Prognostic value of first polar body morphology on fertilization rate and embryo quality in intracytoplasmic sperm injection

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The association between oocyte morphology and subsequent fertilization rate and embryo quality in intracytoplasmic sperm injection (ICSI) is subject to considerable controversy. This retrospective study was carried out to investigate a possible prognostic value of first polar body morphology with regard to fertilization rate and embryo quality. A total of 70 consecutive ICSI cases was included in this study. The results showed that classification based on first polar body morphology revealed a significant correlation with fertilization rate (P < 0.025) and embryo quality (P < 0.001). Cytoplasmic criteria showed no correlation in this respect. Present data indicate that ICSI of oocytes with intact well-shaped first polar bodies yields higher fertilization rates and higher quality embryos.

Key words: embryo quality/fertilization/first polar body/intracytoplasmic sperm injection/oocyte morphology

Introduction

With the introduction and establishment of intracytoplasmic sperm injection (ICSI) in assisted reproduction technology, substantial fertilization and implantation rates, even in couples with severe male infertility, can be achieved (Palermo et al., 1992; Van Steirteghem et al., 1993).

The outcome of ICSI treatment is not only affected by patient parameters but also by embryo variables (Serhal et al., 1997), though no correlation could be demonstrated between oocyte morphology and fertilization rate or embryo quality (De Sutter et al., 1996; Balaban et al., 1998). However, recent data revealed a significant association between oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality (Xia, 1997).

In summary, oocyte features and their influence on fertilization rate and embryo quality are controversial in ICSI. However, this influence is accepted in conventional in-vitro fertilization (IVF) (Veeck, 1988; Bedford and Kim, 1993).

To our knowledge, the prognostic value of first polar body on fertilization rate and embryo quality has not yet been examined in ICSI.

Materials and methods

The study included 66 patients who underwent 70 consecutive ICSI treatments. All women were ≦40 years of age (average age: 31.2; range: 22–40). The couples were referred to the ICSI programme for either male infertility (50 cases, <5 × 10⁶ progressively motile spermatzoa) or failure of at least two previous IVF cycles (16 cases).

Ovarian stimulation was performed (Smitz et al., 1987) with human menopausal gonadotrophin following down-regulation of the pituitary with a gonadotrophin-releasing hormone agonist. When the lead follicle reached a mean diameter of 19 mm and serum oestradiol appeared adequate, 5000–10 000 IU human chorionic gonadotrophin (HCG) were injected to induce ovulation. Oocyte retrieval was carried out transvaginally under ultrasound guidance 36 h after HCG administration.

After a 3 h incubation in BM1 medium (NMS Bio-Medical, Praroman, Switzerland) at 37°C in a 6% CO₂ atmosphere, the collected eggs were briefly exposed to 80 IU/ml hyaluronidase to facilitate mechanical removal of the surrounding cumulus cells. Thus, accurate assessment of first polar body morphology, and consequently of nuclear maturity, was possible. At the same time morphological anomalies of the cytoplasm were recorded. Only the most frequent cytoplasmic anomalies were used for statistical analysis, i.e. dark incorporations, dark cytoplasm (inclusive granularity) or zona pellucida, refractile bodies, irregular shape.

All semen samples were obtained by masturbation and analysed as recommended (Kruger et al., 1986; World Health Organization, 1992). Ejaculates were washed and centrifuged in sperm preparation medium. A Mini swim-up technique was used to obtain a sufficient number of progressively motile spermatozoa for injection.

Before ICSI treatment was performed (immediately after removal of cumulus cells), using an inverted microscope (>200 magnification) and Hoffman Modulation Contrast optics, oocytes were placed in drops of BM1 medium in a Petri dish. Then the ICSI procedure was carried out by means of micromanipulators as published elsewhere (Van Steirteghem et al., 1993). Particular importance was attached to accurate injection timing (39–40 h after HCG administration). After injection, the oocytes were incubated separately in order to avoid mixing of the different polar body grades (Table I, Figure 1).

Fertilization rate and embryo grade (according to Veeck, 1990) per polar body grade were assessed 16–18 h and 40–42 h after ICSI by an independent observer. The criterion for accepting fertilization as normal was the presence of two pronuclei as well as two polar bodies.

Table 1. Oocyte grading according to first polar body morphology

<table>
<thead>
<tr>
<th>Grade</th>
<th>Shape of first polar body</th>
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<tbody>
<tr>
<td>1</td>
<td>Ovoid or round, smooth surface</td>
</tr>
<tr>
<td>2</td>
<td>Ovoid or round, rough surface</td>
</tr>
<tr>
<td>3</td>
<td>Fragmented</td>
</tr>
<tr>
<td>4</td>
<td>Large*</td>
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*Large perivitelline space.
Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). The statistical methods used were the Mann-Whitney U-test and the χ²-test. Significance was defined as P < 0.05.

Results

Overall, 70 punctures resulted in 610 oocytes (8.7 ± 4.1 per cycle). Forty-eight (7.9%) oocytes remained in prophase I and 18 (2.9%) in metaphase I, while 544 (89.2%) were mature. Of these, six metaphase II (MII) oocytes were excluded from further analysis because they were found to have multiple vacuoles. Thus 538 eggs were considered for ICSI.

A total of 238 (44.2%) oocytes had at least one cytoplasmic anomaly. None of the morphological classes separately revealed a significant association with fertilization rate. Using a published protocol (De Sutter et al., 1996) all oocytes were divided into three categories: oocytes without any anomaly, oocytes displaying one, and oocytes with more than one anomaly (Table II). No significant difference between any of the cytoplasmic anomalies and fertilization rate was found.

On the other hand, gradation with regard to first polar body morphology revealed a significant association with fertilization rate (Table II, P < 0.025). The appearance of morphological anomalies was not related to polar body morphology (Table III, P > 0.05).

Thirty-nine (7.2%) eggs degenerated after ICSI, 48 (8.9%) MII oocytes failed to fertilize, while 15 (2.8%) oocytes displayed three pronuclei. All in all, 436 eggs were considered for embryo quality assessment.

A significant association was found between oocyte grading based on the morphology of the first polar body and embryo quality (P < 0.001). Embryo stage (2–6 cells on day 2) was not related to first polar body appearance (data not shown). Morphological anomalies did not influence embryo quality as assessed by the proportion of embryos with <25% fragmentation (Table II).

Discussion

The introduction and establishment of ICSI (Palermo et al., 1992; Van Steirteghem et al., 1993) provided substantial fertilization rates in cases of dramatically diminished sperm parameters. The success of this procedure is facilitated by enzymatic and mechanical removal of the surrounding cumulus cells ensuring accurate assessment of oocyte maturity and quality.

It is generally recognized that ovarian stimulation using a long protocol may affect oocyte quality because maturation of abnormal oocytes (which otherwise would have become atretic)
Changes in cytoplasmic appearance during completion of reduction embryo quality because it is known that ageing of the... 187 79 42.3 In conventional IVF, atypical morphology of...

Table III. Relationship between first polar body (PB) grading and appearance of morphological anomalies

<table>
<thead>
<tr>
<th>PB grade</th>
<th>No. of oocytes</th>
<th>≥1 anomaly</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>174</td>
<td>71</td>
<td>40.8</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>79</td>
<td>42.3</td>
</tr>
<tr>
<td>3</td>
<td>159</td>
<td>70</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*Only the most frequent anomalies were separately recorded (see text), which accounts for the difference in the total number recorded (307) and the total number of oocytes showing any anomaly (320 above).

Percentages with different superscripts in a column differ significantly: a,b,c P < 0.025, d,e,f P < 0.001 (χ²-test).

ICSI = intracytoplasmic sperm injection; Deg = degeneration of oocyte; PN = pronucleus.

can occur (Serhal et al., 1997). A higher number of mature eggs per oocyte collection can compensate for this drawback (Greenblatt et al., 1995). However, chromosomal disorder has been reported for up to 40% of all eggs collected (Van Blerkom and Henry, 1992). In addition, more than half of the oocytes show morphological anomalies (De Sutter et al., 1996). This could be related to a progesterone and oestradiol deficiency which is closely connected to maturation of the nucleus and cytoplasm (Thibault, 1977). Oocytes recruited from slow-developing follicles may not have adequate hormonal supply at the time of ovulation induction. In the present study, the hormonal supply seemed to be optimal which was manifested in a lower percentage of cytoplasmic anomalies. In contrast to conventional IVF, for which cytoplasmic inclusions, especially refractive bodies, are known to correlate with lower fertilization rates (Veeck, 1990), ICSI results are not influenced by cytoplasmic anomaly (Serhal et al., 1997; Balaban et al., 1998).

De Sutter et al. (1996) reported that so-called ‘ideal’ oocytes (those without inclusions and granular cytoplasm) represent only 34% of all collected oocytes. Although the present study showed a higher percentage of oocytes without anomalies, it produced similar results concerning morphological aspects and ICSI results. Cytoplasmic anomalies showed no correlation with ICSI results (neither separately nor in combination). Changes in cytoplasmic appearance during completion of cytoplasmic maturation are unlikely to influence fertilization rate and embryo quality. Since cytoplasmic anomalies and appearance of first polar body were not related (P < 0.05), it may be speculated that first polar body morphology provides an acceptable prognostic value with regard to fertilization rate and embryo quality.

In conventional IVF, atypical morphology of first polar body (fragmentation, irregularity in size) may result in slightly reduced fertilization rates (Veeck, 1990). Thus, it seems reasonable to suspect similar results in ICSI. Surprisingly, only few data on this aspect are available. In the literature, the prognostic value of first polar body morphology on embryo quality in ICSI was assessed either in combination with other criteria (Xia, 1997) or not at all (De Sutter et al., 1996; Balaban et al., 1998). Hence, discrepancies in reports on the prognostic value of morphological features of the oocyte on fertilization rate and embryo quality may be due to the different approaches to oocyte evaluation. This is the first investigation on a larger number of oocytes focusing exclusively on the relationship of the first polar body and microinjection results.

In contrast to natural cycles, nuclear and cytoplasmic maturation seem to act asynchronously in IVF and ICSI (Sundstrom and Nilsson, 1988). Extrusion of the first polar body, which indicates the end of meiotic maturation, can easily be controlled. In the worst case, suboptimal nuclear maturation may lead to a complete failure of first polar body extrusion. Rapid maturation to metaphase II before retrieval and prolonged arrest in this state before fertilization is believed to contribute to a degenerative effect on the first polar body (Eichenlaub-Ritter et al., 1995). Ovarian stimulation may result in recruitment of metaphase II oocytes with varying duration of nuclear maturation which is likely to be manifested in varying grades of first polar bodies. This suboptimal nuclear maturation may reduce embryo quality because it is known that ageing of the
oocyte can result in spindle damage and loss of chromosome scattering (Pickering et al., 1988; Martini et al., 1997).

In conclusion, the present data indicate that first polar body should be considered as a prognostic factor with regard to egg quality. This has also been shown by a significantly lower fertilization rate in grades 3 and 4. However, higher numbers of oocytes per grade will need to be examined in order to provide a clearer picture of this relationship. Our data suggest that in ideal circumstances oocytes showing an enlarged first polar body (grade 4) should not be considered for transfer.

Firstly, the present study revealed that such polar bodies were associated with an enlarged perivitelline space and, secondly, all recorded grade 4 oocytes showed multiple morphological anomalies which may be associated with an increased frequency of aneuploidy (Van Blerkom and Henry, 1992). For future studies it would be interesting to clarify the chromosomal situation and the implantation potential of embryos derived from oocytes with varying first polar bodies. However, the preliminary data concerning the prognostic value of first polar body morphology for the rates of implantation and pregnancy are encouraging (Ebner et al., 1999).

References


