First polar body morphology before ICSI is not related to embryo quality or pregnancy rate

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BACKGROUND: The aim of this study was to analyse the relationship between the first polar body (1st PB) morphology and the fertilization rate, cleavage rate, embryo quality, pregnancy and implantation rate. METHODS: This was a retrospective study on 167 consecutive cycles undergoing assisted reproduction with ICSI. The 1st PB morphology was evaluated at the moment of ICSI in the 596 injected oocytes and it was coded as intact or fragmented. The fertilization rate, cleavage rate, embryo quality (three grades), pregnancy rate, implantation rate and the time elapsed between oocyte retrieval and ICSI were evaluated. The 1st PB morphology was checked twice (denudation and ICSI) in a random sample of 180 oocytes in order to verify the effect of the in vitro culture. RESULTS: No significant relationship was found between the 1st PB morphology and the fertilization rate (P = 0.703), cleavage rate (P = 0.055), embryo quality (P = 0.673), pregnancy rate (P = 0.201) and implantation rate (P = 0.511). A significant positive relationship (P = 0.006) was found between the frequency of the 1st PB fragmentation and the time elapsed between denudation and ICSI. The pregnancy rate was significantly higher (P = 0.008) when oocytes were injected between 5 and 7 h after retrieval rather than earlier or later. CONCLUSIONS: Our data suggest that the embryo quality, pregnancy rate and implantation rate are not related to the 1st PB fragmentation. The time which elapses between the oocyte retrieval and ICSI should be maintained at ~6 h in order to obtain optimal results.

Key words: embryo quality/first polar body/pregnancy rate/sperm injection

Introduction

ICSI is an assisted reproductive technique which has enabled many couples to achieve pregnancy. The introduction of ICSI generated a wide range of information about oocyte morphology because this technique requires the denuding of the oocytes from the cumulus cells and permits the oocyte to be observed during the process of fertilization. The removal of the cumulus cells from the oocyte prior to ICSI allows investigation of many factors: the shape of the oocyte, the colour and the granularity of the cytoplasm, the regularity and the thickness of the zona pellucida, the size of the perivitelline space, the presence of vacuolization, the presence of the germinal vesicle and the absence or the presence of the first polar body (1st PB) and its morphology. The influence of all these oocyte features on the fertilization rate, embryo quality, pregnancy and implantation rate is still controversial when using ICSI (De Sutter et al., 1996; Serhal et al., 1997; Xia, 1997; Balaban et al., 1998; Hidekazu et al., 2000; Kahraman et al., 2000; Meriano et al., 2001). The establishment of simple criteria, to be used with light microscopy, to help the embryologists to choose the embryo(s) for transfer is fundamental for improving the pregnancy rate and reducing the incidence of multiple pregnancies. First PB morphology is believed to indicate the postovulatory age of the oocyte (Eichenlaub-Ritter et al., 1995). Recent data report that the elective transfer of embryos selected on the basis of the 1st PB morphology results in a higher fertilization rate and better quality embryos with an increased implantation and pregnancy rate (Ebner et al., 1999, 2000). These same authors recently found a significant relationship between 1st PB morphology and blastocyst formation, implantation and ongoing pregnancy (Ebner et al., 2002). The present retrospective study was carried out in a group of patients in which the microinjection was given over a wide span of time after hCG administration and it was aimed at checking the correlation between 1st PB morphology and embryo quality, pregnancy and implantation rate and at evaluating the role of the duration of the oocyte culture before microinjection on developmental potential/pregnancy rate.

Materials and methods

Patients and ovarian stimulation

A total of 167 consecutive ICSI cycles were included in the present study. The couples were referred to the ICSI programme for male
infertility or for previous fertilization failure. The mean ± SD age of the patients was 35.4 ± 3.9 years (range 26–46). Ovarian stimulation was induced as previously reported (Porcu, et al., 1994).

**Oocytes**

Supernumerary oocytes are cryopreserved in our centre to avoid ethical, legal and religious problems related to embryo freezing. For this reason, a maximum of six oocytes per patient were inseminated while the rest were prepared for cryopreservation (Fabbri et al., 2001). All the oocytes were cultured in Universal IVF Medium (MediCult, Denmark) at 37°C in a 5% CO₂ atmosphere. They were denuded by brief exposure to hyaluronidase at a final concentration of 40 IU/ml (MediCult) and mechanically cleaned from their surrounding cumulus cells by aspiration using a denuding pipette with a diameter of 170–140 μl (Denuding Flexi-Pet™; Cook, Australia). Nuclear maturity was evaluated and metaphase II oocytes were randomly selected for insemination. The classification of 1st PB morphology was performed using an inverted microscope (Eclipse TE-300; Nikon, Japan) by the same operator as previously reported (Ebner et al., 1999): round or ovoid with smooth surface = grade 1; round or ovoid with rough surface = grade 2; fragmented = grade 3; broken into two parts = grade 4; huge and extruded in a large perivitelline space = grade 5. In the analysis of the data reported, a simpler classification was used by grouping grades 1 and 2 as intact polar bodies and grades 3 and 4 as fragmented polar bodies (Figure 1) (Ebner et al., 2002).

A total of 1239 oocytes were retrieved (mean ± SD number per patient: 7.4 ± 3.9; range 1–21). A total of 136 oocytes were discarded (germinal vesicle, empty zone, abnormal) and 507 (metaphase I or II) were cryopreserved as supernumerary oocytes (mean number per patient ± SD: 3.0 ± 3.7; range 0–16). A total of 596 metaphase II oocytes were injected (mean ± SD number per patient: 3.6 ± 1.1; range 1–6).

**Semen treatment**

All semen samples were freshly ejaculated. The samples were collected in Flushing Medium (MediCult) supplemented by 0.5% human albumin (CAF-DCF, Belgium). After liquefaction and evaluation of the basal parameters, the samples were prepared by discontinuous density gradient centrifugation (Pure Sperm; Nidacon, Int. AB, Sweden). Concentration, motility and morphology were evaluated after treatment. The samples were maintained at 37°C until ICSI and utilized at a final concentration of 1 × 10⁶/ml motile sperm.

**Effect of the duration of in vitro culture on the 1st PB fragmentation**

The 1st PB morphology was checked twice (1st observation at the moment of denudation and 2nd observation at the moment of injection) in a group of 180 randomly selected oocytes (49 cycles). For analysis, the time between the two observations was divided into three intervals: 1 h 20 min to 2 h 30 min; 2 h 31 min to 3 h 30 min; 3 h 31 min to 5 h 30 min. The percentage of intact 1st PB which showed fragmentation in the second observation was calculated (fragmentation rate).

**ICSI and fertilization**

ICSI was performed as previously reported (Van Steirteghem et al., 1993), utilizing an inverted microscope at × 20 magnification with a Hoffman modulation contrast (Eclipse TE-300 Nikon, Japan). 35°

![Figure 1](https://academic.oup.com/humrep/article-abstract/19/10/2334/589043 by guest on 10 January 2019)

**Figure 1.** First polar body (PB) morphology: grade 1: round or ovoid with smooth surface (A); grade 2: round or ovoid with rough surface (B); grade 3: fragmented (C); grade 4: broken into two parts (D) (×20). In the analysis of the data the 1st PB morphology was coded into two categories: intact polar body: grade 1 and 2 (A + B); fragmented polar body: grade 3 and 4 (C + D).
angled micropipettes were utilized (Cook, Australia). Oocytes were cultured individually after injection. ICSI was performed after a mean time of 6 h 02 min. The minimum time after retrieval was 2 h 40 min and the maximum time was 9 h 15 min. This time was divided into three intervals: ≤5, >5, ≤7, >7 h.

The fertilization assessment was performed 16–18 h after ICSI and it was defined as the presence of two pronuclei and the 2nd PB.

**Embryos**

Zygotes and embryos were cultured individually in IVF Medium (MediCult, Denmark) in 4-well dishes (Nunclon; Nunc, Denmark). The embryo quality was evaluated prior to transfer (40–42 h after ICSI) on day 2 (2–6 cells) and divided into five grades: 1 = regular cells, no fragments; 2 = regular or irregular cells, 0–10% fragments; 3 = regular or irregular cells, 11–30% fragments; 4 = regular or irregular cells, 31–45% fragments; 5 = irregular cells, 46–60% fragments. In the analysis of the reported data, embryos were grouped into three categories: A = grade 1 or 2; B = grade 3; C = grade 4 or 5.

**Statistical analysis**

Mean values, SD and frequencies were used as descriptive statistics. The Mann–Whitney test, the Kruskal–Wallis test, the Mantel–Haenszel linear by linear $\chi^2$-test, the Yates corrected $\chi^2$-test, and the Spearman rank correlation were applied. Statistical analyses were performed by running the SPSS/PC+ statistical package on a personal computer. Two-tailed $P < 0.05$ was considered statistically significant.

**Results**

The distribution of the 1st PB morphology in the 596 inseminated oocytes was as follows: 445 (74.7%) intact polar bodies; 151 (25.3%) fragmented polar bodies. No oocyte showed the 1st PB of grade 5.

A total of 449 zygotes (75.3% fertilization rate) were produced and 429 embryos developed (95.5% cleavage rate). Of these, 244 embryos were of quality A (56.9%), 116 of quality B (27.0%) and 69 of quality C (16.1%). The 1st PB morphology ($P = 0.301$), the fertilization rate ($P = 0.465$), the cleavage rate ($P = 0.497$) and the embryo quality ($P = 0.525$) were not significantly related to the age of the patients. Moreover, no significant relationship was found between the 1st PB morphology and the fertilization rate ($P = 0.703$), the cleavage rate ($P = 0.055$) and the embryo quality ($P = 0.673$) (Table I).

The oocyte distribution, according to the time elapsed between oocyte retrieval and ICSI, showed that the majority (356, 59.7%) were injected between 5 and 7 h post-retrieval, while 105 (17.6%) and 135 (22.7%) were injected at ≤5 and >7 h post-retrieval respectively. No significant relationship was found between the time elapsed from retrieval until ICSI and fertilization rate ($P = 0.696$), cleavage rate ($P = 0.968$) and embryo quality ($P = 0.424$) (Table II). The same results were obtained considering intact and fragmented PB separately (fertilization rate: $P = 0.963$, $P = 0.917$; cleavage rate: $P = 0.653$, $P = 0.012$; embryo quality: $P = 0.412$, $P = 0.823$ respectively). Finally, the morphology was also not predictive for fertilization rate, cleavage rate and embryo quality when the three subsets of time were analysed separately (fertilization rate: $P = 1.000$, $P = 0.835$ and $P = 0.845$; cleavage rate: $P = 1.000$, $P = 0.148$ and $P = 0.305$; embryo quality: $P = 0.829$, $P = 0.494$ and $P = 0.896$ in oocytes injected at ≤5 h, between 5 and 7 h and >7 h respectively).

A total of 406 embryos were transferred in 165 cycles (mean ± SD 2.5 ± 1.0). One patient had fertilization failure, and one, because of a severe ovarian syndrome, had no transfer but four frozen embryos. We obtained 43 pregnancies (overall pregnancy rate/transfer: 26.1%) and 49 gestational sacs with presence of embryonic cardiac activity (implantation rate: 12.1%). The mean age ± SD of the pregnant patients (35.2 ± 4.1) was not significantly different from the non-pregnant patients ($P = 0.993$). The number of transferred embryos was significantly higher in patients who became pregnant ($3.0 ± 0.7$ versus $2.3 ± 1.0$ in non-pregnant; $P < 0.001$) whereas it was not significantly related to the number of implanted embryos ($3.0 ± 0.9$ in the six

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Table I. Distribution of fertilization rate, cleavage rate, and embryo quality according to 1st polar body (PB) morphology

<table>
<thead>
<tr>
<th></th>
<th>Intact 1st PB</th>
<th>Fragmented 1st PB</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate</td>
<td>333/445</td>
<td>74.8</td>
<td>116/151</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>314/333</td>
<td>94.3</td>
<td>115/116</td>
</tr>
<tr>
<td>Embryo quality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>178/314</td>
<td>56.7</td>
<td>66/115</td>
</tr>
<tr>
<td>B</td>
<td>89/314</td>
<td>28.3</td>
<td>27/115</td>
</tr>
<tr>
<td>C</td>
<td>47/314</td>
<td>15.0</td>
<td>22/115</td>
</tr>
</tbody>
</table>

Table II. Distribution of fertilization rate, cleavage rate, and embryo quality according to the time elapsed between oocyte retrieval and ICSI

<table>
<thead>
<tr>
<th>Time elapsed between oocyte retrieval and ICSI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5 h</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>78/105</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>74/78</td>
</tr>
<tr>
<td>Embryo quality</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>50/74</td>
</tr>
<tr>
<td>B</td>
<td>18/74</td>
</tr>
<tr>
<td>C</td>
<td>11/74</td>
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</tbody>
</table>
Table III. Distribution of pregnancies and implanted embryos according to 1st polar body (PB) morphology

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Fragmented</th>
<th>Mixed</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Pregnancies</td>
<td>20/88</td>
<td>22.7</td>
<td>3/18</td>
<td>16.7</td>
</tr>
<tr>
<td>Implanted embryos</td>
<td>22/206</td>
<td>10.7</td>
<td>8/28</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Time elapsed

<table>
<thead>
<tr>
<th>Fragmentation rate</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h 20 min to 2 h 30 min</td>
<td>2/46</td>
<td>4.3</td>
</tr>
<tr>
<td>2 h 31 min to 3 h 30 min</td>
<td>11/84</td>
<td>13.1</td>
</tr>
<tr>
<td>3 h 31 min to 5 h 30 min</td>
<td>8/30</td>
<td>26.7</td>
</tr>
<tr>
<td>Total</td>
<td>21/160</td>
<td>13.1</td>
</tr>
</tbody>
</table>

Table IV. Frequency of the 1st polar body intact at the first observation (denudation) which was fragmented at the second observation (ICSI) reported according to the time elapsed between denudation and ICSI

- Twin pregnancies versus 3.1 ± 0.6 in the 37 single pregnancies; \( P = 0.905 \).

The results of pregnancy and implantation rates according to the 1st PB morphology were evaluated by dividing the 165 embryo transfers into three groups: embryos developed from oocytes with intact 1st PB, embryos developed from oocytes with fragmented 1st PB, and embryos developed from oocytes with mixed 1st PB morphology (Table III). No significant relationship was found between 1st PB morphology and either pregnancy or implantation rate (\( P = 0.201 \), \( P = 0.511 \) respectively).

Finally, the pregnancy rate/transfer, according to the time elapsed between oocyte retrieval and ICSI, was also calculated. A significantly (\( P = 0.008 \)) higher pregnancy rate was obtained when oocytes were injected between 5 and 7 h (34 pregnancies out of 98 transfers, 34.7%) versus 16.1% (5/31) when ICSI was performed \( \leq 5 \) h after retrieval, and 11.1% (4/36) when \( > 7 \) h elapsed before ICSI. This result was not biased by the number of transferred embryos since such a parameter was not significantly (\( P = 0.372 \)) different among the three groups having a different time lapse between oocyte retrieval and ICSI.

**Aging effect of in vitro culture on 1st PB morphology**

In the group of 180 oocytes which were checked twice, 20 (11.1%) and 41 (22.8%) 1st PB were fragmented at the first and second observations respectively. Therefore, the fragmentation rate in this time interval was 13.1% (21/160). Furthermore, a significantly positive relationship (\( P = 0.006 \)) was found between the frequency of the 1st PB fragmentation and the time elapsed between denudation and ICSI (Table IV); in particular, the fragmentation rate of the oocytes in which the time interval was \( > 3 h \) 30 min was 26.7%. No significant relationship between the embryo quality and the 1st PB morphology (evaluated both at the 1st and the 2nd observation) was found in this subgroup of 180 oocytes (\( P = 0.571 \) and \( P = 0.896 \) respectively).

**Discussion**

The oocytes retrieved after an ovarian stimulation show different grades of 1st PB morphology because they have different duration of nuclear maturation. ICSI is performed on a metaphase II (MII) oocyte because the extrusion of the 1st PB indicates that the oocyte has developed to meiosis II. The absence of the 1st PB indicates that the oocyte is still immature (MI), but it could indicate also an overmaturity caused by a rapid degeneration (Eichenlaub-Ritter et al., 1995). It is known that the ageing of the oocytes can cause spindle damage and chromosome defects (Pickering et al., 1988; Martini et al., 1997).

The prognostic value of 1st PB morphology for embryo quality obtained after ICSI was assessed alone or in combination with other criteria. In the literature, it is emphasized that synchrony between cytoplasmic and nuclear maturity is a fundamental aspect for the successful development and vitality of the embryos (Xia et al., 1997). Furthermore, it is reported that higher quality embryos are obtained from oocytes having the 1st PB intact and a normal perivitelline space as if to suggest that oocytes with these characteristics are those with the best synchrony between cytoplasmic and nuclear maturation (Xia et al., 1997). Furthermore, the encouraging results of Ebner et al. (1999, 2000) suggest a significant prognostic role of the 1st PB morphology.

This evidence, together with the simplicity and feasibility of the evaluation of the 1st PB morphology, has stimulated us to evaluate this parameter with reference to ICSI outcome. Since only a few oocytes are inseminated at our centre and the supernumerary oocytes are stored, the finding of a simple oocyte feature predicting embryo quality would be of great relevance. Our centre has routinely used this strategy for \( > 3 \) years, but recently a law in Italy on assisted fertilization, which allows for the insemination of a maximum of three oocytes per patients, has been approved. All centres will have to follow this procedure and, therefore the early identification of prognostic parameters to be able to identify the oocytes with the greatest probability of implantation will become an absolute necessity.

Our results show that the age of the patients is not related to the distribution of 1st PB morphology. The age of patients is neither related to the fertilization and cleavage rates nor to the embryo quality, pregnancy rate and implantation rate.

No relationship between 1st PB morphology, fertilization and cleavage rates and embryo quality was found.
The pregnancy and implantation rate was also not related to the 1st PB morphology when the embryo transfers were divided on the basis of the classification of the 1st PB morphology obtained at the 1st observation.

The interval of time during which a human oocyte is fertilizable in vivo is limited and, therefore, the time that it spends in culture before being fertilized in vitro is an important factor. Analysing the results of our study on the basis of this factor, we see that there was a wide span of time between retrieval and ICSI and the majority (60%) of ICSI were performed 1–3 h later than recommended (Ebner et al., 1999, 2000). It has been reported in the literature that there are no differences in results when the ICSI is carried out rapidly (1–2 h after retrieval) or later (5–6 h after retrieval) (Van de Velde et al., 1998). In a previous study in which the 1st PB morphology was not considered, we found no significant differences in the fertilization rate, cleavage rate, embryo quality, pregnancy rate and implantation rate when comparing two groups of patients in which ICSI was performed within or after 4 h after retrieval (Ciotti et al., 1997). In another study, poorer results using oocytes having an immature polar body were reported (Xia et al., 1997). Recently it was reported that the ability for complete activation and normal development was achieved by human oocytes during the MII arrest stage. MII oocytes require further time for complete ooplasm maturation in order to be readily activated by the sperm (Eichenlaub-Ritter et al., 2003; Balakier et al., 2004).

In the present study, no correlation was found between fertilization rate, cleavage rate and embryo quality according to the time elapsed from retrieval until ICSI, considering all the oocytes together or dividing them according to their 1st PB morphology. Instead, there was a significant difference in the percentage of pregnancies correlated with time passed between the retrieval and the ICSI, considering three time intervals ($\leq 5$, $> 5–7$, $\geq 7$ h). The best result was obtained in the middle group and the worst in the group having the longest time interval. From our data it can be deduced that it is beneficial to wait a few hours for the complete cytoplasmic in vitro maturation of the oocytes before ICSI, and an interval of optimal waiting can be identified. The lower pregnancy rate at early ICSI could be explained by studies which utilize the novel spindle imaging method (Oldenbourg, 1996, 1999). In fact, human oocytes with a polar body can show the absence of a birefringent spindle because they are still at telophase I or prometaphase I stage (Eichenlaub-Ritter et al., 2003). By contrast, it does not seem to be advisable to wait too many hours from retrieval in order to prevent in vitro ageing and all the negative consequences which ensue. At times, for various reasons, it is difficult to ensure that the injection is done within a suitable time interval such as, for example, cases in which the male has difficulty in producing a seminal liquid. Serious delays in carrying out the ICSI might compromise the final outcome of the procedure. This is a very important element which must be taken into account when dealing with microinjection of thawed oocytes because the time spent in vitro before freezing has to be added to that spent after thawing. Certainly in the near future, the employment of the spindle imaging method will be of crucial value to define the correct injection time of each single fresh or thawed oocyte.

We also verified the ageing effect of in vitro culture on 1st PB morphology and we found a positive relationship with the time elapsed in culture. The morphology of the 1st PB changes after a few hours of in vitro culture, and thus it can vary according to the moment in which the observation is carried out. However, we did not find any relationship between either the first or the second observation and embryo quality. Therefore, we could not find any predictive value for the morphology of the 1st PB.

In conclusion, our data do not agree with previous results which report a better fertilization rate, embryo quality, pregnancy and implantation rate in relation to 1st PB morphology (Ebner et al., 1999, 2000). Our results agree in part with a recent study (Ebner et al., 2002) in which no significant difference was found between 1st PB morphology and the fertilization rate. The authors believed that this was due to the simpler classification utilized (intact and fragmented PB). In addition, it should be noted that our data were also analysed with a five-grade classification (Ebner et al., 1999) but our results did not change (data not shown). Moreover our study is completely in agreement with a recent study (Verlinsky et al., 2003) in which PB morphology is not correlated with the percentage of fertilization, embryo quality, blastocyst survival and outcome. Furthermore, PB morphology does not show a correlation with the genotype analysed for aneuploidy in patients who underwent preimplantation genetic diagnosis.

The analysis of the literature shows that this topic is controversial and the results reported are often conflicting probably due to the small number of samples in each study.

From our results, we cannot conclude that 1st PB morphology is a prognostic factor of embryo quality and subsequent pregnancy and implantation rate. Even though a larger study should be performed, we believe that 1st PB morphology is a ‘fragile’ parameter because it is susceptible to ageing and, in our opinion, using 1st PB morphology as primary prognostic factor of oocyte quality is hazardous but, as suggested (Ebner et al., 2003), it may be good practice to include such an evaluation among the several morphological features that aid embryologists in the difficult work of embryo selection at the different stages of preimplantation development.

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References


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Polar body morphology and embryo quality

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