Article

Predicting embryo quality: mRNA expression and the preimplantation embryo

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Abstract

To overcome the low implantation rate (10–20%) following IVF in humans, more than two embryos are commonly replaced, potentially leading to high order multiple pregnancies with associated significantly elevated risks. Selecting the most viable embryos and transferring fewer of them could reduce this risk. Prolonged culture of embryos *in vitro* to the blastocyst stage may expose the embryo to hazards not normally encountered in the female reproductive tract. Recent studies comparing bovine oocyte maturation, fertilization and embryo culture *in vivo* and *in vitro* have demonstrated that the origin of the oocyte is the main factor affecting blastocyst yield, while the post-fertilization culture environment is crucial in determining blastocyst quality, measured in terms of cryotolerance and relative transcript abundance, irrespective of the origin of the oocyte. Production of embryos *in vitro*, particularly when using an extended period of in-vitro culture may predispose the embryo to phenomena such as 'large offspring syndrome', which is probably linked to altered gene expression, particularly of imprinted genes. Post-fertilization culture environment clearly has a profound effect on the relative abundance of gene transcripts within the embryo. Culture under sub-optimal conditions for even one day can lead to perturbations in the pattern of expression.

Keywords: *assisted reproduction, blastocyst, development, embryo culture, gene expression, in-vitro fertilization*

Introduction

It is now more than 25 years since the birth of the first IVF baby, but success rates are still disappointingly low, with only 1:5 couples going home with a child. The advent of techniques such as intracytoplasmic sperm injection (ICSI) and surgical sperm retrieval has meant that fertilization can now be achieved in almost all couples. However, apparently normal embryos are often replaced into the mother but fail to implant.

In human assisted reproduction treatment a trade-off exists between the risk of multiple pregnancy and the prospects for pregnancy itself. One way of tipping the balance against multiple pregnancies would be to select the most viable embryos for transfer and to transfer fewer of them. Two approaches towards this objective are: to select the fastest cleaving embryos for transfer at day 2 or 3 (Shoukir *et al*., 1997; Lonergan *et al*., 1999; Sakkas *et al*., 2001; Fenwick *et al*., 2002; Salumets *et al*., 2003); or the prolonged culture of embryos *in vitro* for ~5 days to the blastocyst stage, by which time the least viable have succumbed, leaving the most competent for transfer (Gardner *et al*., 1998a,b, 2000).

In humans, blastocyst culture has been reported to substantially increase the implantation rate per embryo transferred (Gardner *et al*., 1998a; Huisman *et al*., 2000; Milki *et al*., 2000) although this has been questioned (Kolibianakis and Devroey, 2002). The safety of prolonged culture in humans has not been firmly established, especially in light of the numerous studies from domestic ruminants of abnormalities apparently associated with perturbations induced by in-vitro culture (Menezo *et al*., 1999; Sinclair *et al*., 2000; Sinclair and Singh, 2004).

Such extended culture allows the embryos to 'select' themselves by growth to the blastocyst stage. In addition, it would help synchronize embryonic stage with the female tract. However, blastocyst formation itself does not fully reflect the viability of the embryo (Tsirigotis, 1998; Jones and Trounson, 1999) and not all blastocysts are of equal quality (Rizos *et al*., 2002b,c, 2003).

Indeed, blastocyst development is only one step along the road to the production of a live offspring and, as pointed out by McEvoy *et al.* (2000), attainment of that stage is more a reflection of past achievement than a guarantee of future ability to implant and give rise to an offspring.

Embryo production *in vitro*

Generally, for the production of embryos in human assisted reproduction treatment, a matured oocyte is recovered from the pre-ovulatory follicle and inseminated shortly afterwards, and the resulting zygote is cultured for 1 or 2 days before being transferred back to the same donor. By contrast, production of domestic animal embryos *in vitro* is essentially a three-step process involving in-vitro maturation (IVM) of immature oocytes recovered from antral follicles, IVF, and subsequent culture of the in-vitro-derived zygote to the blastocyst stage, at which point they are transferred to surrogate recipients. In terms of efficiency, in cattle approximately 90% of immature oocytes undergo nuclear maturation *in vitro* from prophase I to metaphase II (the stage at which they would be ovulated *in vivo*); and about 80% undergo fertilization and cleave at least once, to the 2-cell stage. However, only 30–40% reach the blastocyst stage. Thus, the major fall-off in development occurs during the last part of the process (in-vitro culture), between the zygote and blastocyst stages, suggesting that post-fertilization embryo culture is the most crucial point in the process in terms of determining blastocyst yield. However, it is known now that this is not the case; there is unequivocal evidence demonstrating that events further back along the developmental axis (i.e., the quality of the oocyte) are crucial in determining the proportion of immature oocytes that form blastocysts and that in fact the post-fertilization culture environment, within certain limits, does not have a major influence on the capacity of the immature oocyte to form a blastocyst (Rizos *et al.*, 2002c, 2003).

There is considerable evidence supporting the notion that the post-fertilization culture environment is crucial in determining the quality of the blastocyst, assessed in terms of cryotolerance and gene expression pattern. For example, by culturing in-vitro-produced bovine zygotes *in vivo* in the ewe oviduct, it is possible to dramatically increase the quality of the resulting blastocysts, measured in terms of cryotolerance, to a level similar to that of totally in-vivo-produced embryos (Galli and Lazzari, 1996; Enright *et al.*, 2000; Rizos *et al.*, 2002c) (**Figure 1a**). Furthermore, in the reciprocal experiment, the culture *in vitro* of in-vivo-produced bovine zygotes results in blastocysts of low cryotolerance (Rizos *et al.*, 2002c) (**Figure 1b**). In other words, the culture of 'poor quality' zygotes, produced by IVM and

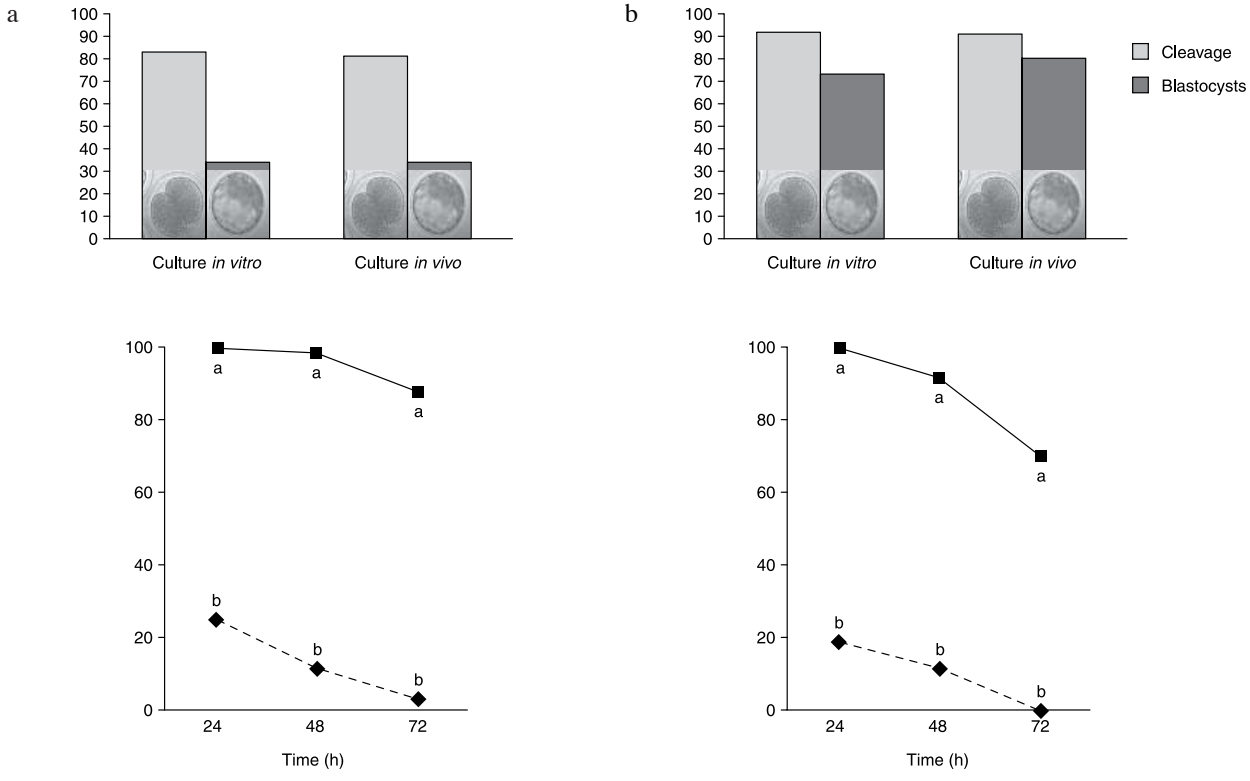

Figure 1. Development and survival after cryopreservation of bovine blastocysts produced following (**a**) maturation and fertilization *in vitro* and culture either *in vitro* or *in vivo*, or (**b**) maturation and fertilization *in vivo* and culture either *in vivo* or *in vitro*. Note that blastocyst yield following in-vitro maturation (IVM) and IVF was approximately 35% compared with approximately 80% following in-vivo maturation and fertilization and that this was not affected by culture environment. In addition, note that the survival of in-vivo (solid line) cultured blastocysts after cryopreservation was significantly higher than those cultured *in vitro* (dashed line), irrespective of the origin of the oocyte. Data from Rizos *et al.* (2002c).