Evaluating Viability of IVP Embryos

Barry D. Bavister

Freeport-McMoRan Professor of Conservation and Reproductive Biology, University of New Orleans, Dept. of Biological Sciences and Audubon Center for Research of Endangered Species New Orleans, Louisiana, USA

In vitro produced (IVP) embryos produced by in vitro fertilization (IVF) often exhibit wide variations in developmental competence and viability, considerably more than are exhibited by embryos that develop *in vivo*. These anomalies in IVP embryos may be due to heterogeneity of oocyte quality, suboptimal culture conditions, disturbances in gene expression, or most likely a combination of these factors (Ho *et al.*, 1994; Roth *et al.*, 1994; McKiernan and Bavister, 1998; Hasler, 1998; Schramm and Bavister, 1999; Doherty *et al.*, 2000; Hyttel *et al.*, 2000; Niemann and Wrenzycki, 2000; Wrenzycki *et al.*, 2001). In research studies or in clinical applications with domesticated animals, cats, non-human primates and humans, oocytes used for IVF are usually collected from a heterogeneous cohort of ovarian follicles that include oocytes which normally might not be ovulated and/or are deficient in developmental competence. Moreover, although major improvements in culture media for oocyte maturation and embryo culture have been made in the last decade or so, IVF technology is still far from perfect (Bavister, 1995; Thompson and Peterson, 2000; Gardner *et al.*, 2000; Vanroose *et al.*, 2001). It is now clear that gene expression, including the critical transition from maternal to embryonic genome control, is subject to perturbations by culture media components, although the mechanism of this disturbance is not understood.

Thus, there are two principal concerns with respect to IVP technology. We need to further improve IVP procedures, especially culture media formulations, in order to raise the average quality of embryos; and also to improve methods for selecting the most viable embryos for transfer. For embryo selection, the dilemma is to evaluate embryos in ways that are rapid, simple, accurate and non-invasive. At present, the method that best fits all of these criteria is embryo development timing. While the timing of embryo development in vivo mostly proceeds

according to a precise schedule in all species, IVP embryos commonly show heterogeneity in their development timing, concomitant with variability in developmental competence. Those embryos that develop in a timely manner have the highest developmental competence and viability post-transfer, as shown in studies with a variety of species, such as rodents, cattle, and primates (McKiernan and Bavister, 1994; Hasler, 1998). Using timing of human embryo development during a limited time window, cleavage stage embryos were selected for transfer that supported pregnancy rates equal to those obtained with blastocyst transfers (Racowsky *et al.*, 2000). Research studies on the timing of embryo development and its relationship to embryo viability will be discussed.

References

1. Bavister, B.D. (1995) Culture of preimplantation embryos: facts and artifacts. *Hum. Reprod. Update*, 1, 91-148.
2. Doherty, A.S., Mann, M.R., Tremblay, K.D., Bartolomei, M.S. & Schultz, R.M. (2000) Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol. Reprod.*, 62, 1526-1535.
3. Gardner, D.K., Pool, T.B. & Lane, M. (2000) Embryo nutrition and energy metabolism and its relationship to embryo growth, differentiation, and viability. *Semin. Reprod. Med.*, 18, 205-218.
4. Hasler, J.F. (1998) The current status of oocyte recovery, *in vitro* embryo production, and embryo transfer in domestic animals, with an emphasis on the bovine. *J. Anim. Sci.*, 76, 52-74.
5. Ho, Y., Doherty, A.S. & Schultz, R.M. (1994) Mouse preimplantation embryo development *in vitro*: effect of sodium concentration in culture media on RNA synthesis and accumulation and gene expression. *Mol. Reprod. Dev.*, 38, 131-141.
6. Hyttel, P., Viuff, D., Laurincik, J., Schmidt, M., Thomsen, P.D., Avery, B., Callesen, H., Rath, D., Niemann, H., Rosenkranz, C., Schellander, K., Ochs, R.L. & Greve, T. (2000) Risks of in-vitro production of cattle and swine embryos: aberrations in chromosome numbers, ribosomal RNA gene activation and perinatal physiology. *Hum. Reprod.*, 15, 87-97.

7. McKiernan, S.H. and Bavister, B.D. (1994) Timing of development is a critical parameter for predicting successful embryogenesis. *Hum. Reprod.* 9:2123-2129.
8. McKiernan, S.H. & Bavister, B.D. (1998) Gonadotropin stimulation of donor females decreases post-implantation viability of cultured 1-cell hamster embryos. *Hum. Reprod.*, 13, 724-729.
9. Niemann, H. & Wrenzycki, C. (2000) Alterations of expression of developmentally important genes in preimplantation bovine embryos by *in vitro* culture conditions: implications for subsequent development. *Theriogenology*, 53, 21-34.
10. Racowsky, C., Jackson, K.V., Cekleniak, N.A., Fox, J.H., Hornstein, M.D. & Ginsburg, E.S. (2000) The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. *Fertil. Steril.*, 73, 558-564.
11. Roth, T.L., Swanson, W.F. & Wildt, D.E. (1994) Developmental competence of domestic cat embryos fertilized *in vitro* versus *in vitro*. *Biol. Reprod.*, 51, 441-451.
12. Schramm, R.D. & Bavister, B.D. (1999) Onset of nucleolar and extranucleolar transcription and expression of fibrillarin in macaque embryos developing *in vitro*. *Biol. Reprod.*, 60, 721-728.
13. Thompson, J.G. & Peterson, A.J. (2000) Bovine embryo culture *in vitro*: new developments and post-transfer consequences. *Hum. Reprod.*, 15, 59-67.
14. Vanroose, G., Van Soom, A. & de Kruif, A. (2001) From co-culture to defined medium: state of the art and practical considerations. *Reprod. Domest. Anim.*, 36, 25-28.
15. Wrenzycki, C., Herrmann, D., Keskintepe, L., Martins, A.J., Sirisathien, S., Brackett, B. & Niemann, H. (2001) Effects of culture system and protein supplementation on mRNA expression in pre-implantation bovine embryos. *Hum. Reprod.*, 16, 893-901.