The effect of nutrition and environment on the preimplantation embryo

Content

1. Preimplantation embryo development is dynamic and responsive to external signals, conferring ‘plasticity’ in form or phenotype
2. Preimplantation embryos sense and adapt to their environment either in vivo dependent upon maternal nutrition and lifestyle or in response to in vitro culture
3. Embryonic responses are beneficial in the short-term to ensure survival and optimal developmental progression yet increase the risk of ill health later in life through to adulthood

Objectives

1. To be aware that maternal nutrition or in vitro environment influence embryonic development, gene expression and metabolism
2. To acknowledge mechanisms by which the preimplantation embryo can sense and react to the environment it develops in
3. To understand the concept that developmental plasticity is advantageous to maximise survival chances at an increased risk of later disease should prevailing conditions change

Ethical issues

1. Can we blame our parents for ill health in the next generation?
2. How can effective interventions to suppress disease risk be developed and tested?

Introduction

The environment profoundly affects the developing conceptus. Observations in the human and experimental data derived from various animal models provide insurmountable evidence for this concept (eg 1-3). In an evolutionary context, such a concept would make sense as predictive adaptations to the environment anticipated later in life would confer a competitive advantage and benefit survival to reproductive age. However, should predictions be wrong and prevailing conditions change, the individual becomes 'maladapted' and increased risk of adult onset diseases will result, particulary metabolic syndrome, diabetes and cardiovascular dysfunction (1). Supporting evidence for this concept of matching or mis-matching environments comes from animal data. For example, in rodent models, maternal undernutrition exclusively during the preimplantation period followed by switching back to normal nutrition thereafter 'tricks' the embryo into making the wrong responses, preparing for a deprived environment. As a consequence, fetal and postnatal overgrowth and associated metabolic and cardiovascular problems follow (4-6). Different developmental periods have shown varying sensitivity to environmental cues and the preimplantation period is amongst the most sensitive.

The preimplantation period

The preimplantation period encompasses the time from fertilisation to blastocyst development (Figure 1) when cells undergo the transition from being totipotent to their first differentiation before the embryo implants into the uterus. During this period, the embryo undergoes a number of key transitions: 1) switching from development regulated by maternally inherited proteins and mRNA messages to embryonic control by activating the newly formed embryonic genome; 2) morphological re-organisation and the first cell specification into inner cell mass (ICM, giving rise to the embryo proper) and trophectoderm (TE, giving rise to extraembryonic lineages such as placenta); 3) a change of metabolic and nutrient preferences; 4) preparation for implantation by signalling at the materno-embryonic interface and developing an invasive phenotype (3,7).

Developmental plasticity and environmental challenges

It has become clear that the developmental processes mentioned above are dynamic events allowing for a high degree of developmental plasticity rather than following a pre-determined static 'programme'. For example, it has long been observed that embryos taken out of their normal in vivo environment within the mother and placed into in vitro culture delay their developmental progress and change their gene expression and metabolic profiles according to the in vitro conditions (8). However, depending on the species, most still retain their capacity to implant and continue developing into viable offspring after transfer back into the mother.

In vitro culture media usually only consist of a buffered salt solution with a limited diversity of nutrient and growth factor supplements. Thus, it could be argued that in vitro culture is a very drastic change in what the embryo is provided with compared to the 'normal' conditions in vivo and, therefore, not being physiologically relevant. Given that around 12,500 children (HFEA, 2006) are currently born each year from infertility treatment in the UK alone, does exposure to in vitro conditions at this sensitive period in development cause reason for concern? Since most assisted conception children have not yet reached adulthood and follow-up studies are limited, it is difficult to reach a firm conclusion. However, a number of reports on increased

birth defects and altered metabolic and cardiovascular physiology associated with IVF/ICSI treatments have been published (2,9,10) (Box 1). More recently, animal models show that more subtle environmental changes such as, for example, slightly altering single media supplements in vitro or specific maternal diets or body conditions in vivo, also influence development and adverse adult outcomes (4-6,11-13). Such developmental plasticity underlies the biological principle of adaptive responses to predicted conditions. Measurable changes can become evident before implantation and may be transient or long-lasting.

Developmental plasticity of the preimplantation embryo

It is now clear that despite not yet being in direct contact with maternal systemic blood supply, the preimplantation embryo is able to sense and react to environmental cues, adapting to prevailing conditions. Underlying mechanisms are still subject to intense investigation (Figure 2). In the preimplantation embryo, processes known to adapt to various environmental cues include cell lineage distribution between ICM and TE in the blastocyst (4,13), mitochondrial function (13,14), cellular energy sensors and signalling system sensitivity (AMPK, mTOR, 15), metabolism (eg amino acid turnover, carbohydrate metabolism 13,16,17) and nutrient transport (7). At mRNA level, an even larger array of cell functions and cellular pathways have been identified to be influenced by environmental cues (reviewed in 8).

Why is the preimplantation embryo so sensitive to its environment?

At one level, the preimplantation embryo is likely to be sensitive to environmental cues as it only consists of a small number of cells which will give rise to all the tissues of the conceptus later on; this finite pool of cells is therefore vulnerable by virtue of their limited supply and essential future contribution. Secondly, putting it simply, the preimplantation embryo's main task is to generate two pools of cells capable of forming the embryo (future fetal) and placental components of the conceptus. From an evolutionary perspective, it would be an advantage for these early cells to have a 'blue-print' of the necessary growth and phenotypic characteristics required for fetal and placental components to function optimally in the nutritional environmental circumstances that the mother finds herself in. It appears likely, therefore, that optimal functioning, can be better regulated early by the way it is constructed from the blastocyst stage onwards rather than by later modification to an existing structural organisation. However, if early development possesses a high degree of plasticity, why does it not revise adaptations if and when the environmental circumstances change? It is possible that this is exactly what happens for many cells so that adaptations become limited to specific tissue and organ systems rather than necessarily affecting the whole organism. Another major question is how environmental changes experienced by cells only during the preimplantation period might be 'memorised' and lead to long-term changes in the phenotype of derivative cells later in development or after birth? The answer appears to reside in a rapidly moving research area, that of epigenetics (see below) and provides a third reason why preimplantation embryos might be vulnerable to their environment.

Epigenetic mechanisms

All cells within the body have the same genetic make-up. Which genes are expressed and which ones are silenced is, at least in part, regulated by chemical modifications to the chromatin, so-called epigenetic mechanisms (reviewed in 18). Epigenetic switches control gene activity in a tissue-specific manner. Stable epigenetic marks such as

DNA CpG methylation are inherited by the daughter cells across the cell cycle. Within the early preimplantation embryo such stable epigenetic marks are mostly erased before being gradually re-established upon cavitation into the blastocyst in a lineage-specific manner. How long epigenetic control stays flexible is subject to debate and, most likely, depends on the specific gene, its activity and function, and, potentially, on the external cues experienced. Overall, it is conceivable that both, transient and more permanent epigenetic deregulation could easily occur during the preimplantation period. Such a deregulation would be likely to have widespread consequences across different tissue types and organs since it happened at a stage when one cell would still give rise to a large part of an organism. Consistent with this idea it has been shown in in vitro culture or diet models that organs from all three germ layers and extraembryonic lineages (placenta) can display altered expression of specific genes coinciding with changes in promoter methylation (12,19-21).

How can we interfere with this biological principle?

Finally, the question arises, can we prevent or intervene with adverse developmental programming, and if so, when do we need to do this? Since the detailed knowledge about underlying mechanisms is still only emerging suggesting a number of different drug target candidates, little is known about effective intervention or prevention strategies. First evidence that prevention is possible is derived from animal studies whereby some of the postnatal phenotypes could be alleviated by supplementing a maternal low-protein diet with folic acid, a methyl-group donor (22). Given the global effect such a treatment could have on DNA methylation patterns, this can just be viewed as a proof-of-principle approach and more targeted interventions are required. It is also likely to be of limited value for reversing already established alterations once they have stabilised. Other examples are postnatal treatment with leptin, growth hormone or statins (23-25) which could alleviate adverse offspring phenotypes after maternal dietary challenge in animal models.

Future directions

Insurmountable evidence now confirms the biological principle that life experiences in utero provoke responses to prevailing conditions in the developing organism. Such adaptations already occur during the preimplantation period before a woman knows she is pregnant. Although these adaptations are beneficial in the short-term enabling the best possible start into life, they can come at a cost of compromised later health. Given the global trend of an ageing human population, this may present the medical profession with a considerable burden in the future. A global effort is required by scientists, medical professions and political decision makers to work together to develop strategies to tackle such a worldwide health risk. Such strategies will also have to address ethical issues arising from the concept that parental lifestyle experiences and choices can profoundly impact on the health of the next generation. The old adage that a pregnant mother eats for two has taken on new meaning and especially around the time of conception.

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Figure legends

Figure 1:
Human preimplantation embryos. After fertilisation and cleavage, blastomeres flatten during compaction before segregation into inner cell mass and trophectoderm at cavitation to the blastocyst.

Figure 2:
Environmental influence on the preimplantation embryo. In vitro conditions or maternal influences in vivo formulate environmental cues the embryo adapts to utilising different mechanisms. This developmental plasticity confers an advantage in the short-term but can have long-term adverse consequences into later development and adult life.

The human pre-implantation embryo

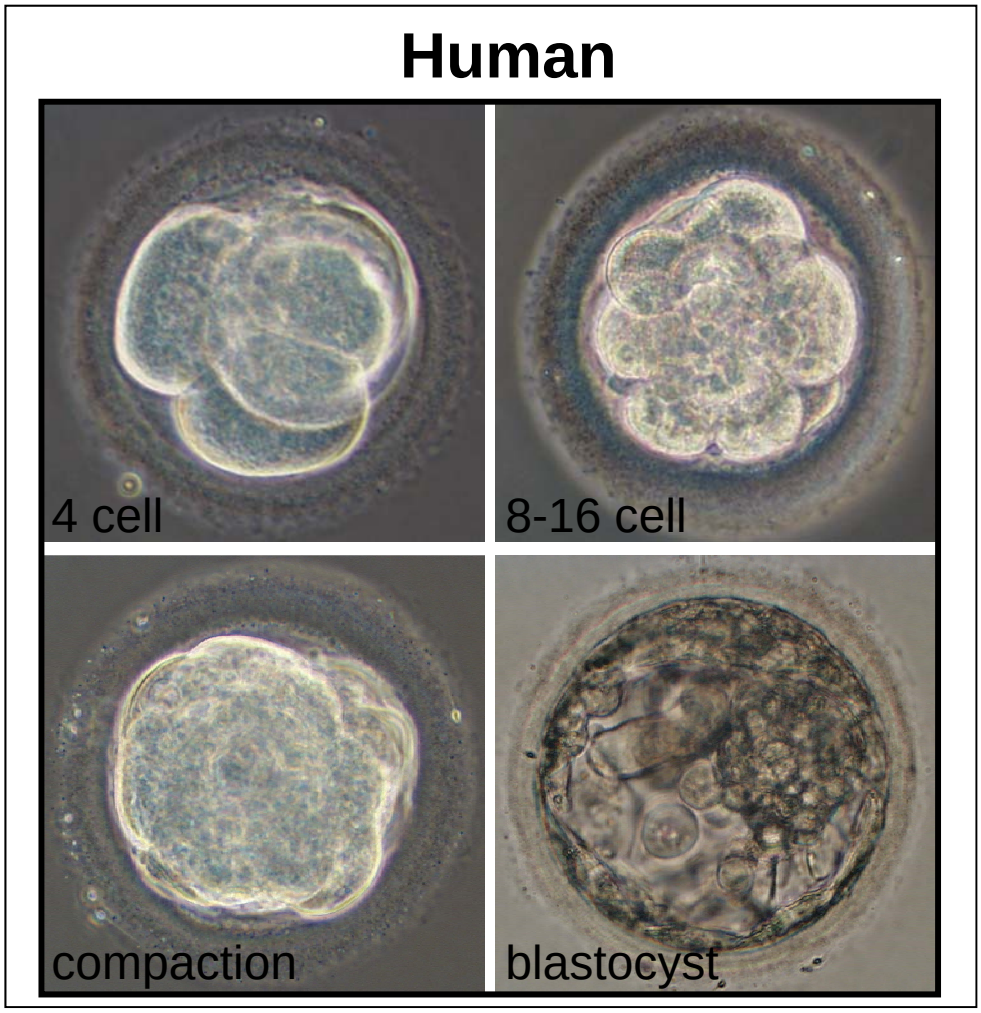

Figure 1

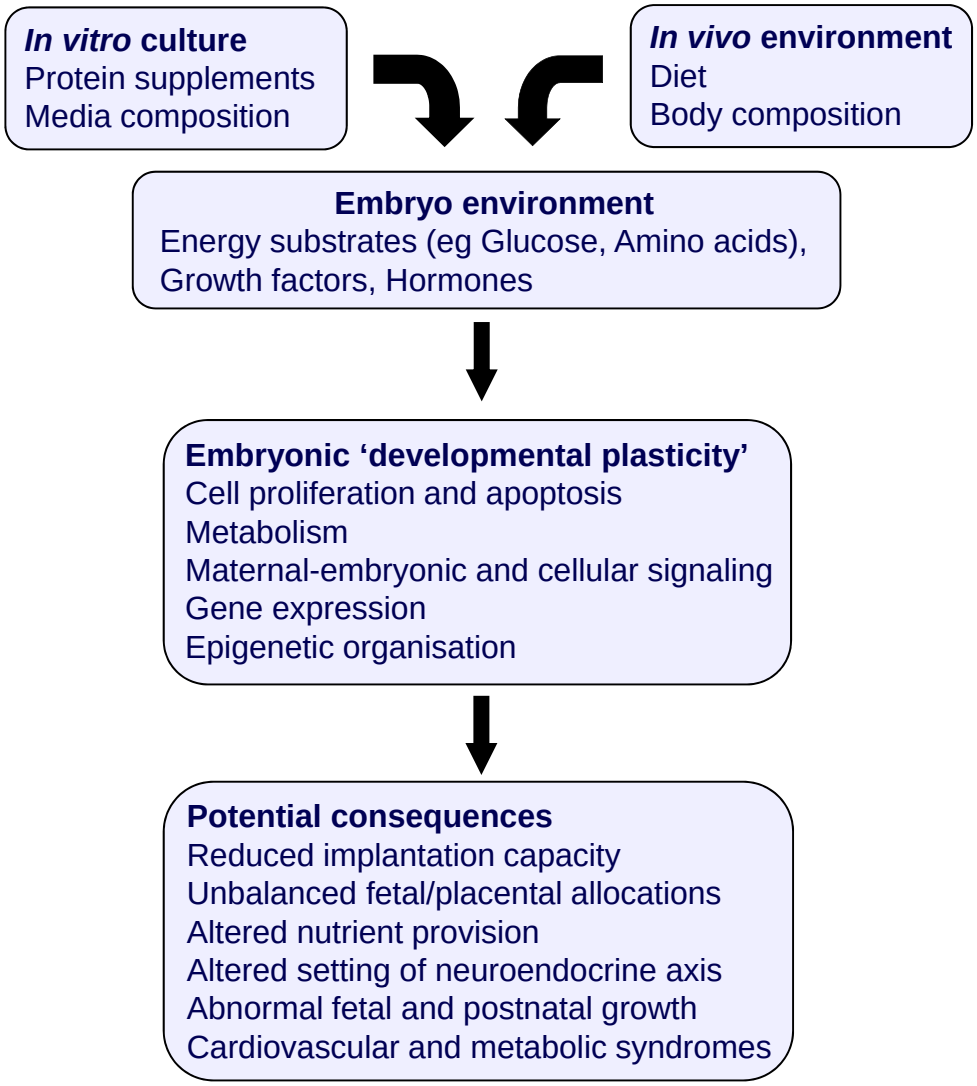
In vitro culture
Protein supplements
Media composition
In vivo environment
Diet
Body composition
Embryo environment
Energy substrates (eg Glucose, Amino acids),
Growth factors, Hormones
Embryonic 'developmental plasticity'
Cell proliferation and apoptosis
Metabolism
Maternal-embryonic and cellular signaling
Gene expression
Epigenetic organisation
Potential consequences
Reduced implantation capacity
Unbalanced fetal/placental allocations
Altered nutrient provision
Altered setting of neuroendocrine axis
Abnormal fetal and postnatal growth
Cardiovascular and metabolic syndromes

Figure 2

BOX 1: Examples of offspring phenotype in response to preimplantation environmental cues

IVF children

Increased systolic and diastolic blood pressure
Increased fasting glucose
Increased DHEAS and LH concentrations in girls
Increased late infancy growth with altered serum profiles
Perturbed body fat composition
Thyroid dysfunction
Increased risk of imprinting disorders is debated
Impact on behaviour and neuromotor development is debated
No effect apparent on allergies

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Maternal diet or in vitro culture animal models

Increased blood pressure (Kwong, Watkins, Sinclair)
Increased anxiety-related behaviour (Ecker, Watkins)
Increased obesity risk (Watkins)
Decreased insulin sensitivity (Sinclair)
Altered immune function (Sinclair)

Animal Rerences:
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