Review

Predictive factors for embryo implantation potential

Since 1992, Dr Borini has been Clinical Director of the Assisted Reproduction Centre 'Tecnobios Procreazione', Bologna, Italy, and of a number of associated IVF clinics. After graduating in Medicine and Surgery at the University of Bologna, Bologna, Italy, in 1986, he completed his residency in Obstetrics and Gynaecology at the University of Bologna in 1991. He then attended the University of California Irvine, Irvine, California, as Research Fellow from October 1989 to May 1991. His research areas are embryo implantation, oocyte freezing and multiple induction of ovulation. Dr Borini has published more than 160 research papers and acts as Chairman of CECOS ITALY.

Dr Andrea Borini

Andrea Borini[1,3], Cristina Lagalla[1], Monica Cattoli[1], Elena Sereni[1], Raffaella Sciajno[1], Carlo Flamigni[2], Giovanni Coticchio[1]

[1]Tecnobios Procreazione, Via Dante 15, Bologna 40125; [2]University of Bologna, Bologna 40125, Italy

[3]Correspondence: Tel: +39 051 2867511; Fax: +39 051 2867512; e-mail: borini@tecnobiosprocreazione.it

Abstract

In spite of recent improvements in IVF, pregnancy rates have not increased significantly and one of the major problems remains the high multiple pregnancy rate. Better criteria are therefore necessary to establish the viability of a transferable embryo. Early prognosis of the developmental fate of the oocyte would help in selecting the best embryos to transfer, but non-invasive selection at the oocyte stage (extracytoplasmic and intracytoplasmic morphology) has proved to be of little prognostic value. Recently, it has been shown that follicular vascularization appears to be predictive of oocyte developmental fate, making it a good first-step approach for selection. Observation of pronuclei patterns at the zygote stage appears to offer an additional prognostic tool, correlating well with IVF outcome. Morphological evaluation of the embryo at days 2–3 remains the most used and valid method of selection, even though it is not sufficient to select embryos with the higher implantation potential. Blastocyst culture is another possible strategy for selecting the best embryos with reduced risk of aneuploidies, though not all major chromosomal aberrations are excluded by prolonged in-vitro culture. In summary, selecting the best embryo for transfer is a decision that should be based on choices made during the different stages of assisted reproductive technologies.

Keywords: *blastocyst culture, embryo implantation, embryo transfer, IVF, oocyte quality, perifollicular vascularity*

Introduction

In mammalian species, the vast majority of preimplantation embryos give rise to a fully formed individual. This is in clear contrast with the human species, in which it is estimated that in the fertile population, only a small proportion of ovulated oocytes (about 20–30%) undergo fertilization and develop to term. This state of affairs means that in IVF therapy, supernumerary embryos need to be generated in order to maximize the chances of success of each treatment cycle and more than one embryo needs to be transferred, with an increasing risk of multiple births (Shoukir *et al*., 1997). Unfortunately, morphological attributes do not fully reflect the developmental potential of the human preimplantation embryo (Munné and Cohen, 1998). As a consequence, the choice of embryos suitable for transfer via routine microscopic evaluation, especially when performed at a single developmental stage, cannot ensure a viable pregnancy, while exposing the patient to the risk of a high-order pregnancy. More accurate selection criteria are clearly required (Steer *et al*., 1992a; Shoukir *et al*., 1997; Van Blerkom, 1997). In most clinical cases, embryos are morphologically assessed and chosen for transfer on day 2–3. Alternative selection procedures have been proposed, such as pronuclear stage morphology, timing of first cell cleavage (Shoukir *et al*., 1997), evaluation of chromosomal status (Gianaroli *et al*., 2003) and extended embryo culture (Gardner and Schoolcraft, 1999a; Gardner *et al*., 1998b, 2000a,b). Given that the fate of the embryo is largely dependent on the oocyte from which it originates (Albertini *et al*., 2003), studies have been conducted to investigate a possible association between oocyte morphology and developmental potential (Serhal *et al*., 1997; Xia, 1997; Ebner *et al*., 2000). Attempts have also been made to examine whether the developmental or clinical outcome of the oocyte can be predicted by specific features, such as size or vascularization, in the pre-ovulatory follicle in which

oogenesis occurs (Bhal *et al.*, 2001; Borini *et al.*, 2004a,b). This paper aims to review such studies, presenting the laboratory as well as clinical evidence.

Laboratory aspects

Oocyte morphology

Since the introduction of ICSI (intracytoplasmic sperm injection), it has been possible to evaluate a wide range of information on oocyte morphology. This technique requires the removal of cumulus cells from the oocyte with a view to selecting for microinjection only those oocytes with an extruded polar body I (PBI) and presumably therefore in metaphase II.

Different morphological characteristics of the oocyte have been investigated including shape, zona pellucida thickness, size of the perivitelline space, granularity of the cytoplasm, presence of vacuoles and incorporations, e.g. refractile bodies, and shape of the PBI. Attempts have been made to relate these features to IVF outcome, but available data remain controversial.

It is assumed that mature oocytes of good morphology have a clear, moderately granulate cytoplasm, a small perivitelline space, an intact PBI and a colourless zona pellucida (Xia, 1997; Ebner *et al.*, 2000), but in fact more than half of all collected oocytes show at least one morphological abnormality (Ebner *et al.*, 2001a), which can be classified as cytoplasmic or extracytoplasmic (Ebner *et al.*, 2001b).

Cytoplasmic abnormalities

The category of cytoplasmic abnormalities includes increased granularity or discoloration of the cytoplasm, uneven cytoplasmic appearance, perhaps corresponding to irregular aggregation of the smooth endoplasmic reticulum, vacuolization and incorporations such as refractile bodies.

It has been shown that, to a certain extent, specific dysmorphic phenotypes in oocytes are likely to be a normal occurrence (Meriano *et al.*, 2001), while some of them may reflect intrinsic defects that might adversely affect oocyte competence (Van Blerkom and Henry, 1992). In this regard, oocytes with abnormal cytoplasmic organization or atypical inclusions seem to occur under conditions of hypoxia generated by poorly or under-vascularized follicles (Van Blerkom, 1997), and relate to regions of oocyte cytoplasm where the number of active mitochondria is fewer than in oocytes coming from well-oxygenated follicles where the active mitochondria are evenly distributed (Van Blerkom *et al.*, 1998). Serhal *et al.* (1997) reported that while oocyte cytoplasmic granularity and inclusions (vacuoles, refractile bodies) do not jeopardize fertilization after ICSI and the quality of the resulting embryo, the transfer of embryos derived from oocytes carrying these cytoplasmic abnormalities does coincide with a massive decrease in pregnancy and implantation rates. A retrospective study by Loutradis *et al.* (1999) confirmed that oocytes with poor morphology (dark cytoplasm, many vacuoles or fragments in the perivitelline space) lead to poor quality embryos and consequently to lower pregnancy rates (5.5 versus 29.4%). The authors also related oocyte quality to serum oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) administration, suggesting that patients with a higher serum oestradiol concentration generate oocytes of higher quality and consequently a higher proportion of good quality embryos, achieving higher pregnancy rates after ICSI.

Extracytoplasmic abnormalities

This category includes zona pellucida thickness, oocyte shape irregularity, perivitelline space with debris or enlarged abnormal oolemma and zona pellucida and PBI morphology.

It has been reported that some of these features are associated with a decreased survival rate after ICSI, but not with fertilization and embryo quality (Ebner *et al.*, 2001b).

Some studies have been performed assessing different oocyte features simultaneously. Xia *et al.* (1997) conducted a study in which oocytes were graded into four groups on the basis of the status of PBI, size of perivitelline space and cytoplasmic inclusions as shown in **Table 1**. The results showed that, for oocytes without cytoplasmic inclusions, fertilization rate and embryo development beyond the 2-cell stage were significantly lower in the oocytes of grade 1–2 (poor) than those in oocytes at grade 3–4 (good). Grade 4 oocytes without inclusions gave the highest proportion (66.7%) of good quality embryos. It was also observed that a higher proportion of grade 1–2 oocytes (44.7%) was obtained from patients older than 35 years; furthermore, a higher proportion of oocytes containing cytoplasmic inclusions was seen in patients with female factor infertility (24.9%) and over 35 years (26.5%) compared with patients below 35 years with male factor infertility. While this study indicates that oocyte quality is significantly related to fertilization rate and embryo quality after ICSI, Balaban *et al.* (1998) did not observe any difference in fertilization, embryo quality and pregnancy/implantation rates between oocytes with different forms of abnormalities, including cytoplasmic granularity and refractile bodies, and those with no irregularity. It is possible that different stimulation protocols and evaluation criteria may have contributed to the divergence in the literature regarding the extent to which oocyte morphology, at the light microscopy level, is related to ICSI outcome (Balaban *et al.*, 1998).

PBI morphology

The idea that PBI morphology could reflect the functional status of the oocyte was initially suggested in a report describing PBI fragmentation as an indication of post-ovulatory ageing (Eichenlaub-Ritter *et al.*, 1995). In more recent years, assessment of PBI morphology has been suggested as valuable criterion for predicting oocyte developmental ability. Ebner *et al.* (1999, 2000, 2002) have repeatedly found a positive association between polar body morphology and oocyte quality. They classified PBI morphology into five grades (**Table 2**) (Ebner *et al.*, 1999). In these studies, it was reported that oocytes with an intact PBI, grade 1 and 2, generate better quality embryos with a higher blastocyst formation rate. It was also found that the transfer of embryos from oocytes selected on the basis of PBI morphology leads to increased pregnancy and implantation rates.

However, some authors have been unable to confirm a correlation between PBI morphology and oocyte quality. A