kadoch IJ, Frydman R. Perinatal outcome and developmental studies on children born after IVF. Hum Reprod Update 2002; 8:117–128.
19. Gosden R, Trasler J, Lucifero D, Faddy M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. Lancet 2003; 361:1975–1977.
20. Doyle P, Beral V, Maconochie N. Preterm delivery, low birthweight and small-for-gestational-age in liveborn singleton babies resulting from in-vitro fertilization. Hum Reprod 1992; 7:425–428.
21. Bergh T, Ericson A, Hillensjo T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982–95: a retrospective cohort study. Lancet 1999; 354:1579–1585.
22. Tarlatzis BC, Grimbizis G. Pregnancy and child outcome after assisted reproduction techniques. Hum Reprod 1999; 14(suppl 1):231–242.
23. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. N Engl J Med 2002; 346:725–730.
24. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med 2002; 346:731–737.
25. Barker DJP. Mothers, Babies and Health in Later Life, 2nd ed. Churchill Livingstone: Edinburgh; 1998.
26. Godfrey KM, Barker DJP. Fetal programming and adult health. Public Health Nutr 2001; 4:611–624.
27. McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Robertson IS. Dietary excesses of urea influence the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. Anim Reprod Sci 1997; 47:71–90.
28. McEvoy TG, Robinson JJ, Ashworth CJ, Rooke JA, Sinclair KD. Feed and forage toxicants affecting embryo survival and fetal development. Theriogenology 2001; 55:113–129.
29. Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 2000; 127:4195–4202.
30. Edwards LJ, McMillen IC. Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo-pituitary adrenal axis in sheep during late gestation. Biol Reprod 2002; 66:1562–1569.
31. Edwards LJ, McMillen IC. Periconceptional nutrition programs development of the cardiovascular system in the fetal sheep. Am J Physiol Regul Integr Comp Physiol 2002; 283:R669–R679.
32. Gallaher BW, Breier BH, Keven CL, Harding JE, Gluckman PD. Fetal programming of insulin-like growth factor (IGF)-I and IGF-binding protein-3: evidence for an altered response to undernutrition in late gestation following exposure to periconceptual undernutrition in the sheep. J Endocrinol 1998; 159:501–508.
33. Wynn M, Wynn A. Nutrition around conception and the prevention of low birthweight. Nutr Health 1988; 6:37–52.
34. Ashworth CJ, Antipatis C. Micronutrient programming of development throughout gestation. Reproduction 2001; 122:527–535.
35. Barker DJP, Osmond C, Rodin I, Fall CHD, Winter PD. Low weight gain in infancy and suicide in adult life. Brit Med J 1995; 311:1203.
36. Burd L, Severud R, Kerbeshian J, Klug MG. Prenatal and perinatal risk factors for autism. J Perinat Med 1999; 27:441–450.
37. Ecker DJ, Stein P, Xu Z, Williams CJ, Kopf GS, Bilker WB, Abel T, Schultz RM. Long-term effects of culture of preimplantation mouse embryos on behavior. Proc Natl Acad Sci U S A 2004; 101: 1595–1600.
38. Lucifero D, Chaillet JR, Trasler JM. Potential significance of genomic imprinting defects for reproduction and assisted reproductive technology. Hum Reprod Update 2004; 10:3–18.
39. Reik W, Santos F, Dean W. Mammalian epigenomics: reprogramming the genome for development and therapy. Theriogenology 2003; 59: 21–32.
40. DeChiara TM, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. Cell 1991; 64:849–859.
41. Wang ZQ, Fung MR, Barlow DP, Wagner EF. Regulation of embryonic growth and lysosomal targeting by the imprinted Igf2/Mpr gene. Nature 1994; 372:464–467.
42. Guillemot F, Nagy A, Auerbach A, Rossant J, Joyner AL. Essential role of Mash-2 in extraembryonic development. Nature 1994; 371: 333–336.
43. Hiby SE, Lough M, Keverne EB, Surani MA, Loke YW, King A. Paternal monoallelic expression of PEG3 in the human placenta. Hum Mol Genet 2001; 10:1093–1100.
44. Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, Stewart F, Kelsey G, Fowden A, Sibley C, Reik W. Placental-specific IGF-II is a major modulator of placental and fetal growth. Nature 2002; 417:945–948.
45. 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 U S A 2002; 99:7490–7495.
46. 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 2003; 547:35–44.
47. Howell CY, Bestor TH, Ding F, Latham KE, Mertineit C, Trasler JM, Chaillet JR. Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. Cell 2001; 104:829–838.
48. Doherty AS, Bartolomei MS, Schultz RM. Regulation of stage-specific nuclear translocation of Dnmt1o during preimplantation mouse development. Dev Biol 2002; 242:255–266.
49. 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.
50. 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.
51. Blondin P, Farin PW, Crosier AE, Alexander JE, Farin CE. In vitro production of embryos alters levels of insulin-like growth factor-II messenger ribonucleic acid in bovine fetuses 63 days after transfer. Biol Reprod 2000; 62:384–389.
52. 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.
53. Kwong WY, Miller DJ, Wild AE, Osmond C, Fleming TP. Effect of maternal low protein diet on imprinted gene expression in the rat preimplantation embryo. Pediatr Res 2003; 53:46A.
54. Dean W, Bowden L, Aitchison A, Klose J, Moore T, Meneses JJ, Reik W, Feil R. Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes. Development 1998; 125:2273–2282.
55. Trounson A, Anderiesz C, Jones G. Maturation of human oocytes in vitro and their developmental competence. Reproduction 2001; 121: 51–75.
56. Sutton ML, Gilchrist RB, Thompson JG. Effects of in vivo and in vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. Hum Reprod Update 2003; 9:35–48.
57. Thomas FH, Walters KA, Telfer EE. How to make a good oocyte: an update on in vitro models to study follicle regulation. Hum Reprod Update 2003; 9:541–555.
58. Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CO, Woad KJ, Webb R, Sinclair KD. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. Biol Reprod 2001; 64:1624–1632.
59. Leese HJ, Tay JI, Reischl J, Downing S. Formation of fallopian tube fluid: role of a neglected epithelium. Reproduction 2001; 121:339–346.
60. Gardner DK, Lane M, Calderon I, Leeton J. Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. Fertil Steril 1996; 65:349–353.
61. Tay JI, Rutherford AJ, Killick SR, Maguiness SD, Partridge RJ, Leese HJ. Human tubal fluid: production, nutrient consumption and response to adrenergic agents. Hum Reprod 1997; 12:2451–2456.
62. Leese HJ. What does an embryo need? Hum Fertil (Camb) 2003; 6: 180–185.

63. Hardy K, Hooper MAK, Handyside AH, Rutherford AJ, Winston RML, Leese HJ. Non-invasive measurement of glucose and pyruvate uptake by individual human oocytes and preimplantation embryos. Hum Reprod 1989; 4:188–191.
64. Houghton FD, Thompson JG, Kennedy CJ, Leese HJ. Oxygen consumption and energy metabolism of the early mouse embryo. Mol Reprod Dev 1996; 44:476–485.
65. Kleeman DO, Walker SK, Seamark RF. Enhanced fetal growth in sheep administered progesterone during the first three days of pregnancy. J Reprod Fertil 1994; 102:411–417.
66. Kleeman DO, Walker SK, Hartwich KM, Fong L, Seamark RF, Robinson JS, Owens JA. Fetoplacental growth in sheep administered progesterone during the first three days of pregnancy. Placenta 2001; 22:14–23.
67. Kawamura K, Sato N, Fukuda J, Kodama H, Kumagai J, Tanikawa H, Nakamura A, Honda Y, Sato T, Tanaka T. Ghrelin inhibits the development of mouse preimplantation embryos in vitro. Endocrinology 2003; 144:2623–2633.
68. Leese HJ. Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. Bioessays 2002; 24:845–849.
69. Lane M, Gardner DK. Selection of viable mouse blastocysts prior to transfer using a metabolic criterion. Hum Reprod 1996; 11:1975–1978.
70. Gardner DK, Leese HJ. Assessment of embryo viability prior to transfer by the noninvasive measurement of glucose uptake. J Exp Zool 1987; 242:103–105.
71. Chi MM, Pingsterhaus J, Carayannopoulos M, Moley KH. Decreased glucose transporter expression triggers BAX-dependent apoptosis in the murine blastocyst. J Biol Chem 2000; 275:40252–40257.
72. Hinck L, Thissen JP, De Hertogh R. Identification of caspase-6 in rat blastocysts and its implication in the induction of apoptosis by high glucose. Biol Reprod 2003; 68:1808–1812.
73. Lea RG, McCracken JE, McIntyre SS, Smith W, Baird J. Disturbed development of preimplantation embryo in the insulin-dependent diabetic BB/E rat. Diabetes 1996; 45:1463–1470.
74. Jimenez A, Madrid-Bury N, Fernandez R, Perez-Garnelo S, Moreira P, Pintado B, de la Fuente J, Gutierrez-Adan A. Hyperglycemia-induced apoptosis affects sex ratio of bovine and murine preimplantation embryos. Mol Reprod Dev 2003; 65:180–187.
75. Colton SA, Pieper GM, Downs SM. Altered meiotic regulation in oocytes from diabetic mice. Biol Reprod 2002; 67:220–231.
76. Carayannopoulos MO, Schlein A, Wyman A, Chi M, Keembiyehetty C, Moley KH. GLUT9 is differentially expressed and targeted in the preimplantation embryo. Endocrinology 2004; 145:1435–1443.
77. Leunda-Casi A, De Hertogh R, Pampfer S. Decreased expression of fibroblast growth factor-4 and associated dysregulation of trophoblast differentiation in mouse blastocysts exposed to high D-glucose in vitro. Diabetologia 2001; 44:1318–1325.
78. Mihalik J, Rehak P, Koppel J. The influence of insulin on the in vitro development of mouse and bovine embryos. Physiol Res 2000; 49: 347–354.
79. Kaye PL, Gardner HG. Preimplantation access to maternal insulin and albumin increases fetal growth rate in mice. Hum Reprod 1999; 14:3052–3059.
80. Leese HJ, Isherwood-Peel G. Early embryo nutrition and disorders in later life. In *Fetal Programming: Influences on Development and Disease in Later Life*. Eds PMS O'Brien, T Wheeler, DJP Barker, 1999:104–116. RCOG Press: London.
81. Nasr-Esfahani MH, Aitken JR, Johnson MH. Hydrogen peroxide levels in mouse oocytes and early cleavage stage embryos developed in vitro or in vivo. Development 1990; 109:501–507.
82. Johnson MH, Nasr-Esfahani MH. Radical solutions and cultural problems: could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos in vitro? Bioessays 1994; 16:31–38.
83. Takahashi M, Keicho K, Takahashi H, Ogawa H, Schultz RM, Okano A. Effect of oxidative stress on development and DNA damage in in-vitro cultured bovine embryos by comet assay. Theriogenology 2000; 54:137–145.
84. Orsi NM, Leese HJ. Protection against reactive oxygen species during mouse preimplantation embryo development: role of EDTA, oxygen tension, catalase, superoxide dismutase and pyruvate. Mol Reprod Dev 2001; 59:44–53.
85. Olson SE, Seidel GE Jr. Culture of in vitro-produced bovine embryos with vitamin E improves development in vitro and after transfer to recipients. Biol Reprod 2000; 62:248–252.
86. Robertson P, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in β-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 2003; 52:581–587.
87. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002; 23:599–622.
88. Van Winkle LJ. Amino acid transport regulation and early embryo development. Biol Reprod 2001; 64:1–12.
89. Van Winkle LJ, Dickinson HR. Differences in amino acid content of preimplantation mouse embryos that develop in vitro versus in vivo: in vitro effects of five amino acids that are abundant in oviductal secretions. Biol Reprod 1995; 52:96–104.
90. Houghton FD, Hawkhead JA, Humpherson PG, Hogg JE, Balen AH, Rutherford AJ, Leese HJ. Non-invasive amino acid turnover predicts human embryo developmental capacity. Hum Reprod 2002; 17:999–1005.
91. Lane M, Gardner DK. Differential regulation of mouse embryo development and viability by amino acids. J Reprod Fertil 1997; 109: 153–164.
92. Lane M, Gardner DK. Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. J Reprod Fertil 1994; 102:305–312.
93. Lane M, Gardner DK. Ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and subsequently alters fetal development in the mouse. Biol Reprod 2003; 69:1109–1117.
94. Porter R, Humpherson P, Fussing P, Cameron I, Osmond C, Fleming TP. Metabolic programming of the blastocyst within the uterine environment. Pediatr Res 2003; 53:46A.
95. Lamb VK, Leese HJ. Uptake of a mixture of amino acids by mouse blastocysts. J Reprod Fertil 1994; 102:169–175.
96. Meijer AJ, Dubbelhuis PF. Amino acid signalling and the integration of metabolism. Biochem Biophys Res Commun 2004; 313:397–403.
97. Tokunaga C, Yoshino K. Yonezawa K. mTOR integrates amino acid- and energy-sensing pathways. Biochem Biophys Res Commun 2004; 313:443–446.
98. Hentges KE, Sirry B, Gingeras AC, Sarbassov D, Sonenberg N, Sabatini D, Peterson AS. FRAP/mTOR is required for proliferation and patterning during embryonic development in the mouse. Proc Natl Acad Sci U S A 2001; 98:13796–13801.
99. Zhang X, Shu L, Hosoi H, Murti KG, Houghton PJ. Predominant nuclear localization of mammalian target of rapamycin in normal and malignant cells in culture. J Biol Chem 2002; 277:28127–28134.
100. Martin PM, Sutherland AE. Exogenous amino acids regulate trophectoderm differentiation in the mouse blastocyst through an mTOR-dependent pathway. Dev Biol 2001; 240:182–193.
101. Martin PM, Sutherland AE, Van Winkle LJ. Amino acid transport regulates blastocyst implantation. Biol Reprod 2003; 69:1101–1108.
102. Petrie L, Duthie SJ, Rees WD, McConnell JM. Serum concentrations of homocysteine are elevated during early pregnancy in rodent models of fetal programming. Br J Nutr 2002; 88:471–477.
103. Rees WD. Manipulating the sulfur amino acid content of the early diet and its implications for long-term health. Proc Nutr Soc 2002; 61:71–77.
104. Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 1998; 12:949–957.
105. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 2002; 132(suppl):2393S–2400S.
106. Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S, Ozil JP. Egg-to-embryo transition is driven by differential responses to Ca(2+) oscillation number. Dev Biol 2002; 250:280–291.
107. Bos-Mikich A, Whittingham DG, Jones KT. Meiotic and mitotic Ca^{2+} oscillations affect cell composition in resulting blastocysts. Dev Biol 1997; 182:172–179.
108. Gordo AC, Wu H, He CL, Fissore RA. Injection of sperm cytosolic factor into mouse metaphase II oocytes induces different developmental fates according to the frequency of [Ca(2+)](i) oscillations and oocyte age. Biol Reprod 2000; 62:1370–1379.
109. Ozil JP, Huneau D. Activation of rabbit oocytes: the impact of the Ca^{2+} signal regime on development. Development 2001; 128:917–928.
110. Christians E, Campion E, Thompson EM, Renard JP. Expression of the HSP 70.1 gene, a landmark of early zygotic activity in the mouse embryo, is restricted to the first burst of transcription. Development 1995; 121:113–122.
111. Fontanier-Razzaq N, McEvoy TG, Robinson JJ, Rees WD. DNA damaging agents increase gadd153 (CHOP-10) messenger RNA levels in bovine preimplantation embryos cultured in vitro. Biol Reprod 2001; 64:1386–1391.
112. Stojanov T, O'Neill C. In vitro fertilization causes epigenetic modi-

fications to the onset of gene expression from the zygotic genome in mice. Biol Reprod 2001; 64:696–705.
113. Stojanov T, Alechna S, O'Neill C. In vitro fertilization and culture of mouse embryos in vitro significantly retards the onset of insulin-like growth factor-II expression from the zygotic genome. Mol Hum Reprod 1999; 5:116–124.
114. Ho Y, Wigglesworth K, Eppig JJ, Schultz RM. Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. Mol Reprod Dev 1995; 41: 232–238.
115. Niemann H, Wrenzycki C. Alterations of expression of developmentally important genes in preimplantation bovine embryos by in vitro culture conditions: implications for subsequent development. Theriogenology 2000; 53:21–34.
116. Rizos D, Lonergan P, Boland MP, Arroyo-Garcia R, Pintado B, de la Fuente J, Gutierrez-Adan A. Analysis of differential messenger RNA expression between bovine blastocysts produced in different culture systems: implications for blastocyst quality. Biol Reprod 2002; 66:589–595.
117. Miller DJ, Eckert JJ, Lazzari G, Durathon-Richoux V, Sreenan J, Morris D, Galli C, Renard JP, Fleming TP. Tight junction mRNA expression levels in bovine embryos are dependent upon the ability to compact and in vitro culture methods. Biol Reprod 2003; 68: 1394–1402.
118. Spanos S, Rice S, Karagiannis P, Taylor D, Becker DL, Winston RM, Hardy K. Caspase activity and expression of cell death genes during development of human preimplantation embryos. Reproduction 2002; 124:353–363.
119. Metcalfe AD, Hunter HR, Bloor DJ, Lieberman BA, Picton HM, Leese HJ, Kimber SJ, Brison DR. Expression of 11 members of the BCL-2 family of apoptosis regulatory molecules during human preimplantation embryo development and fragmentation. Mol Reprod Dev 2004; 68:35–50.
120. Bloor DJ, Metcalfe AD, Rutherford A, Brison DR, Kimber SJ. Expression of cell adhesion molecules during human preimplantation embryo development. Mol Hum Reprod 2002; 8:237–245.
121. Ghassemifar MR, Eckert JJ, Houghton FD, Picton HM, Leese HJ, Fleming TP. Gene expression regulating epithelial intercellular junction biogenesis during human blastocyst development in vitro. Mol Hum Reprod 2003; 9:245–252.
122. Hardy K. Cell death in the mammalian blastocyst. Mol Hum Reprod 1997; 3:919–925.
123. Hardy K, Spanos S. Growth factor expression and function in the human and mouse preimplantation embryo. J Endocrinol 2002; 172: 221–236.
124. Moley KH. Hyperglycemia and apoptosis: mechanisms for congenital malformations and pregnancy loss in diabetic women. Trends Endocrinol Metab 2001; 12:78–82.
125. Jurisicova A, Rogers I, Fasciani A, Casper RF, Varmuza S. Effect of maternal age and conditions of fertilization on programmed cell death during murine preimplantation embryo development. Mol Hum Reprod 1998; 4:139–145.
126. Peters JM, Wiley LM, Zidenberg-Cherr S, Keen CL. Influence of periconceptional zinc deficiency on embryonic plasma membrane function in mice. Teratog Carcinog Mutagen 1993; 13:15–21.
127. Tsunoda Y, McLaren A. Effect of various procedures on the viability of mouse embryos containing half the normal number of blastomeres. J Reprod Fertil 1983; 69:315–322.
128. Rands GF. Size regulation in the mouse embryo. II. The development of half embryos. J Embryol Exp Morphol 1986; 98:209–217.
129. Tam PP. Postimplantation development of mitomycin C-treated mouse blastocysts. Teratology 1988; 37:205–212.
130. Power MA, Tam PPL. Onset of gastrulation, morphogenesis and somitogenesis in mouse embryos displaying compensatory growth. Anat Embryol 1993; 187:493–504.
131. Hishinuma M, Takahashi Y, Kanagawa H. Post-implantation development of demi-embryos and induction of decidual cell reaction in mice. Theriogenology 1996; 45:1187–1200.
132. Papaioannou VE, Ebert KM. Mouse half embryos—viability and allocation of cells in the blastocyst. Dev Dynam 1995; 203:393–398.
133. Papaioannou VE, Mkandawire J, Biggers JD. Development and phenotypic variability of genetically identical half mouse embryos. Development 1989; 106:817–827.
134. Fleming TP. A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. Dev Biol 1987; 119:520–531.
135. Pickering SJ, Maro B, Johnson MH, Skepper JN. The influence of cell contact on the division of mouse 8-cell blastomeres. Development 1988; 103:353–363.
136. Fleming TP, Wilkins A, Mears A, Miller DJ, Thomas F, Ghassemifar MR, Fesenko I, Sheth B, Kwong WY, Eckert JJ. The making of an embryo: short-term goals and long-term implications. Reprod Fert Dev 2004; (in press).
137. Richter KS, Harris DC, Daneshmand ST, Shapiro BS. Quantitative grading of a human blastocyst: optimal inner cell mass size and shape. Fertil Steril 2001; 76:1157–1167.
138. Van Soom A, Boerjan ML, Bols PE, Vanroose G, Lein A, Coryn M, de Kruif A. Timing of compaction and inner cell allocation in bovine embryos produced in vivo after superovulation. Biol Reprod 1997; 57:1041–1049.
139. Beckman DA, Lloyd JB, Brent RL. Investigations into mechanisms of amino acid supply to the rat embryo using whole-embryo culture. Int J Dev Biol 1997; 41:315–318.
140. O'Regan D, Welberg LL, Holmes MC, Seckl JR. Glucocorticoid programming of pituitary-adrenal function: mechanisms and physiological consequences. Semin Neonatol 2001; 6:319–329.
141. Langley-Evans SC. Fetal programming of cardiovascular function through exposure to maternal undernutrition. Proc Nutr Soc 2001; 60:505–513.
142. Bertram CE, Hanson MA. Prenatal programming of postnatal endocrine responses by glucocorticoids. Reproduction 2002; 124:459–467.
143. Lesage J, Blondeau B, Grino M, Breant B, Dupouy JP. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinology 2001; 142:1692–1702.
144. Brown RW, Diaz R, Robson AC, Kotelevtsev YV, Mullins JJ, Kaufman MH, Seckl JR. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. Endocrinology 1996; 137:794–797.
145. Langley-Evans SC, Phillips GJ, Benediktsson R, Gardner DS, Edwards CR, Jackson AA, Seckl JR. Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. Placenta 1996; 17:169–172.
146. Bertram C, Trowern AR, Copin N, Jackson AA, Whorwood CB. The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension in utero. Endocrinology 2001; 142:2841–2853.
147. Whorwood CB, Firth KM, Budge H, Symonds ME. Maternal undernutrition during early to midgestation programs tissue-specific alterations in the expression of the glucocorticoid receptor, 11beta-hydroxysteroid dehydrogenase isoforms, and type 1 angiotensin ii receptor in neonatal sheep. Endocrinology 2001; 142:2854–2864.
148. Moritz KM, Dodic M, Wintour EM. Kidney development and the fetal programming of adult disease. Bioessays 2003; 25:212–220.
149. Hollingsworth SA, Deayton JM, Young IR, Thorburn GD. Prostaglandin E_2 administered to fetal sheep increases the plasma concentration of adrenocorticotropin (ACTH) and the proportion of ACTH in low molecular weight forms. Endocrinology 1995; 136:1233–1240.
150. Godfrey KM. The role of the placenta in fetal programming—a review. Placenta 2002; 23(suppl A):S20–S27.
151. Thompson JG, Peterson AJ. Bovine embryo culture in vitro: new developments and post-transfer consequences. Hum Reprod 2000; 15(suppl 5):59–67.
152. Bertolini M, Anderson GB. The placenta as a contributor to production of large calves. Theriogenology 2002; 57:181–187.
153. Zernicka-Goetz M. Patterning of the embryo: the first spatial decisions in the life of a mouse. Development 2002; 129:815–829.