Embryo viability and metabolism: obeying the quiet rules

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BACKGROUND: It has been proposed that preimplantation embryo viability during culture and following embryo transfer is associated with a ‘quiet’ metabolism. Viable embryos may be better equipped to contend with damage to the genome, transcriptome and proteome, or they may possess less damage than non-viable embryos. METHODS: Much of the data for the quiet embryo hypothesis was obtained in the human and mouse. In this article, evidence is reviewed suggesting that the quiet hypothesis may equally be applied to reproduction in livestock, which can provide good models for the human. RESULTS: Data, particularly for the sheep and cow, suggest that a quiet metabolism during early embryo development is consistent with successful embryo development. Conversely, an ‘active’ metabolism is associated with sub-optimal outcomes in later life. CONCLUSIONS: The challenge is to identify the range of values for a given marker within which an embryo has a high chance of giving rise to healthy offspring. We also speculate on the ways in which such a metabolic profile might be encouraged and the implications for weight loss in obese women prior to conception.

Keywords: quiet metabolism; embryo viability; embryo transfer; preimplantation development; IVF

Introduction

Leese (2002) proposed that cell viability is associated with a metabolism that is ‘quiet’ rather than ‘active’. In this proposition, the most viable cells exhibit a ‘quieter’ metabolism, because they are required to expend less energy rectifying damage to the genome, transcriptome and proteome. We further speculate that this quiet metabolism represents the basal turnover required for normal cellular and developmental processes and that this is reflected in a minimal or reduced rate of consumption of oxygen and nutrients compared to that by other cells in a population. All cells will expend energy to repair damage as part of normal ‘housekeeping’ processes. However, as cell viability is compromised due to external stresses, e.g. radiation, mutagens, carcinogens, teratogens, further energy will be expended in an attempt to accommodate the requisite repair processes. This will lead to an elevation in metabolic processes, manifested as an ‘active’ metabolism likely to generate increased levels of Reactive Oxygen Species with potentially deleterious consequences for the cell.

Quiet metabolism

The quiet metabolism hypothesis was largely derived from data on preimplantation mammalian embryos that may be generated in vivo, or by IVF, and grown in culture to the blastocyst stage. During the cleavage stages, metabolism may be assayed non-invasively, the embryos replaced in culture and their development to the blastocyst stage monitored. Such assays typically comprise measurements of oxygen, pyruvate and glucose consumption, lactate production and the turnover (sum of depletion and appearance) of amino acids (Gopichandran and Leese, 2003; Sturmey and Leese, 2003). This quiet metabolism paradigm is exemplified by recent data on amino acid turnover by surplus cleavage stage human embryos which had been frozen and thawed, where those embryos with the capacity to develop to the blastocyst stage had a lower amino acid turnover than those which arrested (Stokes et al., 2007). In a related article, we have put forward a catalogue of molecular processes and events that might alter embryo viability and dictate the appearance of a quiet metabolism phenotype (Baumann et al., 2007). Notable amongst such processes are DNA/RNA and protein synthesis, which, in somatic cells such as thymocytes, typically account for about 12 and 20%, respectively, of total energy consumption (Buttgereit and Brand, 1995; Buttgereit et al., 2000). A further process which contributes in a major way to energy utilization is the operation of the sodium pump enzyme: Na⁺,K⁺-ATPase which, in the bovine blastocyst, has been estimated to account for between 10.5 and 44% of oxygen consumption (Leese et al., 2001). It is possible that less viable embryos may be more permeable to sodium...
ions and have to devote more energy to sodium pumping, contributing to an ‘active’ phenotype.

We speculate that compromised cells have to increase such processes as they attempt to maintain development. Some compromised cells may be able to ‘repair’ such damage and continue to develop, however, it is likely that a proportion will become overwhelmed with the need to devote large amounts of energy to repair processes and go down the default pathway of apoptosis. In this context, it is significant that the extent of apoptosis is much greater for in vitro-produced embryos than those conceived in vivo (Pomar et al., 2005), reflecting the lower quality of the former compared with the latter.

We believe that the concept of quiet metabolism may be applied at other levels of organization and in the remainder of this commentary, we consider some instances from livestock reproduction studies where unquiet, or ‘active’, metabolism during early embryo development has been associated with later sub-optimal outcomes. Finally, we consider briefly some implications for human Assisted Reproduction Technologies (ART) and the means whereby quiet metabolism can be fostered.

‘Active’ metabolism
Evidence for active metabolism in livestock early embryos was provided in a study where superovulated donor ewe diets containing 3% urea (high urea, HU) were observed to generate elevated ammonium concentrations in vivo. The resultant embryos proved less capable of surviving to term following transfer to conventionally fed recipients than did embryos from conventionally fed (control, C) donor ewes (McEvoy et al., 1997). In a complementary experiment reported in the same paper, Day 3 embryos collected from analogous C and HU ewes differed to the extent that the latter were more advanced and more metabolically active. However, the HU donor-derived embryos tended to have inferior survival rates during subsequent culture in vitro, hinting that dietary-mediated up-regulation of metabolism was harmful rather than beneficial to embryos so affected. The more active metabolism noted among HU donor-derived Day 3 embryos persisted to morula/blastocyst stages despite all embryos having been cultured for 72 h in a benign in vitro environment. At morula/blastocyst stages, HU donor-derived embryos exhibited glucose consumption indices (pmol/embryo/3 h) that were up to 2.8 times the levels found among C donor-derived blastocysts. Intuitively, such excessive activity might be expected to lead to burn-out where embryos, unable to sustain the metabolic pace, fail to survive: a consequence of which would be compromised pregnancy rates, as indeed occurred in the study. Alternatively, if up-regulation was sustained, perhaps as a consequence of abnormal developmental programming or cues, the outcome might, e.g. be fetal oversize. It is notable, therefore, that transfer of HU donor-derived embryos in the same study resulted not only in a low incidence of pregnancies but, in one instance, the birth of an oversize lamb.

One likely consequence of active metabolism, unless devoted almost solely to ‘running repairs’ in adverse conditions, is accelerated development of embryos, a process that can be counter-productive. As well as undermining synchrony between an embryo and its environment, there is some evidence that ‘advanced’ embryos could be predisposed to more aberrant development. Kuran et al. (1999), surveying the outcomes of large offspring studies in sheep (total of 219 singleton pregnancies), concluded that embryos transferred as early/mid blastocysts incurred a lower incidence of fetal oversize (38%) than same-age expanding/expanded blastocysts (58%) or hatched blastocysts (80%). More recent data from Powell et al. (2006) support the contention that accelerated development of embryos can predispose them to exhibit notable aberrations during subsequent development. For example, circumstances associated with excessive dietary nitrogen provision to donor ewes yielded a set of outcomes that included accelerated early embryo development, low survival following embryo transfer, and altered fetal development among survivors. By contrast, Kakar et al. (2005) showed in ovine embryos that reduced energy availability, as may occur when the activity of methylmalonyl-CoA mutase is compromised, was associated with an increase in the number of cells per blastocyst and an improvement in morphology in the absence of any difference in ovulation rate.

A key to ensuring appropriate development, both in vivo and in vitro, is the provision of conditions that foster ‘quiet metabolism’ and match developmental stage to age. For in vitro development, avoiding, neutralizing or minimizing of stressors is crucial. In vivo development too can be promoted by having these same strategies in place. However, one must also consider the status of the donor animal, which can influence the developmental potential of oocytes and derivative embryos (McEvoy et al., 2001, 2003). Features as diverse as day length, diet and donor response to superovulatory regimens help formulate the developmental ‘communiqué’ dictating an oocyte’s or embryo’s agenda in the hours or days before and after fertilization. Avoidance of cues that can trigger an undesirable trajectory should become easier as we learn more about the ‘programming’ influences of specific nutrients, metabolites, hormones and other factors in different circumstances.

Species-to-species variations lead us to propose that the concept of quiet metabolism is not about ‘one size fits all’ but rather that, for any given set of circumstances that has evolved to achieve Nature’s reproductive goals, there is an optimal range of embryonic activity consistent with successful developmental progression. This concept is well illustrated by recent data from (Lopes et al., 2007a,b) who demonstrated that in vivo-derived cattle embryos with oxygen consumption values either below 0.78 nl/h or above 1.10 nl/h were linked to pregnancy incidences of 48 and 25%, respectively, following transfer to recipients whereas all of those with indices between those values resulted in successful establishment of pregnancy. Although cautioning that many other factors, such as the calibre of recipients, influence pregnancy outcomes, the authors did state that, for key physiological parameters such as respiration, ‘we frequently observe an optimal range, and adverse conditions as well as a poor prognosis are likely to occur when the observed parameter is below or above this range’. They went on to state ‘Very low respiration rates would correlate with adverse alterations in oxidative phosphorylation and in other oxygen-related processes, and consequently with reduced embryo viability and increased mortality.
Moreover, a very high respiration rate could represent a manifestation of metabolic stress, which is associated with reduced embryo viability.

As already stated, there is no expectation on our part that a single set of universal boundaries can be deemed applicable to any metabolic parameter in all circumstances. Rather, in cases of contrasting developmental norms, a given level of gene expression or cellular function might be deemed too ‘active’ in pre-implantation embryos of one species, but suitably ‘quiet’ in those of another. For example at one extreme, we might find the suspended animation pertinent to a roe deer’s embryonic diapause at the blastocyst stage (Lambert et al., 2001; Lopes et al., 2004) while at the other extreme, embryos of wild and domesticated South American camelids (e.g. guanaco and llama), must exhibit an impressive degree of trophic acceleration to ensure maternal recognition of pregnancy as early as 8–10 days post-mating (McEvoy et al., 1994). The crux for each mammalian subject, regardless of what happens in other species, is that when perturbing embryos by generating or growing them in vitro or altering their environment in situ, a major determinant of their fate will be our ability to identify and provide those circumstances and agents that favour ‘quiet metabolism’ consistent with normal healthy development.

Conclusions and implications for the efficacy and safety of human ART

We have presented evidence for the hypothesis that a ‘quiet’ embryo, in metabolic terms, is associated with a viable phenotype. If this proposition is true, there are two implications for the efficacy and safety of ARTs. First, interpretation of data from a number of studies on putative/possible markers of embryo competence may need to be revised. These experiments have, understandably, tended to take as their premise the notion that ‘up-regulation’ of genes involved in de novo protein synthesis, production of key metabolic enzymes, or metabolite uptake, is indicative of a healthy embryo. We maintain that this is not necessarily the case; rather, the challenge is to identify the ‘range’ of values for a given marker within which an embryo has a high probability of giving rise to healthy offspring. Our contention is that this range is likely to be in the quiet region of the scale. Secondly, and as stated in the original ‘Quiet embryo hypothesis’ (Leese, 2002): ‘Culture media should promote embryo metabolism which is relatively quiet rather than relatively active. This is likely to require that the concentrations of nutrients be kept fairly low, corresponding to the situation in the female reproductive tract (Leese, 1998)’ and in line with a previous conclusion that ‘the in vitro development of preimplantation embryos is best served by providing a combination of substrates which lead them to adopt a metabolic pattern akin to that which, to the best of our knowledge, is exhibited in the female tract’ (Leese, 1991). Such a strategy is also likely to lead embryos to utilize endogenous nutrients; something that they may have evolved to do (Leese and Ferguson, 1999; Leese, 2003, Ferguson and Leese, 2006). The need to maintain early embryos in systems designed to minimize stress has also been considered by Lane and Gardner (2005).

Finally, it is tempting to speculate that the concept of ‘quiet’ versus ‘active’ metabolism may be useful in accounting for the association in humans between maternal obesity periconceptually and compromised reproductive outcomes for the mother, fetus and offspring. The data discussed above show that even in the case of fit-for-purpose domestic ruminant animals in lean or moderate body condition, a high level of feeding prior to and during conception leads to adverse developmental outcomes; observations we have linked to the metabolism of the early embryo. Among humans, an unfavourably high body mass index can be an additional aggravating factor and, consistent with this, Nelson and Fleming (2007) recommend that women with a body mass index above 35 kg/m² lose weight prior to conception. One detrimental consequence of the almost invariably high plane of nutrition of obese women and those with diabetes (Moley, 2001) might be the nutritional supra-enrichment of the reproductive environments (follicular, oviductal and uterine) and the undesirable up-regulation of genes involved in metabolism this may induce in the embryo. It should be noted, of course, that such up-regulation would not always occur due to variations in genotype and in adaptive or pre-conditioning influences. Indeed, this varied responsiveness could explain some of the seeming inconsistencies noted by Bellver et al. (2006) in a review of literature relating to the impact of obesity on assisted reproduction outcomes.

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