IDENTIFICATION OF VIABLE EMBRYOS BY NON-INVASIVE MEASUREMENT OF AMINO ACIDS

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The major challenge facing human *In Vitro* Fertilisation (IVF) and related techniques is to devise a rigorous, non-invasive test to select single embryos for transfer and overcome the problem of multiple births, which pose serious risks for mother and child (http://www.oneatatime.org.uk/).

A recent development which holds considerable promise is the non-invasive measurement of 'amino acid turnover'. In this method, individual human embryos at the cleavage stage of development are cultured with a close to physiological mixture of amino acids. Some amino acids are depleted from the medium while others appear. When the subsequent development of the embryos is recorded, those in which the depletion and appearance (i.e. 'turnover') of amino acids lies within a lower range have a higher viability in terms of their capacity to develop to the blastocyst stage in culture (Houghton et al *Hum Reprod* 2002; 17:999-1005) or give rise to a pregnancy following embryo transfer in clinical IVF (Brison et al *Hum Reprod* 2004; 19:2319-24). The same criterion, with regard to development in culture, applies to cryopreserved embryos (Stokes et al *Hum. Reprod* 2007; 22:829-35). Recent work by Sturmey et al (*Mol Reprod Dev* 2010; 77:285-96) has shown that non invasive amino acid profiling now offers the possibility of predicting prospectively the ability of single bovine zygotes to develop to the blastocyst stage. This same study also highlighted differences in amino acid profiles between male and female embryos.

One explanation for these data is in terms of the 'Quiet Embryo Hypothesis' (Leese *Bioessays*. 2002; 24:845-849) which proposed that viable embryos have a 'quieter' metabolism than those which fail to develop. The term 'quiet' was used since it emphasized the range of values consistent with developmental competence (Leese et al *Hum Reprod* 2007; 22:3047-50). The hypothesis was developed by Baumann et al (*Mol Reprod Dev* 2007; 74:1345-53) who speculated that the origin of this relationship was linked to molecular and cellular damage; that 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 (such as amino acids) to repair processes; colloquially termed 'running repairs'. By contrast, embryos with higher levels of damage had a larger demand for nutrients and were thus metabolically more active. As a test of the hypothesis, Sturmey et al (*Hum. Reprod* 2009*;* 24:81-91) compared the amino acid turnover of cattle, pig and human blastocysts with levels of DNA damage in each individual cell of the embryo. For each species, there was a strong, positive correlation between amino acid turnover and DNA damage, consistent with the Quiet Embryo Hypothesis. Further studies reported by Morris et al (Teagasc, Research Centre, Athenry, Co. Galway Project No. 4627 Beef Production Series No. 40) compared rates of protein synthesis in bovine *in vivo* derived zygotes (which are of high quality in terms of their ability to develop to blastocysts and give rise to a pregnancy) with those produced *in vitro* (which are of lower quality). The *in vivo*-derived zygotes exhibited a lower rate of protein synthesis; an expensive process in energy terms, than the in vitro despite the protein content of the resulting blastocysts being similar in the two types of embryo; a conclusion in line with the Quiet Embryo Hypothesis.

Amino acid profiling is likely to be one of a number of biomarkers which relate an embryo's phenotype to its developmental potential. Moreover, such variables need not be concerned with metabolism; they could cover the whole range of cellular functions and have their own characteristic distributions; however, amino acid metabolism has proved to be a convenient marker for the early embryo because it may be measured non-invasively, exploits the wide range of amino acid functions and is therefore likely to generate data which differ considerably between individual embryos. In order to translate these findings into the IVF clinic, there needs to be a large-scale clinical trial, first to define retrospectively which amino acid profile(s) correlates most closely with a successful pregnancy outcome and then to test this finding prospectively. In reality it is likely the amino acid profile(s) will be combined with conventional morphological embryo scores to generate an algorithm predictive of embryo viability.