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Abstract

This study was undertaken in order to gain insight into the morphology of the first polar body (PB1) and the two pronuclei (2PN) in ICSI patients, specifically the nucleolar precursor bodies (NPB). Whether early abnormalities in these structures are related to the ovarian reserve of infertile women was also studied. Eighty consecutive infertile women were prospectively investigated throughout their first ICSI cycles. Basal ovarian reserve studies were performed in all women. Cycles were evaluated with respect to PB1 and 2PN morphology of the transferred embryos. Cycles that had at least one transferred embryo with normal PB1 and 2PN morphology had significantly better basal ovarian reserve parameters compared with cycles in which all transferred embryos had abnormal PB1 and 2PN morphology. Moreover, the normal morphology group performed significantly better throughout the ovarian stimulation, compared with the abnormal morphology group. Furthermore, the clinical implantation and pregnancy rates were significantly higher in the normal compared with the abnormal morphology group, corresponding to 20.7% versus 10.6% and 42.4% versus 18.2%, respectively. The study concluded that the morphology of the PB1 in metaphase II oocytes as well as that of the NPB within the 2PN zygotes seems to be related to the ovarian reserve in infertile women.

Keywords: first polar body morphology, ICSI, nucleolar precursor bodies, ovarian reserve, pronuclear zygote morphology

Introduction

Since the successful introduction of human IVF-embryo transfer in 1978, the search for a viable embryo with the utmost potential for implantation remains a key factor for success in assisted reproduction. The continuing pursuit for the identification of embryonic criteria that could increase the pregnancy rate while at the same time decreasing multiple pregnancy rates has been the most important quest of every assisted reproduction technology unit. In the last quarter century, and in today’s clinical setting, embryo selection has remained primarily based on morphological parameters of the early developing embryos. Throughout this period, the main criteria used are embryo morphology at 48–72 h after oocyte retrieval, especially homogeneity and fragmentation of blastomeres, as well as the cleavage rate of the embryos during culture. Nevertheless, there is still a big discrepancy between the number of embryos transferred and the number of live offspring conceived. Furthermore, multiple pregnancy rates, with their elevated perinatal mortality and morbidity, are still unacceptably high.

During the last decade, the understanding of the underlying genetic and molecular aspects of oocyte and early embryo development has improved considerably. Accordingly, several investigators, through meticulous and thorough studying of the oocyte, zygote and early embryo, have suggested additional early morphological criteria that could distinguish between
potentially viable and non-viable embryos. These criteria have been suggested based on fundamental biological events in the oocyte and in the very early embryo that could help to define an embryo with increased potential for implantation (Ebner et al., 2003, 2006; Scott, 2003b).

Developmental selection criteria that have been explored in the last few years include the morphology of the first polar body (PB1) (Xia, 1997; Ebner et al., 1999, 2002), the perivitelline space configuration (Xia, 1997; Farhi et al., 2002), and the appearance of the cytoplasm (Xia, 1997; Ebner et al., 2005) in the metaphase II (MII) oocyte. In addition, meiotic spindle visualization, using birefringent polarizing light technology, has been suggested as an early means to evaluate the reproductive potential of the oocyte (Cook et al., 2003). Moreover, the position and size of the two pronuclei (2PN) (Scott and Smith, 1998; Ludwig et al., 2000; Scott et al., 2000), the number, size and distribution of nucleolar precursor bodies (NPB) within the 2PN (Scott and Smith, 1998; Tesarik and Greco, 1999; Ludwig et al., 2000; Scott et al., 2000; Tesarik et al., 2000, Wittemert et al., 2000; Balaban et al., 2001; Montag et al., 2001; Scott 2003a), the cytoplasmic appearance of the zygote (Scott, 2003a; Ebner et al., 2005) and the orientation of the nuclei in relation to the polar bodies (Scott, 2003a; Gianaroli et al., 2007) have been investigated. In addition, the presence of multinucleation in the blastomeres of embryos at 48–72 h of culture (Van Royen et al., 2003) has also been examined. Comitantly, other assisted reproduction technology modalities of treatment have been suggested, specifically blastocyst culture and transfer. However, this modality of treatment is still in debate and has not yet gained wide acceptance (Damario and Rosenwaks, 2004).

The development of the first polar body and the 2PN are fundamental biological events in the physiology of oocyte-zygote development and the evaluation of the morphology of these structures is a crucial step in the assisted reproduction laboratory routine. Moreover, both are simple, non-invasive and microscopically reproducible observations that are straightforward to teach and learn. Studying the detailed morphology of these early structures, and not simply recording at their mere presence or absence, could increase knowledge of the quality of the early embryo. Indeed, recent studies have shown that normally developed PB1 (Xia, 1997; Ebner et al., 1999, 2002) as well as normal position and morphology of the 2PN including the development of NPB (Scott and Smith, 1998; Tesarik and Greco, 1999; Ludwig et al., 2000; Scott et al., 2000; Tesarik et al., 2000, Wittemert et al., 2000; Balaban et al., 2001; Montag et al., 2001, Scott, 2003a) are associated with good quality embryos and favourable pregnancy rates. Therefore, these parameters were suggested as a means of early embryo selection in order to increase implantation rates and decrease multiple pregnancy rates.

This prospective study was conducted to explore this concept one step further, by examining whether early morphological abnormalities in PB1 and 2PN, specifically the NPB, are related to the ovarian reserve of women undergoing intracytoplasmic sperm injection (ICSI) treatment.

Materials and methods

Patients

Eighty consecutive infertile women attending the Poriya Reproductive Medicine Unit were prospectively investigated throughout their first ICSI treatment cycle. Women were referred to ICSI treatment for male factor infertility. Only cases using fresh ejaculated sperm were included. All women were regularly menstruating with both ovaries intact, and had a normal uterine cavity as determined by hysterosalpingography and/or hysteroscopy.

Basal ovarian reserve studies

Basal ovarian reserve studies, including serum FSH and oestradiol (E2) in addition to LH levels, were obtained on day 3 of a natural cycle, 1 month prior to initiating IVF and embryo transfer treatment and following at least 3 months of no hormonal therapy. On the same day, one clinician blinded to the clinical data evaluated the ovarian volume of both ovaries using a transvaginal scan (TVS). Ovarian volume was determined by employing a two-dimensional endovaginal probe with a frequency of 7 MHz (Acuson 128-P-10, Mountain View, CA, USA). Ovarian volumes were calculated as the volume of an ellipsoid, i.e. length × width × depth × π/6. The total basal volume of both ovaries was evaluated in each patient.

Treatment protocol

The long protocol for IVF-embryo transfer, starting with gonadotrophin releasing hormone agonist (GnRHα) on day 21 of the cycle, was employed in each patient. Down-regulation was achieved after i.m. administration of GnRHα (Decapeptyl CR 3.75 mg; Ferring, Malmo, Sweden) and was confirmed by serum oestradiol concentration of ≤30 pg/ml. Superoxelution was commenced with i.m. human menopausal gonadotrophin (HMG) (Menogon; Ferring, Malmo, Sweden) 4 ampoules per day for the first 5 days. Menogon dosage in each patient was tailored thereafter, in accordance with serum oestradiol concentration and transvaginal scanning (Acuson 128-P-10) of follicular development. Human chorionic gonadotrophin (HCG) (Pregnyl; Ferring) 10,000 IU, was administered when the transvaginal scan showed ≥2 follicles with diameter of 18–20 mm and serum oestradiol concentration ≥400 pg/ml. Transvaginal oocyte retrieval was performed 34–36 h after HCG administration under ultrasound guidance. The treatment of oocytes, sperm and embryos, as well as the embryo transfer technique performed were performed as routinely carried out in the study unit. Luteal support was administered in all patients via i.m. injection of progesterone in oil (Gestone; Paines and Byrne Limited, Greenford, United Kingdom) 50 mg/day.

Hormone assays

Sera obtained for basal FSH and LH measurements were analysed by microparticle enzyme immunoassay (AxSYM®; Abbott, Abbott Park, IL, USA). The intra-assay and inter-assay coefficients of variation were <5% and <11%, respectively, for FSH and <7% and <8%, respectively, for LH. Serum oestradiol and progesterone concentrations were assayed by solid-
phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000; DPC, Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation were <10% and <16%, respectively, for oestradiol and <18% and <22%, respectively, for progesterone.

**First polar body morphology**

The cumulus–oocyte complexes (COC) were incubated for 3–4 h following retrieval in P1 medium (Scientific Irvine, Santa Ana, CA, USA) with 5% human serum albumin (Scientific Irvine) at 37°C in a 5% CO₂ atmosphere. Oocyte maturity and first polar body development was evaluated following a 20-second exposure to hyaluronidase (20 IU/ml; Scientific Irvine) to facilitate mechanical removal of the cumulus cells. Prior to ICSI, oocytes were rotated so that both the side view and the top view of PB1 were observed. First polar body morphology was examined in all patients 38–39 h following HCG administration, i.e. 3–4 h following oocyte retrieval. The presence of PB1 designated the gamete as an MII mature oocyte. Normal appearance of the PB1 was defined in accordance with Ebner et al. (2000) as oval or ovoid in appearance, intact and with a smooth surface. However, abnormal PB1 was defined as having a rough surface, fragmented or very large in volume (Ebner et al., 2000).

**ICSI procedure**

All women underwent a routine ICSI treatment and no additional interventions were performed throughout the study. The ICSI procedure was carried out as described by Van Steirteghem et al. (1993) with the use of an inverted microscope applying ×200 magnification (Olympus IX70, Tokyo, Japan) and Hoffman modulation contrast (Modulation Optics Inc., Greenvale, NY, USA). The ICSI procedure was performed using Eppendorf micromanipulators (Eppendorf HQ, Hamburg, Germany) employing commercially available holding and injection pipettes (Humagen Fertility Diagnostics, Charlottesville, VA, USA). The oocytes were injected in micro-droplets of modified human tubal fluid, HEPES buffered (Irvine Scientific), supplemented with human serum albumin 5% (Irvine Scientific) under light mineral oil (Irvine Scientific). The timing of injection was 39–40 h following HCG administration, approximately 4–5 h following oocyte retrieval.

**Pronuclear zygote morphology**

Fertilization and evaluation of 2PN morphology was performed 16–18 h following the ICSI procedure (i.e. 50–54 h after HCG administration). The presence of 2PN and two PB characterized normal fertilization. Three different morphological criteria were considered when examining the normality of 2PN development in accordance with the criteria published by Scott et al. (2000). First, homogeneity in the size of the 2PN; second, their alignment; and the third criterion evaluated NPB morphology within the 2PN. A normal 2PN zygote was described when the 2PN were centrally located, almost equal in size and aligned next to each other, and when the NPB morphology was similar with respect to number, size and distribution. Abnormal 2PN development was determined when the 2PN were not equal in size, when they were not aligned and when the NPB morphology within was not similar with respect to number, size and distribution.

**Embryo grading**

Embryo transfer during the study period was performed 48–72 h following oocyte retrieval. Embryo grading before transfer was performed in accordance with the classical criteria of blastomere homogeneity and the degree of anucleated fragments (Veeck, 1991).

**Group selection**

The study was performed in a prospective manner in a university-affiliated reproductive medicine unit. Biologists in the IVF laboratory, as well as the ovarian ultrasound clinician were blinded to the clinical data. Women were scheduled for evaluation in accordance with the PB1 and 2PN morphology of their transferred embryos. Two groups were defined. The first included cycles that had a transfer of at least one embryo with normally developed PB1 and normally developed 2PN, including the NPB morphology (normal morphology group). The second group included cycles that had a transfer of embryos where none had a normally developed PB1 and 2PN (abnormal morphology group). Subsequently, the basal ovarian reserve parameters of these two groups, their performance during ovarian stimulation and their ICSI results were assessed and compared.

**Statistical analysis**

Data were analysed using the SPSS software, Release 13 (SPSS Inc. 2004. SPSS for Windows, Ver. 13, SPSS Inc. Chicago, Illinois, USA). Mann–Whitney two samples test (unpaired, non-parametric), were used wherever appropriate. Significance was interpreted as $P < 0.05$. All data are presented as the mean ± SD.

**Results**

The prospective investigation included evaluation of 763 oocytes, 595 MII oocytes, 443 2PN zygotes and 429 embryos (from 2PN zygotes) in 80 ICSI cycles. Overall, 57.2% of the MII oocytes had abnormal PB1 morphology. Moreover, among the 443 2PN zygotes examined, in 3.1% the 2PN were not aligned, in 3.7% the 2PN were not equal in size and 43.5% the 2PN had abnormal NPB morphology.

Following the end of the study, complete data were available for 66 cycles. Each of the normal morphology group (at least one embryo transferred with normally developed PB1 and 2PN) and the abnormal morphology group (none of the embryos transferred with normally developed PB1 and 2PN) comprised 33 cycles. The major characteristics of these two groups were comparable (Table 1).

As expected, the proportion of normal/total PB1 morphology was significantly higher ($P < 0.0001$) in the normal morphology group compared with the abnormal morphology group, 0.54 ± 0.25 and 0.23 ± 0.26, respectively. Likewise, the proportion of normal NPB morphology was significantly higher ($P < 0.001$) in the normal morphology group compared with abnormal morphology group, 0.61 ± 0.22 and 0.35 ± 0.36, respectively. Minor differences were noted concerning the proportion of...
normal 2PN size (homogeneity) and position (alignment); however, this did not differ significantly between the two groups (Table 2).

Interestingly, the normal morphology group had significantly better basal ovarian reserve parameters compared with the abnormal morphology group (Table 3). Moreover, the normal morphology group performed significantly better throughout ovarian stimulation compared with the abnormal morphology group. The maximal serum oestradiol concentration on HCG day was significantly higher in the study compared with controls, 2435 ± 1106 and 1467 ± 608 pg/ml, respectively. In addition, the number of ≥14 mm follicles, oocytes, MII oocytes, zygotes and embryos was significantly higher in the study group compared with the control group (Table 3).

The mean number of transferred embryos was comparable between the two groups, 2.6 ± 1.0 and 2.8 ± 0.9, respectively. However, the clinical implantation and pregnancy rates were significantly higher in the normal morphology group compared with the abnormal morphology group, corresponding to 20.7 versus 10.6% ($P < 0.03$) and 42.4 versus 18.2% ($P < 0.02$), respectively.

### Table 1. Patients’ characteristics in normal and abnormal PB1 morphology groups.

<table>
<thead>
<tr>
<th></th>
<th>Normal morphology</th>
<th>Abnormal morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.0 ± 5.5</td>
<td>32.2 ± 6.4</td>
</tr>
<tr>
<td>Years of infertility</td>
<td>6.1 ± 3.9</td>
<td>4.6 ± 2.8</td>
</tr>
<tr>
<td>BMI</td>
<td>25.2 ± 5.0</td>
<td>24.2 ± 4.1</td>
</tr>
<tr>
<td>No. previous cycles</td>
<td>3.2 ± 2.0</td>
<td>3.1 ± 2.8</td>
</tr>
</tbody>
</table>

There were no significant differences between the two groups.

### Table 2. First polar body and proportion of 2PN normal development in normal and abnormal morphology groups.

<table>
<thead>
<tr>
<th></th>
<th>Normal morphology</th>
<th>Abnormal morphology</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>PB1</td>
<td>0.54 ± 0.25</td>
<td>0.23 ± 0.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2PN position</td>
<td>0.96 ± 0.08</td>
<td>0.93 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>2PN size</td>
<td>0.96 ± 0.01</td>
<td>0.93 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>NPB</td>
<td>0.61 ± 0.22</td>
<td>0.35 ± 0.36</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Proportion of normal/total.

### Table 3. Basal ovarian reserve and ovarian stimulation results in the normal and abnormal morphology groups.

<table>
<thead>
<tr>
<th></th>
<th>Normal morphology</th>
<th>Abnormal morphology</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>7.0 ± 4.6</td>
<td>5.7 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>58 ± 33</td>
<td>74 ± 59</td>
<td>NS</td>
</tr>
<tr>
<td>Total ovarian volume (cm³)</td>
<td>17.2 ± 11.7</td>
<td>13.0 ± 10.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Day 3 FSH (mIU/ml)</td>
<td>6.2 ± 2.1</td>
<td>7.9 ± 3.6</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Oestradiol on HCG day (pg/ml)</td>
<td>2435 ± 1106</td>
<td>1467 ± 608</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Progesterone on HCG day (ng/ml)</td>
<td>0.91 ± 0.46</td>
<td>0.88 ± 0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Total HMG ampoules</td>
<td>46.5 ± 20.5</td>
<td>49.4 ± 13.5</td>
<td>NS</td>
</tr>
<tr>
<td>HMG treatment (days)</td>
<td>11.5 ± 2.3</td>
<td>11.2 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>No. of ≥14 mm follicles</td>
<td>12.5 ± 5.2</td>
<td>7.7 ± 4.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>12.4 ± 4.0</td>
<td>6.9 ± 4.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. of MII oocytes</td>
<td>10.1 ± 3.8</td>
<td>5.6 ± 3.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. of 2PN oocytes</td>
<td>7.7 ± 3.5</td>
<td>4.7 ± 3.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. of zygotes</td>
<td>7.4 ± 3.4</td>
<td>4.6 ± 3.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. of embryos</td>
<td>12.1 ± 2.3</td>
<td>12.0 ± 2.6</td>
<td>NS</td>
</tr>
</tbody>
</table>
Discussion

This prospective study clearly demonstrates that abnormal development of the PB1 as well as abnormal NPB morphology is a frequent occurrence in women undergoing ICSI treatment due to male factor infertility. Among the 595 MII oocytes evaluated, 57.2% of these had an abnormally developed PB1. Moreover, among the 443 zygotes (2PN) examined, 43.5% had an abnormally developed NPB morphology.

The most important finding of this study is that the morphology of the PB1 in MII oocytes as well as the NPB within the 2PN zygotes seems to be related to the ovarian reserve of infertile women. Women with normal PB1 and NPB transferred embryos had significantly better basal ovarian reserve parameters and responded superiorly to the ovarian stimulation compared with women with abnormal PB1 and NPB transferred embryos. Moreover, the normal morphology group achieved significantly higher clinical implantation and pregnancy rates compared with the abnormal morphology group.

The finding that embryo selection according to the morphology of PB1 and NPB significantly increases clinical pregnancy is in agreement with previously reported studies (Scott and Smith, 1998; Ebner et al., 1999; Tesarik and Greco, 1999; Ludwig et al., 2000; Wittermer et al., 2000; Balaban et al., 2001; Cooke et al., 2003; Scott 2003a). However, previous studies have examined the importance of each of these two early morphological structures remotely and independently of each other. As far as is known, this study is the first to examine the relationship of both of these structures in combination to the clinical pregnancy rate and to ovarian reserve. Furthermore, the question of which of these two early structures, the PB1 or the NPB morphology, contributes more to embryo implantation potential, is of vital importance and should be investigated in a prospective targeted study.

It should also be noted that the association between PB1 morphology and oocyte quality as well as pregnancy rate is still controversial in the literature. Ciotti et al. (2004) in a retrospective study and De Santis et al. (2005) in a prospective study did not find that PB1 morphology contributed to the identification of embryos with high developmental ability. Further studies are needed in order to settle this issue.

This study was performed in male factor infertility couples undergoing ICSI treatment. This was primarily to allow thorough examination of the morphology of the PB1 following cumulus cell removal. It may well be argued that this model is not an optimal one to evaluate ovarian reserve in infertile patients. However, since male factor infertility was a common feature, and equally distributed, in all patients in the study and the control groups, there was no conflict in examining the ovarian reserve of the female partners. Moreover, investigators have shown that the transition between dependence on maternal transcripts and proteins inherited in the oocyte and embryonic gene expression in the human preimplantation embryo occurs at the 4–8-cell stage (Braude et al., 1998; Taylor et al., 1998). In other words, activation of the human embryonic genome occurs at day 2–3 during in-vitro culture. Therefore, it is reasonable to assume that, prior to day 3, embryo development is primarily governed by maternal transcripts with a possible paternal influence. Paternal influence on embryo development and NPB morphology has been suggested in one ICSI study in patients with non-obstructive azoospermia, using testicular sperm and round spermatids (Kahraman et al., 2002). In the present study only fresh ejaculated sperm was employed during ICSI. On the whole, it seems that the basis of this study is solid. Morphological abnormalities of the PB1 and 2PN, specifically the NPB, could be examined and related to the ovarian reserve in ICSI patients. Developmental abnormalities of paternal origin are assumed to appear later in embryo growth, following the 4–8-cell stage.

In this study, three different morphological criteria concerning 2PN development were examined. Interestingly, no significant difference was found between normal and abnormal morphology groups in relation to homogeneity of size as well as apposition/alignment of the 2PN. These findings are reinforced by the findings of Tesarik and Greco (1999). They found that asymmetry in size of the 2PN is the result of oocyte fertilization by an immature sperm and may thus be related to incomplete nuclear protein transition in the male gamete. In addition, lack of 2PN apposition has been linked to an abnormal sperm-derived centriole and its associated microtubule-organizing region (Schatten, 1994). In the present study only NPB was found to be significantly different between normal and abnormal morphology groups. All told, it could be assumed that size homogeneity and alignment of 2PN development are sperm-related events, whereas NPB morphological development is primarily an oocyte-dependent occurrence. These findings need to be re-examined in prospective targeted studies.

It is essential to bear in mind that both PB1 and NPB development are crucial checkpoints in the development of the mature oocyte and zygote. Their appearance has been correlated with developmental potential, which has its basis in crucial biological events. Clearly, the extrusion of the PB1 designates the completion of the first meiotic division, whereas the appearance of the NPB in both pronuclei provide a good indication of the events of fertilization, the completion of the second meiotic division and cell cycle events leading to the first mitotic division (Scott, 2003a; Ebner et al., 2003). The NPB are part of the nucleoli, which develop at sites on DNA. The nucleoli are the site of RNA synthesis and ribosomal gene transcription. The production of ribosomal RNA is necessary for protein synthesis to occur. Concomitantly, the chromatin in each pronucleus and NPB, which are closely associated, undergoes a polarization that seems to be a crucial event in the design of the embryonic axis for subsequent cell determination in the developing embryo (Van Blerkom, 1995). Deviation from any of these strictly interrelated events may be associated with an abnormal pattern of embryo growth, survival and implantation.

Several mechanisms could be suggested to explain the abnormal development of the PB1 and NPB in low ovarian reserve women. These mechanisms have the potential to affect several biological checkpoints adversely during the development of the PB1 and 2PN. Undoubtedly, these mechanisms could be interrelated and connected to each other. However, in order to simplify their illumination and clarification they are considered here as three different mechanisms.

The first is linked to the reduced stromal blood flow in low ovarian reserve women (Pan et al., 2004; Younis et al., 2007).
Reduced oxygen supply to the developing follicle may slow the flow of meiosis-arresting substances from the granulosa cells through gap junctions into the oocyte (Buccione et al., 1990). This could lead to premature oocyte maturation, ultimately causing early signs of oocyte atresia or apoptosis and abnormal PB1 morphology. In addition, reduced ovarian blood supply to the developing follicle resulting in hypoxia has been linked to defects in the oocyte spindle as well as in chromosomes (Van Blerkom et al., 1997). Since the formation of the first meiotic spindle is the time of crossing over, abnormal spindles may lead to a non-disjunction event, which could cause aneuploidy and abnormal PB1 morphology (Scott, 2003a). Moreover, oxygen deficiency may also impinge on intracytoplasmic ATP content and adversely affect the oocyte’s developmental capacity (Van Blerkom et al., 1995), adversely affecting PB1 developmental morphology.

Similarly, it could be assumed that low oxygen content of the oocyte could adversely affect its fertilization potential and zygote formation capability. The second meiotic spindle could be negatively affected during zygote formation, increasing the risk of chromosomal aberrations and aneuploidy, leading to NPB developmental abnormalities.

Taken together, it seems that reduced blood supply to the ovary during controlled ovarian stimulation in low ovarian reserve women could detrimentally affect the cumulus–oocyte complex. This may lead to early signs of apoptosis and/or non-disjunction abnormalities adversely affecting the normal development and morphology of the PB1 and the NPB.

A second explanation is associated with the oocyte maturation process from prophase I to MII stage. Specifically, in this process, both nuclear and cytoplasmic maturation of the oocyte should be completed in a coordinated and synchronized mode to ensure optimal conditions for subsequent fertilization. Disturbances or asynchrony of this process may result in different morphological abnormalities (Ebner et al., 2003). Asynchrony between nucleus and cytoplasm could lead to premature completion of first meiosis before the LH surge. Eventually, this could lead to an early PB1 extrusion, adversely affecting its normal development and morphology. The first polar body undergoes a programmed cell death by apoptosis (Choi et al., 1996) and this is usually complete by approximately 20 h after extrusion (Ortiz et al., 1983). An early extrusion of the PB1 may accelerate its normal programmed cell death and adversely affects its developmental morphology. If the PB1 is beginning to fragment and degrade, it could mean that the oocyte is post–mature, which may lead to abnormal development of the oocyte and adversely affect its potential to develop into a viable embryo.

An increased incidence of premature luteinization in low ovarian reserve women undergoing fertility treatment has been reported previously (Younis et al., 1998, 2001). In this regard, it has been shown that this phenomenon is a non-LH-dependent event and could be related to the ageing process of the cumulus–oocyte complex (Younis et al., 2001). Taken together, it could be reasoned that in low ovarian reserve women there is an early nuclear maturation that surpasses the cytoplasmic development. As a result, there is an early extrusion and subsequently an early disintegration of the PB1, disturbing its normal development.

Likewise, asynchrony of development between nucleus and cytoplasm during oocyte maturation could lead to metabolic and molecular disturbances in the process of fertilization, ultimately disrupting 2PN development, and specifically the NPB morphology.

A third explanation could be related to the genetic control of c-Mos and mitogen activated protein kinase that are involved in formation of the first meiotic spindle in mammalian oocytes (Choi et al., 1996; Verlhac et al., 2000). The formation of the first meiotic spindle is the time of crossing over, and abnormal spindles may lead to non-disjunction events and aneuploidy (Scott, 2003a). Thus, abnormal expression of these two genes may lead to an abnormal first meiotic division and as a result, an abnormally developed PB1. An oocyte with a large or an abnormal PB1 will more than likely be abnormal and may indicate a breakdown of normal spindle formation.

In the same manner, disruption of the normal genetic control of the oocyte could interrupt second meiotic spindle formation leading to additional non-disjunction aberrations. This could lead to abnormal development of NPB within the 2PN. Oocytes that present with NPB patterns in which there is complete inequality in numbers and distribution most likely show abnormal chromatin (Goessens, 1984). Oocytes that present with highly fragmented NPB may be showing signs of ageing and could be destined for early arrest (Guanrete, 1997).

In summary, it is possible that the reduced blood supply to the cumulus–oocyte complex, nuclear and cytoplasmic developmental asynchrony, as well as the disruption of normal genetic control of oocyte maturation, could ultimately lead to chromosomal aneuploidy or accelerated apoptosis. It is well accepted that low ovarian reserve is associated with accelerated follicular depletion and declining infertility as well as an increase in spontaneous abortion rate. Accumulated evidence strongly suggests that the primary cause for these manifestations is an increasing prevalence of chromosomal aneuploidy in ageing oocytes. The incidence of chromosomal aberrations has been reported to be as high as 60–80% in oocytes and embryos in such cases (Battaglia et al., 1996; Magli et al., 1998; Gianaroli et al., 1999).

The authors’ assumption that abnormal NPB development in low ovarian reserve women is related to, and associated with, chromosomal aberrations in the oocyte and/or early embryo is reinforced by several recently published genetic studies. The relationship between abnormal 2PN morphology specifically NPB morphology, and aneuploidy is well documented (Coskun et al., 2003; Gamiz et al., 2003; Gianaroli et al., 2003, 2007; Balaban et al., 2004). Of special interest is the recently published study by Gianaroli et al. (2007) in which the morphology of 2PN zygotes generated from euploid oocytes diagnosed by PB1 analysis was evaluated. In this study it was shown that a scoring system that requires centralized and juxtaposed 2PN, large-size aligned or scattered NPB and PB located in the longitudinal or perpendicular axis of PN, corresponded to the highest proportion of chromosomally normal embryos.

Although several studies have documented a clear relation between chromosomal aneuploidy and NPB morphological abnormalities, this has not been the case concerning PB1 abnormal morphology. As far as is known, the only study that has examined the association between PB1 morphology
and chromosomal aneuploidy was by Verlinsky et al. (2003). This study did not find a correlation between chromosomal aneuploidy and PB1 abnormal morphology. Further studies are required to substantiate these findings.

If the results of Verlinsky et al. (2003) are confirmed, in order to explain the data presented here, it is reasonable to assume that abnormal PB1 morphology in low ovarian reserve cases is related to over-maturity or apoptotic cell death. Indeed, apoptosis as a function of ovarian reserve has been previously shown in women undergoing IVF-ET treatment (Seifer et al., 1996).

In conclusion, it has been demonstrated that abnormal morphology of the PB1 in MII oocytes and NPB in 2PN zygotes is a frequent occurrence in infertile women following ICSI procedures employing fresh ejaculated sperm. It has been shown that the morphology of the PB1 in MII oocytes as well as the NPB within the 2PN zygotes seems to be related to the ovarian reserve of infertile women. It is postulated that abnormal morphology of the PB1 is related to over-maturity and/or apoptosis whereas the abnormal morphology of the NPB is associated with increased chromosomal aneuploidy in ageing oocytes. The results also support the application of PB1 and NPB morphology in the embryo selection decision before embryo transfer in assisted reproduction treatment.

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