0013-7227/05/$15.00/0
Printed in U.S.A.

Endocrinology 146(5):2140–2141

doi: 10.1210/en.2005-0221

Embryo Culture Conditions: What Embryos Like Best

In London this May, there will be an unusual event: the celebration of two million children delivered after *in vitro* fertilization (IVF) procedures. The first "test tube" baby, Louise Brown, was born on 25 July 1978, demonstrating for humans what had already been shown for the mouse and the rabbit, namely that an oocyte could be fertilized by sperm *in vitro*, transferred to the reproductive tract, and establish a successful pregnancy. By the mid-1980s there were a few hundred IVF children, mostly conceived at Bourne Hall, near Cambridge, where Patrick Steptoe, who retrieved the eggs, and Robert Edwards, the embryologist and human IVF pioneer, established their clinic and research laboratory. What was originally labeled as "immoral, unethical and dehumanizing" (http://news.bbc.co.uk./1/hi/health/3093429.stm; Ref. 1) has now become mainstream, transforming the treatment of infertility in both women and men, and bringing the gift of children to countless couples world wide. One birth in 50 in Western societies is now the outcome of IVF.

Despite the triumph of IVF, there remain concerns about the procedures. Although the success of such artificial reproductive technologies (ART) has improved over the last 25 yr, a successful outcome for any couple is not ensured (2). Many embryos simply fail to implant, and there also remains the daunting outcome of multiple pregnancies, a problem that would be most easily overcome by reducing the number of embryos transferred to one. Finally, there remains the worry that IVF babies are at greater risk than natural conception babies for developmental abnormalities (3).

For most species, it is the intrinsic quality of the oocyte before it is fertilized that primarily determines whether a zygote has the potential to progress through pregnancy (4). In general, human oocytes used for IVF are derived from a clutch of eggs collected by endoscopy after ovarian stimulation. Not all these eggs are of equivalent quality. Selection of embryos for transfer after they have divided only once or twice is usually based on their morphological appearance according to criteria that most embryologists recognize as arbitrary and imprecise (5). Accordingly, there has been an increased tendency to culture the embryos for several days before transfer (6). Because embryos that cleave fastest and reach the blastocysts stage earliest are likely to be the most developmentally competent (4), there is an obvious logic to this practice.

Although oocyte quality is crucial for successful IVF, the culture conditions in which the zygote forms and subsequently divides plays a large part in determining whether that oocyte potential can be realized (4). Unfortunately, *in vitro* culture can lead to epigenetic changes in the embryonic genome (7) and also influence gene expression in a global manner (4, 8–10), which together might well result in adverse outcomes on development. The best known example in animal models is the "large offspring syndrome" frequently noted in ruminant species after embryos have been cultured *in vitro* under less than optimal conditions (3, 4). There are also indications that human IVF offspring might have a higher frequency of abnormalities at birth (11, 12). The number of low birth weight IVF babies raises another concern, namely whether these children will demonstrate subsequent overcompensatory adolescent growth, hypertension, and other aspects of FOAD syndrome (fetal origin of adult disease), which has been noted in naturally conceived babies of low birth weight (see Ref. 3).

Culture media are now available that allow embryos to progress to blastocysts at rates comparable with those occurring within the uterus (12), raising the hope that such embryos will be free of the epigenetic marks introduced as a result of the stress of *in vitro* culture. Many of these media are based rather loosely on the concentrations of ions, amino acids, and sugars found in the reproductive tract of the female at the time of egg release, fertilization, and development (13). In the studies of Sjöblom *et al.* (14), for example, the focus of this commentary, a defined medium completely devoid of protein, allowed more than 95% of murine two-cell embryos flushed from the oviduct of naturally bred mice to advance to blastocysts. A key question is whether these blastocysts are of equivalent quality to those naturally conceived and developing *in vivo*. Some recent studies with mouse embryos have been reassuring in this regard in that they have shown that optimizing a culture medium in terms of its ability to promote embryo growth (15) or omitting a suspect ingredient such as fetal bovine serum (16) can avoid certain postnatal developmental and behavioral consequences attributed to the prior *in vitro* embryo culture. On the other hand, these studies also imply that minor deviations from optimal practice can lead to subtle, unintended consequences on the resulting pups.

Oviductal and uterine fluids are certainly more complex than any of the culture media now commonly used for human, rodent, and bovine embryos and contain, among their mysterious ingredients, bioactive factors produced by the mother. Sjöblom *et al.* (14) examined the effects of one such factor, a cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), which is known to be present in the reproductive tract and to have a positive effect on embryo cell number, by including it during culture of their mouse embryos to the blastocyst stage. These cultured embryos were then transferred to recipient females and examined either late in gestation or at various times after birth. Prior culture in regular medium without the cytokine led to lower fetal growth, a more rapid compensatory growth after birth, increased body mass as adults and greater fat deposits in the abdomen compared with controls that had not been cultured as embryos. Males were particularly susceptible to these outcomes. Surprisingly, the embryonic exposure to GM-CSF greatly reduced all these side effects of culture except the adiposity. The lower fetal growth of previ-

Abbreviations: ART, Artificial reproductive technologies; GM-CSF, granulocyte-macrophage colony-stimulating factor; IVF, *in vitro* fertilization.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

ously cultured embryos seems likely to have been caused by a reduction in volume of the placental region responsible for nutrient exchange. Again, GM-CSF reversed most of the effects of prior culture on placental structure. These results suggest that the outcomes of embryo culture in absence of GM-CSF are very likely the result of nutritional restrictions placed on the fetus as a result of a subfunctional placenta. The features of the syndrome, although quite subtle, are surprisingly similar to those noted in offspring of women who experienced famine during their pregnancies (reviewed in Ref. 3). The greater vulnerability of males to maternal nutritional stress has been noted for many species and generally results in a bias of the sex ratio toward females during times of food restriction as the result of fetal loss (reviewed in Ref. 17). It is not clear whether such a skewing occurred in the study of Sjöblom *et al.* (18), although there did not appear to be a reduction in litter size. Nor is it clear whether the affected male mice were hypertensive, as were male rats whose mothers experienced a short restriction of protein intake during early pregnancy.

What does all this mean in the context of human IVF? First, the study emphasizes that a culture medium that promotes excellent growth to blastocyst might not necessarily provide optimal developmental outcomes. Second, it is unlikely that GM-CSF is a magic ingredient that will cure all ills. At least one of the manifestations of prior culture noted by Sjöblom *et al.*, abdominal adiposity, was not reversed by *in vitro* exposure to the cytokine. Third, there are a host of factors, in addition to GM-CSF, present in the complex milieu of the uterine tract before implantation, and several of these have been reported to accelerate embryonic development (19). Finally, what applies to the mouse may not be relevant to other species. An example is the intolerance of bovine embryos to even modest concentrations of glucose (20). Embryos of different species likely have different requirements for optimal development *in vitro* and *in utero*. On the other hand, mammalian embryos exhibit remarkable plasticity and will struggle to form blastocysts under a wide range of culture conditions, although presumably at some adaptive cost to their postgestational development program. Even an embryo conceived normally faces an intimidating battle to survive *in vivo* as it struggles to keep pace with a changing uterine environment. The embryo must tolerate and adapt to nutritional and other stresses and pass what are likely to be quite stringent quality control barriers laid down by the mother to minimize the progression of embryos that she perceives as less than fit (21). It is not surprising that embryonic loss tends to be high in the majority of mammals that have been examined and that clones, whether they are produced naturally or by intent, are not exact images of each other.

ART now has a permanent place in our society. It brings great good and will not be legislated away. Perhaps the best that can be done is to continue to be vigilant about how embryos are created, manipulated, and cultured, and accept the reality that culture media will never mirror exactly the complex and not necessarily felicitous conditions of the reproductive tract. The final reality is that we are all individual, honed in part by the rigors we encountered during the time we were not much more than a tenth of a millimeter in size.

R. Michael Roberts
Departments of Veterinary Pathobiology, Biochemistry, and Animal Sciences
University of Missouri
Columbia, Missouri 65211

Acknowledgments

Received February 23, 2005. Accepted February 24, 2005.

Address all correspondence and requests for reprints to: R. Michael Roberts, Departments of Veterinary Pathobiology, Biochemistry, and Animal Sciences, University of Missouri, Life Sciences Center, 1201 E. Rollins Street, Columbia, Missouri 65211.

References

1. 24 July 2003, BBC (British Broadcasting Corp.) News, UK Edition, http://news.bbc.co.uk./1/hi/health/3093429.stm; Glasgow, Scotland, UK
2. **Schoolcraft W, Surrey E, Gardner D** 2001 Embryo transfer: techniques and variables affecting success. Fertil Steril 76:863–870
3. **Fleming T, Kwong W, Porter R, Ursell E, Fesenko I, Wilkins A, Miller D, Watkins A, Eckert J** 2004 The embryo and its future. Biol Reprod 71:1046–1054
4. **Lonergan P, Rizos D, Gutierrez-Adan A, Fair T, Boland M** 2003 Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Dom Anim 38:259–267
5. **Ebner T, Moser M, Sommergruber M, Tews G** 2003 Selection based on morphological assessment of oocytes and embryos at different stages of preimplantation development: a review. Hum Reprod 9:251–262
6. **Gardner D, Lane M** 2003 Towards a single embryo transfer. Reprod Biomed Online 6:470–481
7. **Mann M, Lee S, Doherty A, Verona R, Nolen L, Schultz R, Bartolomei M** 2004 Selective loss of imprinting in the placenta following preimplantation development in culture. Development 131:3727–3735
8. **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
9. **Rinaudo P, Schultz R** 2004 Effects of embryo culture on global pattern of gene expression in preimplantation mouse embryos. Reproduction 128:301–311
10. **Behr B, Wang H** 2004 Effects of culture conditions on IVF outcome. Eur J Obstet Gynecol Reprod Biol 115(Suppl):S72–S76
11. **Schieve L, Rasmussen S, Buck G, Schendel D, Reynolds M, Wright V** 2004 Are children born after assisted reproductive technology at increased risk for adverse health outcomes? Obstet Gynecol 103:1154–1163
12. **Summers M, Biggers JD** 2003 Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. Human Reprod Update 9:557–582
13. **Gardner D, Lane M** 1998 Culture of viable human blastocysts in defined sequential serum free media. Hum Reprod 13:148–160
14. **Sjöblom C, Roberts C, Wilkland M, Robertson S** 2005 Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. Endocrinology 146:2142–2153
15. **Ecker D, Stein P, Xu Z, Williams C, Kopf G, Bilker W, Abel T, Schultz R** 2004 Long-term effects of culture of preimplantation mouse embryos on behavior. Proc Natl Acad Sci USA 101:1595–1600
16. **Fernández-Gonzalez R, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez M, De Fonseca F, Pintado B, Gutiérrez-Adán A** 2004 Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. Proc Natl Acad Sci USA 101:5880–5885
17. **Rosenfeld CS, Roberts RM** 2004 Maternal diet and other factors affecting offspring sex ratio: a review. Biol Reprod 71:1063–1070
18. **Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP** 2000 Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 127:4195–4202
19. **Paria B, Dey S** 1990 Preimplantation embryo development in vitro: cooperative interactions among embryos and role of growth factors. Proc Natl Acad Sci USA 87:4756–4760
20. **Larson M, Kimura K, Kubisch H, Roberts R** 2001 Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocysts and in the expression of the signaling molecule IFN-τ. Proc Natl Acad Sci USA 98:9677–9682
21. **Roberts R, Xie S, Mathialagan N** 1996 Maternal recognition of pregnancy. Biol Reprod 54:294–302

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**