NEWS AND VIEWS

CONTROVERSIES IN ASSISTED REPRODUCTION AND GENETICS

Human Embryo Viability: What Determines Developmental Potential, and Can It Be Assessed?

INTRODUCTION

The true determinant of human embryo developmental potential is ultimately the normalcy of the gametes from which it is derived. Unfortunately, a significant percentage of gametes are genetically or chromosomally abnormal and, therefore, lack normal developmental competence. It has been estimated that approximately 25% of oocytes from a stimulated cycle are aneuploid (1), the frequency of this abnormality increasing with age (2). Conversely, not all sperm are chromosomally competent. Therefore, a significant percentage of embryos conceived through in vitro fertilization (IVF) were not destined to form a viable embryo or resultant fetus. Following from this, not all pronuclear embryos will reach the blastocyst stage of development in culture, their failure to develop being attributed to factors other than the culture environment. Yet this does not guarantee that all embryos that form blastocysts will be genetically normal.

DEVELOPMENTAL POTENTIAL IN CULTURE

Given that a particular pronuclear embryo conceived through IVF is genetically normal, what does

determine its developmental potential in culture, and equally as important, can it be assessed? To begin this discussion it is important to emphasize that culture conditions cannot improve the inherent developmental potential of an embryo per se. Rather it is evident that suboptimal culture conditions impair developmental potential and reduce viability and that optimal conditions facilitate normal cellular function. So what constitutes the optimal environment for the embryo developing in culture? It is proposed that two key items need to be addressed. First, the environment must fulfill the nutrient requirements of the embryo as it develops. As the embryo undergoes dramatic changes in its physiology and exhibits concomitant changes in its nutrient requirements and utilization (3–5), more than one medium formulation will be required to satisfy the changing demands of the embryo during the preimplantation period (6). Such a proposition not only is consistent with the dynamics of the embryo's physiology, but also mimics the environment to which the embryo is exposed in vivo, i.e., the fluids of the oviduct and uterus vary in their nutritional composition (7). Interestingly, the levels of nutrient available to the embryo as it passes along the female tract mirror the changing requirements of the embryo. Second, it is essential to include in the culture medium components that will help to minimize culture-induced cellular stress in the embryo. Examples of such stress being shifts in intracellular pH, osmotic shock, and perturbations in energy metabolism. It is proposed that the human embryo, and those of other mammalian species, is

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most susceptible to culture-induced stress prior to compaction, i.e., before the generation of the first transporting epithelium and therefore before the embryo can actively regulate its internal environment. The period prior to compaction in the human is also associated with the activation of the embryonic genome and is when the embryo appears to be most susceptible to stress as manifested by the characteristic developmental block around the eight-cell stage in vitro. After compaction the embryo can regulate its internal environment and is not as dependent on external regulators. Therefore, the requirements of the embryo for extracellular regulators are different before and after compaction (4,8), supporting the hypothesis that to maintain the viability of an embryo in culture will require more than one medium formulation. It may come as little surprise that certain regulators of cell function, amino acids, are present at relatively high levels in oviduct fluid. Amino acids have been shown to minimize intracellular pH shifts (8), reduce the impact of ionic/osmotic stress (9,10), and minimize metabolic perturbations (4,11,12). Although the optimal environment for the developing embryo is the female tract, mimicking the entire composition of the female in a culture medium may not necessarily produce the best environment in vitro, due to the stress placed on the embryo in vitro by the artificial nature of its environment. Therefore, other components of culture media, such as ethylenediaminetetraacetic acid (EDTA), which can hardly be considered a physiological molecule, are required in vitro to protect the embryo. EDTA acts by the chelation of potentially harmful divalent cations present in the culture system (13), and by its ability to reduce glycolytic flux (11,14), thereby minimizing the risk of any Crabtree-like effects in the cleavage-stage embryo (11,15).

By taking such factors into account, i.e., satisfying the embryo's nutritional requirements while at the same time protecting the embryo from culture-induced stress, thereby facilitating normal cell function, it has been possible to formulate media capable of supporting the development of the pronuclear human embryo to the blastocyst stage in culture. Examples of these media are G1 and G2 (16), formulated on the principles discussed here. As we learn more about the human embryo it becomes possible to build upon the basic principles on which media like G1 and G2 were formulated. At present in our clinic it is possible to obtain greater than 60% blastocyst development from pronuclear embryos after 4 days of culture (17,18), but, more important, such

blastocysts have been shown to have a high viability. After 120 blastocyst transfers we have established an implantation rate of 50%, with a mean of just over 2.1 blastocysts transferred, which has translated into an ongoing pregnancy rate of over 65%. Such high implantation rates are in contrast to those reported by Bolton *et al.* (19), who obtained 40% blastocyst development but an implantation rate of only 7%. Such differences can be attributed to the fact that Bolton *et al.* (19) used a rather simple culture system and did not allow for the changing requirements of the embryos (8,15). Importantly, it has been shown that culture conditions during the first 48 hr of development have a significant effect on subsequent blastocyst development even though the cell number and quality of embryos on day 3 were the same (18). Furthermore, although it is possible to get high frequencies of blastocyst development in culture under different conditions, this does not necessarily mean that the resultant blastocysts in each culture environment have the same developmental potential, i.e., blastocyst formation does not equate to viability (15). This can be explained by the different requirements of the inner cell mass and the early embryo, i.e., what stimulates the cleavage-stage embryo can actually impair inner cell mass development (20), reinforcing the need for more than one culture environment.

ASSESSMENT OF VIABILITY

The identification of a viable embryo requires the identification of some quantifiable parameter, and in the case of the human embryo this has to be noninvasive. Not even the incubation of embryos with some vital dye can be considered, no matter how harmless this procedure may appear. Embryo morphology has conventionally been the criterion on which embryos have been selected for transfer in human IVF. However, such procedures are notoriously subjective and therefore difficult to quantitate (21). Studies of the hamster embryo showed that the rate of cleavage was more informative in determining which embryos would go on to form a blastocyst in vitro or fetus after transfer (22,23). Two recent reports on the human embryo (24,25) indicate that the timing of the first and second cleavage divisions is related to pregnancy outcome, with the faster cleaving embryos giving rise to significantly more pregnancies. Bearing such data in

mind, it is perhaps time to reconsider the times at which we score embryos derived from IVF.

A parameter that can be directly quantitated non-invasively is nutrient uptake. This procedure has been used to identify viable blastocysts in the mouse (26,27). In both studies glucose was significantly higher in those blastocysts which went to term after transfer. Furthermore, measurement of both glucose uptake and lactate production to assess glycolytic activity could be used to identify prospectively those blastocysts with the highest viability prior to transfer (27). Using such an approach it was possible to increase the fetal rate per blastocyst transfer by a factor of four. Interestingly, a previous attempt to correlate nutrient uptake and embryo viability in the human showed that there was an inverse relationship between pyruvate uptake on days 2 and 3 and subsequent viability (28). However, this study was retrospective and was done only up to the eight-cell stage, i.e., before the embryonic genome is activated. It is therefore plausible that up to the eight-cell stage, one is not assessing true embryo physiology but rather that of the oocyte.

A quantitative measure of membrane integrity is the leakage of enzymes from the cytosol of the blastomere. As the mammalian embryo has an abundance of glycolytic enzymes, the appearance of specific enzymes in the surrounding culture medium can be used as an indirect measure of cell viability (29). However, although this approach applies to a rather extreme circumstance, it may be of use in the assessment of freezing procedures and their affect on membrane integrity.

In conclusion, the use of rate of embryo development to the blastocyst stage, combined with analysis of nutrient uptake and utilization, should provide a reliable indicator of an embryo's viability prior to transfer.

SUMMARY

The understanding of the embryo's nutrient requirements and physiology has led to the development of more physiological culture media, capable of supporting acceptable levels of human blastocyst development in vitro. The success of such media can be attributed to catering to the embryo's changing nutrient requirements, while minimizing culture-induced stress, thereby facilitating normal cell function. Most important, blastocysts derived from such sequential culture systems have a high viability. The ability to identify those blastocysts from within a given cohort which have the highest developmental

potential will lead to further increases in implantation and pregnancy rate. Such an approach should ultimately lead to the routine transfer of a single blastocyst in a given IVF cycle, while being able to maintain a high pregnancy rate.

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David K. Gardner¹

William B. Schoolcraft

Colorado Center for Reproductive Medicine
799 East Hampden Avenue, Suite 300
Englewood, Colorado 80110

¹ To whom correspondence should be addressed.