

# Metabolism of the preimplantation embryo: 40 years on

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## Abstract

This review considers how our understanding of preimplantation embryo metabolism has progressed since the pioneering work on this topic in the late 1960s and early 1970s. Research has been stimulated by a desire to understand how metabolic events contribute to the development of the zygote into the blastocyst, the need for biomarkers of embryo health with which to improve the success of assisted conception technologies, and latterly by the 'Developmental Origins of Health and Disease' (DOHaD) concept. However, arguably, progress has not been as great as it might have been due to methodological difficulties in working with tiny amounts of tissue and the low priority assigned to fundamental research on fertility and infertility, with developments driven more by technical than scientific advances. Nevertheless, considerable progress has been made in defining the roles of the traditional nutrients: pyruvate, glucose, lactate, and amino acids; originally considered as energy sources and biosynthetic precursors, but now recognized as having multiple, overlapping functions. Other nutrients; notably lipids, are beginning to attract the attention they deserve. The pivotal role of mitochondria in early embryo development and the DOHaD concept, and in providing a cellular focus for metabolic events is now recognized. Some unifying ideas are discussed; namely 'stress-response models' and the 'quiet embryo hypothesis'; the latter aiming to relate the metabolism of individual preimplantation embryos to their subsequent viability. The review concludes by updating the state of knowledge of preimplantation embryo metabolism in the early 1970s and listing some future research questions.

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## Introduction

The basic pattern of metabolism in the early mammalian embryo was established in the late 1960s and early 1970s. The key figure in this endeavor was John Biggers working successively at the University of Philadelphia, The Johns Hopkins University and Harvard Medical School; other notable contributors included his graduate students Ralph Brinster and David Whittingham, and colleagues Ray Wales and Wesley Whitten.

The state of knowledge at that time was well-summarized by Biggers & Stern (1973) in a review entitled 'Metabolism of the preimplantation embryo' and in an article published the same year by Brinster (1973), 'Nutrition and metabolism of the ovum, zygote and blastocyst'. In this review, Brinster provided the following description:

Pyruvate appears to be the central energy substrate in those species (mouse, rabbit and monkey) in which energy source requirements of the embryo have been examined. During the first day or two of the embryo's life, the Embden–Meyerhoff pathway (glycolysis) has a very low capability, but after blastocyst formation there is a sharp increase in glycolytic ability. The Krebs cycle is the main source of energy throughout the

preimplantation period. Large increases in oxygen consumption and uptake and incorporation of carbon occur at about the time of blastocyst formation. The embryo goes from a relatively inactive metabolic tissue at ovulation to a rapidly metabolizing tissue at implantation.

Almost 40 years on, this succinct statement remains remarkably accurate; a testimony to the pioneers mentioned above. However, arguably, fundamental research on metabolism in early embryos has not advanced as fast as it might have done. One reason is methodological; despite being the largest cells in the female mammal, eggs and preimplantation embryos are available in small numbers only and in the case of the human, their quality is poor and there are ethical constraints in many countries. Thus, techniques (e.g. for studying enzymology, signal transduction, and embryo proteomics) developed for somatic tissue, where the numbers of cells, by comparison, are virtually limitless are insufficiently sensitive for oocytes and early embryos.

Secondly, although there has been a long search for biomarkers of embryo viability with which to improve the success of assisted reproductive technologies, which

has provided some stimulus to research, developments in human and animal assisted conception have tended to be driven by technical advances rather than fundamental science (Leese & Wittall 2001). As recorded in O'Brien *et al.* (1999):

Advances in assisted conception techniques are being introduced into the clinic before the basic scientific work on how they affect early embryonic development has been carried out.

The statement was made at a Study Group convened in by the Royal College of Obstetricians and Gynaecologists to consider: 'Fetal Programming: Influences on Development and Disease in Later Life'. This area of biology, which began as the 'Fetal Origins' hypothesis of Barker (O'Brien *et al.* 1999), was extended to encompass pre- and periconceptual events and then to the whole time-span of development – whereby conditions (especially nutritional) during early stages in the life-scale can potentially impact on events in later life. As a result, this field of study is now termed: the Developmental Origins of Health and Disease (DOHaD). The reason for this digression is to point out that the DOHaD concept has provided a welcome stimulus to studies in reproductive biology and to human and animal nutrition; indeed, reproductive biologists and nutritionists owe a great debt to Barker (2004) for throwing this lifeline to their respective disciplines which have not been perceived historically as priority areas within the scientific and clinical establishments and have not attracted the funding they deserve.

DOHaD-inspired studies have also contributed to the notion that changes in embryo phenotype, including metabolic, following exposure to novel environments are to a major extent, mediated by epigenetic modification and that there is considerable metabolic plasticity (Chason *et al.* 2011). This appreciation – that epigenetic changes are crucial for normal early development in mammals – is one example of the wider discipline of 'Ecological developmental biology' which provides a valuable framework for studying the means by which environmental factors alter phenotype at all levels of organization, including the whole organism, in animals and plants (Gilbert & Epel 2009). DOHaD issues are now centre-stage and the subject of numerous reports; highly recommended is an authoritative survey by the UK Scientific Advisory Committee on Nutrition (SACN 2011) entitled: 'The influence of maternal, fetal and child nutrition on the development of chronic disease in later life', which delves deeply into the evidence base underlying our understanding of these complex issues.

A further area that has recently brought embryo metabolism to a wider scientific community is that of embryonic stem (ES) cells, which, of course, originate in the inner cell mass (ICM) of the blastocyst. The extent to which the metabolism of ES cells mirrors that of the ICM

and changes with differentiation is a subject of debate (Birket *et al.* 2011, Varum *et al.* 2011).

### Animal models

Before considering the ways in which metabolism has developed as a subject of enquiry in early embryos it is useful to consider briefly the main animal models that have been used in research.

The standard model for studying preimplantation development has been the mouse, which, especially for unraveling developing mechanisms has been unrivaled, with inbred strains providing large numbers of highly homogeneous zygotes, virtually all of which, in skilled hands, will develop into blastocysts. As well as providing a robust model for studying metabolism and its relationship with development, the mouse embryo has provided an exquisite system for testing the effect of dietary or environmental perturbations on future development and contributing to understanding the DOHaD concept. However, this strength is also the major weakness of the mouse model metabolically in that it is especially vulnerable to changes in nutrient provision compared with the oocytes and embryos of domestic animals and most likely of man. This is starkly illustrated by experiments in which oocytes or embryos are deprived of nutrients and their subsequent development monitored. For example, Downs & Hudson (2000) found that mouse oocytes arrested within 15 h of culture under such conditions and Manser and Leese (cited in Ferguson & Leese (2006)) reported a similar finding for mouse zygotes, which degenerated within 10 h. By contrast, rabbit zygotes can complete three cleavage divisions in the complete absence of nutrients (Kane 1987); with analogous results for cattle zygotes reported by Ferguson & Leese (2006). Sturme *et al.* (2009a) accounted for these data in terms of the high endogenous energy; especially, fat content, of the embryos of these large animals, building on earlier work by Kane (1979). Thus, pig oocytes contain 37.3 ng fat/nl volume of oocyte, sheep, 21.2 ng and cow 15 ng compared with 6.25 in the mouse. These differences are reflected at the whole-body level with mice consuming about 120 g food/kg of their body weight per day compared with about 6 g/kg in man. This makes mice and other rodents vulnerable to dietary modification.

In summary, studies on mouse embryos have provided the bedrock of our knowledge of preimplantation metabolism and a sensitive indicator of what potentially may go wrong if the early environment of the embryo is perturbed. Considerable caution is required, however, when extrapolating from the mouse to large animals and the human, which by comparison, has an enormous buffering capacity against metabolic insults. Having said this, it is notable that an elevation of blood pressure; one of the best characterized conditions which arise in the offspring of mice following maternal dietary restriction

during the preimplantation phase (Watkins & Fleming 2009) is replicated in the human, where in a large follow-up study of children conceived through *in vitro* fertilisation (IVF) (average age 12 years) systolic blood pressure was raised by 6 mm Hg (Ceelen *et al.* 2008).

### Energy metabolism

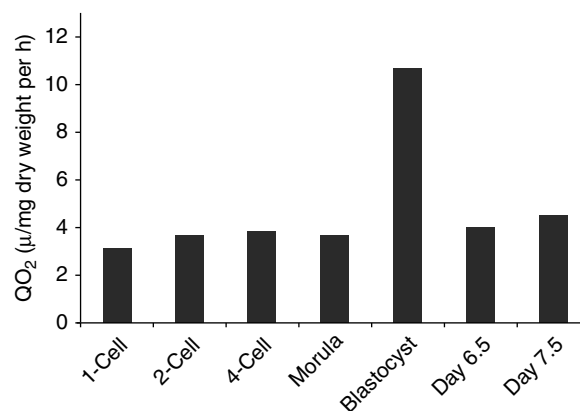
Metabolism has traditionally been considered as having two functions: the first, to provide the energy required by cells to maintain intracellular homeostasis and support specialized functions; and the second, the provision of metabolites for the biosynthesis of cellular constituents and those destined for export. In the context of the preimplantation embryo, more attention has been devoted to the former than the latter with a focus on the major substrates added to embryo culture media; pyruvate, glucose, lactate, and amino acids.

However, as anticipated in a remarkable, authoritative, review by Barnett & Bavister (1996) entitled: 'What is the relationship between the metabolism of the preimplantation embryos and their developmental competence?' these distinctions have become blurred since it became apparent that the traditional nutrients have multiple, overlapping functions, and that other nutrients, particularly, lipids, are beginning to attract attention (Sturmey *et al.* 2006, 2009a, 2009b, Dunning *et al.* 2010, Jungheim *et al.* 2011, Van Hoeck *et al.* 2011, McKeegan & Sturmey 2012). There is also a welcome interest in downstream events, notably, cell signaling; both intracellular and intercellular. This is a fascinating area since preimplantation embryos exhibit considerable autonomy *in vitro* and produce their own trophic factors, while engaging in dialog with the female reproductive tract *in vivo* (Kane *et al.* 1997, Navarette-Santos 2008). O'Neill (2008) has provided an excellent guide to the molecular complexity involved in these interactions.

These developments are now considered in more detail using as examples, research on oxygen consumption and glucose and amino acid metabolism.

### Oxygen consumption

As stated by Brinster (1973) most of the energy in preimplantation embryos, in the form of ATP, is derived by oxidative metabolism. The general pattern in the preimplantation embryo was established by Fridhandler (1961) for the rabbit, Suguwara & Umezu (1961) for the rat and Mills & Brinster (1967) for the mouse. In each case, oxygen consumption was low during cleavage and increased with blastocyst formation; a pattern confirmed over 30 years later by Houghton *et al.* (1996) (Fig. 1) who also showed that in early postimplantation mouse embryos, oxygen uptake reverts to precavitation levels highlighting the striking need of the blastocyst for ATP to power  $\text{Na}^+$ ,  $\text{K}^+$  ATPase activity required for blastocoels cavity formation (Donnay & Leese 1999, Houghton



**Figure 1** Oxygen consumption by early mouse embryos. Reproduced, with permission, from Houghton FD, Thompson JG, Kennedy CJ & Leese HJ 1996 Oxygen consumption and energy metabolism of the early mouse embryo. *Molecular Reproduction and Development* **44** 476–485.

*et al.* 2003) and for protein synthesis as the embryo initiates net growth (Leese 1991).

In a significant development, Manes & Lai (1995) found that 30% of the oxygen consumed by rabbit blastocysts was due to nonmitochondrial processes; a figure confirmed for mouse blastocysts by Trimarchi *et al.* (2000) who also reported that in cleavage stage mouse embryos the figure was ~70%. This remarkable finding has gone largely unnoticed. Two candidates for non-mitochondrial oxygen consumption in somatic cells are i) the generation of reactive oxygen species (ROS) via enzymes such as NADPH oxidases; detectable in mouse blastocysts (Manes & Lai 1995) but not in cleavage stage mouse embryos (Johnson & Nasr-Esfahani 1994), and xanthine oxidase, not detected during mouse preimplantation development (Alexiou & Leese 1992, 1994). However, Alexiou & Leese (1992) showed that purine/pyrimidine salvage pathways, which can limit ROS formation (Gupta *et al.* 2006), were active in early mouse embryos. A second candidate is cell surface oxygen consumption (Herst & Berridge 2007) but this has not been examined in early embryos. The role of high nonmitochondrial oxygen consumption in the preimplantation embryo remains enigmatic.

ROS are very much centre-stage in many areas of biology; from aging to apoptosis. Although formed via enzymes such as those given above, >95% of ROS normally arise as by-products of the mitochondrial electron transport chain. ROS have physiological roles; notably in monitoring mitochondrial dysfunction and triggering cell repair processes or apoptosis (Sahin & DePinho 2010) but 'outside an optimal range result in various developmentally regulated modes of embryo demise' (Bain *et al.* 2011). ROS are referred to colloquially, as 'friend and foe', for a discussion of which, the reader is referred to an excellent review on mammalian sperm metabolism by Storey (2008). The related topic of reactive nitrogen species in the

preimplantation embryo has, by contrast, attracted much less attention, though the data of Manser *et al.* (2004) suggested a physiological role for nitric oxide (NO) in limiting oxygen consumption during mouse preimplantation development and Van Blerkom *et al.* (2008) reported that NO produced by mouse or human cumulus cells regulates mitochondrial polarity in the associated oocytes.

In practical terms, there is strong evidence from a number of species that early embryos benefit from being cultured under conditions which will tend to limit ROS formation, for example, low oxygen concentrations (~5%), similar to those in the oviduct (Fischer & Bavister 1993), and in the presence of EDTA; interventions which were shown to be superior, at least in the mouse, in promoting the development of zygotes to blastocysts compared with the ROS scavengers catalase and superoxide dismutase (Orsi & Leese 2001).

The interest in oxidative stress and the means for its alleviation is one example of the way in which the traditional field of energy metabolism in the early embryo has spawned related areas which have become research topics in their own right. Thus, oxygen and ROS are bound up in a major way with redox status and calcium signaling (Harvey *et al.* 2002, Lopes *et al.* 2010); much of the stimulus for research on which has come from studies on oocyte maturation and fertilization (Dumollard *et al.* 2004) later extended to the early embryo (Dumollard *et al.* 2009). A common denominator for these biochemical events is obviously provided by their association with the mitochondrion. Van Blerkom *et al.* (1984) first showed that mitochondria are re-organized during meiotic resumption, and has continued to be a leader in this field (Van Blerkom 2011) alongside the groups of Cummins (2001), Dale and Wilding (Wilding *et al.* 2009), and Carroll and Swann (Dumollard *et al.* 2004, 2009, Yu *et al.* 2010). Impairment of mitochondrial function during preimplantation development can have long-term implications (Igosheva *et al.* 2010, Wakefield *et al.* 2011).

Such work parallels the general emergence over the past ~30 years of molecular cell biology; the placing in a cellular context of research originally concerned with biochemical and molecular events. Mitochondrial events in early embryos are likely to offer rich pickings for researchers, with much to be done; for example, the elegant study of Lane & Gardner (2005) on the mitochondrial malate–aspartate shuttle is one of few such investigations, and the special properties of mitochondrial DNA (mtDNA; Poulton *et al.* 2010, St John *et al.* 2010) provide an interesting backdrop. Thus, in an intriguing hypothesis that unites genetics and metabolism, Bendich (2010) has proposed that germ cells are maintained in a metabolically quiet state in order to limit mitochondrial activity and ROS formation and protect mtDNA, which is extremely unstable and has limited repair capacity, from degradation.

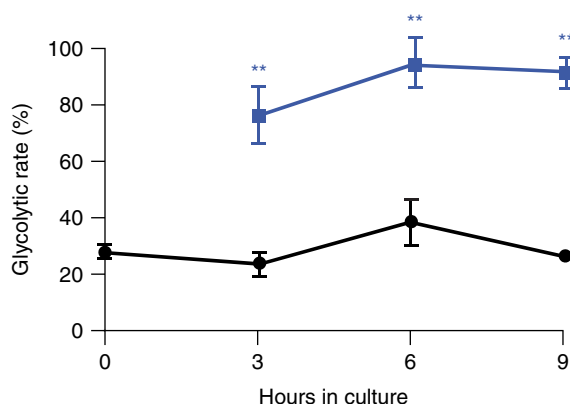
### A word about ATP

It is often reported that ‘increased ATP levels’ are desirable in order to stimulate embryo development; a viewpoint which considers ATP as a ‘store of energy’. In reality, ATP turnover, i.e. the time taken for it all to be replaced, is about 1 min, in the mouse zygote and about 25 s in the blastocyst (Leese 1991). In other words, ATP has a very short-term lifetime and has to be replaced as soon as it is used, as illustrated by the speed with which aerobic cells, tissues, and organisms die if deprived of oxygen! An even more striking example is given by the elite marathon runner who, it has been calculated, uses a colossal 60 kg of ATP during a race (Frayn 2010). Moreover, an increase in the steady state level of ATP could arise from an increase in the rate of production, a decrease in the rate of consumption, or some combination of the two. Without considering such details, it is not possible to know the significance of an increase or decrease in ATP content.

### Glucose metabolism

While the rise in glucose consumption during late preimplantation development is well-known in all species studied including the human (Hardy *et al.* 1989), there has been less discussion on the reason(s) for this. Leese (1995) proposed that acquiring the ability to use glucose and to convert it into lactate enabled the blastocyst to survive the hypoxia that occurs to a varying extent, depending on the species, at implantation. For example, the decidual zone in the rodent is devoid of capillaries and the human conceptus develops in a low oxygen environment during the first trimester (Burton *et al.* 2010). The potential of early embryos to switch to anaerobic glycolysis was strikingly demonstrated in the rat where Brison & Leese (1994) showed that blastocyst development could occur in the absence of oxygen or presence of inhibitors of oxidative phosphorylation; cyanide, antimycin-A, and 2,4-dinitrophenol, with related observations; that transient inhibition of oxidative metabolism promotes *in vitro* development of cattle and pig embryos, reported by Thompson *et al.* (2000) and Macháty *et al.* (2001) respectively. However, perhaps the most tantalizing data on glucose metabolism were provided by Lane & Gardner (1998) on the extent of glycolysis in mouse blastocysts freshly flushed from the uterus and then cultured *in vitro* (Fig. 2).

The glycolytic rate for fresh embryos: 28% glucose converted into lactate, increased in only 3 h *in vitro* to ~76% and ~90% in 6 h. *In vivo*-derived controls flushed at these time intervals retained a low glycolytic rate. Supplementation of the culture medium with amino acids reduced the 3 h glycolytic rate to ~55% and with amino acids and vitamins to 45%. The virtue of using the mouse as a model is the speed with which



**Figure 2** Glycolytic activity of mouse blastocysts immediately following retrieval and following incubation *in vitro*. Reproduced, with permission, from Lane & Gardner 1998 Amino acids and vitamins prevent culture-induced metabolic perturbations and associated loss of viability of mouse blastocysts. *Human Reproduction* 13 991–997. Glycolytic activity of mouse blastocysts after culture in modified mouse tubal fluid (mMTF) medium.  $n=20$  blastocysts examined/time-point. Filled square, represents *in vivo* blastocysts measured immediately after collection; open square, represents blastocysts cultured in mMTF. \*\*Significantly different to *in vivo* developed blastocysts ( $P<0.01$ ).

*in vivo*-derived embryos can be removed from the oviduct or uterus and placed in culture and hopefully retain their native characteristics compared with large animal models, where the interval between flushing and culture will be much longer and they become, essentially, *in vitro* embryos by the time they are assayed. Put another way, the data suggest that the high glycolytic rate of *in vitro* produced embryos and of those derived *in vivo* but subject to delay between flushing and culture is an artifact which arises upon removal from their natural environment. Strong evidence for this proposition is provided by the follow-up data of Lane & Gardner (1996) showing that mouse blastocysts with a high glycolytic rate are less viable posttransfer than their low glycolytic counterparts. The same authors developed this concept to consider the nature of stress in preimplantation embryos and the possible means for its alleviation (Lane & Gardner 2004).

Subtle effects of glucose were proposed by Kaye's group in Australia. It was well-known that mouse preimplantation embryos require at least, a brief exposure to glucose to develop to the blastocyst stage (Chatot *et al.* 1989, Martin & Leese 1995, 1999). It was then discovered that glucose was required for morulae to express the glucose transporter GLUT3, essential for blastocyst formation and that glucose was also responsible for expression of the monocarboxylate transporters Jansen *et al.* (2006, 2008) responsible for coupled transport of a proton with an anion such as pyruvate and lactate; which as well as being involved in the transport of these nutrients, plays a role in [pH]<sub>i</sub> regulation (Fitzharris & Baltz 2009); indirect evidence for which had earlier been obtained by Gibb *et al.* (1997)

and Butcher *et al.* (1998). Exploration of the molecular details of this intriguing story has revealed a ROS-mediated stress response leading to peroxisomal proliferation (Jansen *et al.* 2009). In other words, glucose plays a role as a cell-signaling agent as well as potential metabolic substrate. Furthermore, Sutton-McDowall *et al.* (2010) have described further roles of glucose in the cumulus oocyte complex via metabolism through the pentose phosphate, hexosamine biosynthesis, and polyol pathways.

## Amino acids

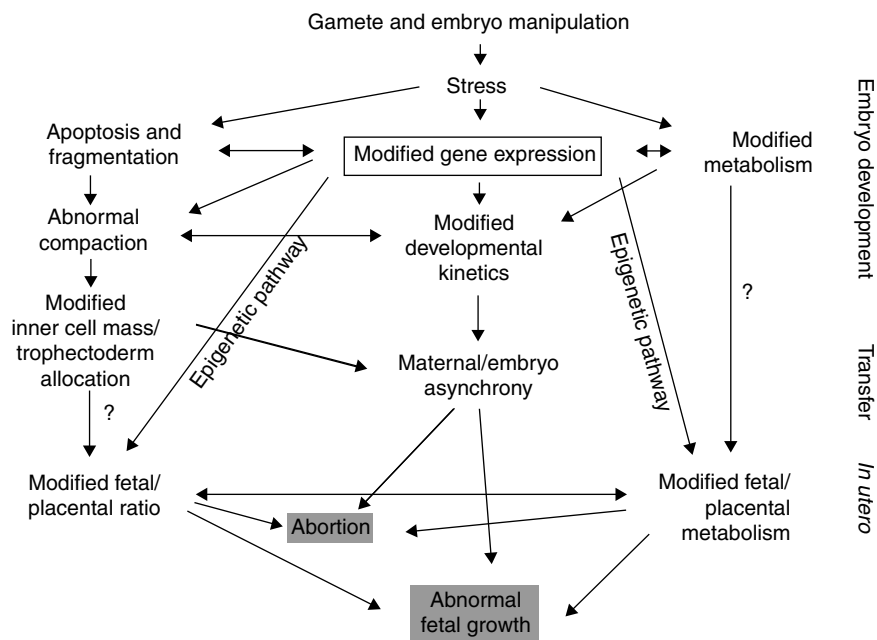
This area has been comprehensively reviewed by Sturme *et al.* (2008) and a brief account only is given here. Amino acids are now added routinely to human embryo culture media but this was not the case during the early years of IVF. The person most responsible for highlighting the importance of amino acids during preimplantation development was Bavister *et al.* (1983), especially for rodent embryos with important contributions from Menezo, especially on amino acid metabolism and its relationship with 1-carbon metabolism (Menezo *et al.* 1989), Rieger *et al.* 1992, Gardner *et al.* 1994 and Thompson *et al.* 1995 on domestic animal embryos, and Hardy and Devreker for human embryos (Devreker *et al.* 2001). Biggers & Summers (2008) have provided authoritative accounts on the contemporary picture of amino acids and embryo culture. Originally thought of as mainly precursors of protein synthesis, amino acids are now acknowledged to have multiple roles, beginning with multiple transporters at the plasma membrane (Van Winkle 2001) and continuing downstream (Martin *et al.* 2003, Kim *et al.* 2011). For a summary, see Fig. 1 of Sturme *et al.* (2008).

## Unifying ideas

### Stress–response models

There have been a number of models and hypotheses which have aimed to provide a unifying framework for considering the metabolism of the oocyte and early embryo. Thus, Lane & Gardner (2004) focused on the maintenance of homeostasis in the face of environmental stress to the early embryo, especially during cleavage, while Thompson *et al.* (2002) proposed a stress-induced causal model to show how perturbation of an embryo's environment can lead to changes in phenotype which may be expressed in the fetus and neonate (Fig. 3). The significance of this latter model lies in the immediacy with which metabolism may be modified; well before any changes in gene expression, and potentially leads directly to abnormal fetal growth.

In this context, Rappolee's group (Xie *et al.* 2011) in a review of the molecular aspects of the stress response in early mammalian development, and contrasting early



**Figure 3** A stress-induced causal model of early embryo perturbation. Manipulation of gametes and embryos results in cellular stress. There are several potential pathways, three of which are shown here, in which stress can modulate cellular function. The response may involve several pathways that are interactive. The degree of response will determine the degree of perturbation, which is lethal at its most calamitous. Reproduced, with permission, from Thompson JG, Kind KL, Roberts CT, Robertson SA & Robinson JS 2002 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? *Human Reproduction* **17** 2783–2786. Adapted from Leese *et al.* (1998).

embryos and ES cells, reported that activity of AMP kinase, a ‘master-switch’ in metabolic homeostasis and response to stress (Chen *et al.* 2006), showed peak activity only 10–30 min after stimulation. This review by Xie *et al.* (2011) is valuable for the way in which it contrasts different stressors and their duration and remarkable for its impressive sweep of the molecular events which follow.

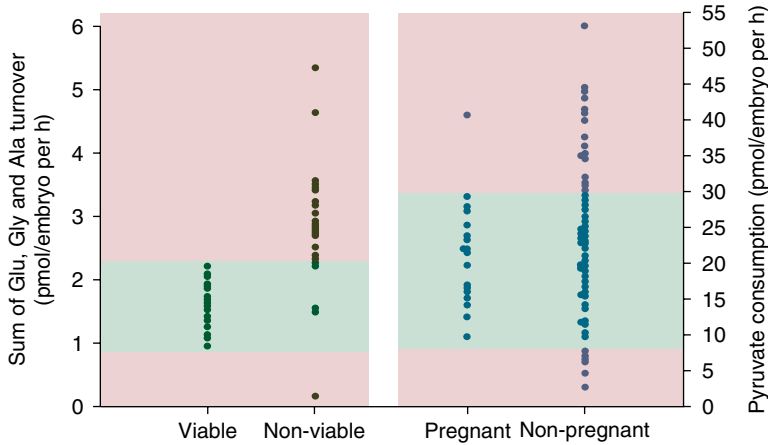
### The quiet embryo hypothesis

The response to stress is a feature of the quiet embryo hypothesis (Leese 2002) but for the most part, this proposition is concerned with the normal state of metabolism during early development. With hindsight, the hypothesis is one statement of the principle that ‘nature is thrifty in all its actions’; that living things are thought to be constrained by a tendency to minimize the expenditure of metabolic cost (Srinivasan (2009) paraphrasing Borelli (1680), who asked why humans and animals walk and run the way they do). In the context of the preimplantation embryo, one can, for example, postulate that by minimizing oxygen consumption (i.e. having a quiet metabolism) from the zygote to morula stage, the embryo may limit the formation of ROS and hence the damage they might cause to cellular and molecular processes at this vulnerable time. Further examples of such ‘functional’ quiet metabolism are considered in Leese *et al.* (2008).

However, the quiet embryo hypothesis is equally concerned with differences between the metabolism of individual preimplantation embryos in relation to their viability. The metabolic marker in which the relationship

with viability is most marked is the ‘Amino Acid Profile’; the pattern of depletion or appearance, measured noninvasively, of a close to physiological mixture of amino acids by single embryos. In the case of surplus cleavage-stage human embryos (Houghton *et al.* 2002) and human zygotes in clinical IVF (Brison *et al.* 2004) the amino acid profiles of those embryos which reached the blastocyst stage or gave a pregnancy were lower (‘quieter’) than those which failed to develop. One explanation for these data was provided by Sturmeijer *et al.* (2009b) who measured the extent of DNA damage in individual human embryos at the blastocyst stage and related this to their amino acid profile. Similar studies were carried out in cow and pig preimplantation embryos. For each species, there was a positive relationship between DNA damage and amino acid profile; the more ‘noisy’ an embryo, the greater was the level of damage and the higher the demand for nutrients such as amino acids and energy for repair processes (discussed in Baumann *et al.* (2007)). By contrast, those embryos with a quieter metabolism were subject to less damage to the genome, transcriptome, and proteome, or were better equipped to deal with damage when it occurred, and thus devoted fewer resources to what may colloquially be termed ‘running repairs’.

Earlier data contributing to the formulation of the quiet embryo hypothesis had been obtained by Conaghan *et al.* (1993) and Turner *et al.* (1994) for the uptake of pyruvate by single embryos in conventional IVF (i.e. with ovarian hyperstimulation) and natural cycle IVF respectively (Fig. 4). However, the distribution patterns of pyruvate uptake between viable and nonviable embryos overlap considerably, illustrating the point that different



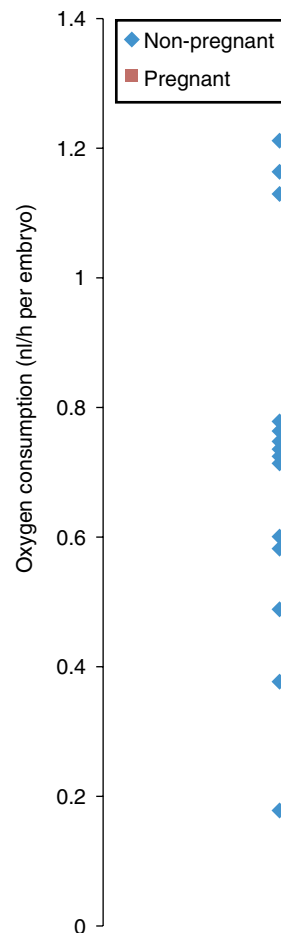
**Figure 4** Comparison of pyruvate and amino acid profiling of early human embryos. Relationships between pyruvate consumption by single human embryos conceived by natural cycle IVF and the outcome of embryo transfer (right hand panel: data re-drawn, with permission, from Turner *et al.* (1994)) and between the turnover of four amino acids by frozen/thawed day 2 human embryos and their capacity to reach the blastocyst stage (left hand panel: data re-drawn, with permission, from Stokes *et al.* (2007)).

biomarkers will have different distributions and potential for use diagnostically to select single embryos for transfer (Leese *et al.* 2007).

Recent data by Gardner *et al.* (2011) showing conclusively that glucose consumption by day 4 or day 5 human embryos which subsequently gave rise to pregnancies following transfer is higher than those which are nonviable apparently contradict the quiet embryo notion. However, as has been discussed, it is a requirement of preimplantation embryos that glucose consumption increases sharply at the blastocyst stage for the embryo to remain viable; entirely consistent with the data of Gardner *et al.* (2011), and what is required as a test of the quiet embryo hypothesis is the overall metabolic cost of this process; the prediction is that this will be lower in high-quality embryos. By way of analogy, if two people carry out the same piece of physical activity (for example on a treadmill or stationary bicycle and analogous to an embryo increasing its glucose consumption), the fit person (analogous to the healthy embryo) will do so with a lower oxygen uptake.

The challenge is therefore to measure energetic efficiency alongside nutrient uptake and relate the data to developmental competence. The most appropriate marker is oxygen consumption, since most of the ATP required during preimplantation development is derived from oxidative phosphorylation. At the time of writing, there is no consensus on the relationship between oxygen consumption and pregnancy potential. For example, findings for human oocytes were reported by Scott *et al.* (2008) which indicated that respiration rates neither too high nor too low were consistent with oocyte viability while Tejera *et al.* (2011) found that human oocytes that generated embryos which implanted had a slightly higher oxygen consumption than those which failed to implant. Ottosen *et al.* (2007) and Sugimura *et al.* (2010) both found that cleavage stage, mouse, and pig embryos respectively with higher oxygen consumption were more likely to reach the blastocyst stage while the data of Lopes *et al.* (2007), given below (Fig. 5), show the distribution of oxygen consumption of single bovine

blastocysts which were then transferred to recipients illustrating the wide variation in oxygen uptake between individual embryos and the lack of any simple relationship between respiratory activity and pregnancy potential. All these findings are not surprising bearing in



**Figure 5** Relationship between respiratory activity (nl/h per embryo) of individual cattle blastocysts produced *in vivo* and the outcome of embryo transfer. Redrawn from Lopes *et al.* (2007). Data kindly supplied by Ana Lopes and reproduced with permission.

mind the data presented earlier on the high proportion of oxygen consumed by nonmitochondrial processes and the requirements of all other cellular functions for ATP.

## Conclusion

To conclude this review, the following is an attempt to update the summary of embryo metabolism presented by Brinster (1973). It is then followed by some future research possibilities.

The Krebs cycle and oxidative phosphorylation provide the main source of energy throughout the preimplantation period. Pyruvate is a central energy substrate during the first cleavage in those species in which energy source requirements of the embryo have been examined, although it is not obligatory for all species (e.g. porcine). Other substrates, notably, amino acids, lactate, and endogenous fatty acids derived from triglyceride, combine with pyruvate to provide embryos with a range of potential energy sources through to, and including, the blastocyst stage. These nutrients have numerous, overlapping, metabolic roles. Prior to the morula stage, glucose consumption and metabolism is low, although some glucose is necessary for intracellular signaling purposes. With blastocyst formation, large increases in oxygen consumption and the uptake and incorporation of carbon occur and there is a sharp increase in glycolysis, at least *in vitro*. The embryo goes from a relatively inactive metabolic tissue at ovulation to a rapidly metabolizing tissue at implantation. Mitochondria play a pivotal role during early development, as well as providing a cellular focus for metabolic events. We are almost totally ignorant of the metabolism of preimplantation embryos *in situ* (in the oviduct and uterus) and understanding of signal transduction within the embryo is in its infancy as is the molecular dialog between embryos in culture and with the maternal tract *in vivo*.

## Future research questions

1. What is the relationship between metabolism and development: what are the metabolic efficiencies of the key molecular/biochemical process required for preimplantation development?
2. What is the metabolism of the early embryo *in vivo*? Metabolic differences between *in vivo* and *in vitro*-derived embryos and the superior quality of the former are well-known (Thompson 1997) but we are ignorant of the metabolism of embryos within the female reproductive tract. One experimental approach might involve imaging embryos through the transparent wall of the mouse ampulla.
3. What is the nature of the molecular dialog between the embryo and the female tract and between individual embryos in culture?
4. What are the mechanisms by which perturbation of metabolism periconceptually compromises the health of the offspring?

## Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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