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CLINICAL ARTICLE

Relationship between first polar body morphology before intracytoplasmic sperm injection and fertilization rate, cleavage rate, and embryo quality

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ABSTRACT

Objectives: To evaluate the influence of the morphology of the first polar body (PB) on intracytoplasmic sperm injection (ICSI) outcomes. *Methods:* The morphology of the first PB was assessed in 3177 metaphase II oocytes and classified as: intact and normal size, fragmented, or enlarged size. The rates of fertilization, cleavage, and embryo quality were evaluated on day 2. *Results:* The rates of fertilization, cleavage, and formation of good quality embryos resulting from the insemination of oocytes with an enlarged first PB (20.7%, 18.7%, and 5.0%, respectively) were significantly lower than those for oocytes with an intact first PB of normal size (70.8%, 62.5%, and 19%, respectively) or a fragmented first PB (69.7%, 60.5%, and 17.1%, respectively). Rates did not differ significantly between oocytes with an intact first PB of normal size and oocytes with a fragmented first PB ($P>0.05$). *Conclusions:* The presence of an enlarged PB is related to poorer rates of fertilization, cleavage, and top quality embryos. However, identification of first PB fragmentation does not seem to interfere with ICSI outcomes.

1. Introduction

The introduction of intracytoplasmic sperm injection (ICSI) as an assisted reproduction technique has provided substantial information about oocyte morphology since the method requires oocyte denudation by eliminating the cumulus oophorus cells. This permits better observation before and during the fertilization process, with the possibility of correlating the parameters of oocyte morphology with embryo quality and viability [1,2]. The removal of cumulus cells before ICSI permits the assessment of many morphological parameters of the oocyte such as oocyte shape, cytoplasm color and granulation, regularity and thickness of the zona pellucida, the size of the perivitelline space, the presence of vacuoles, the presence or absence of the germinative vesicle and of the first polar body (first PB), as well as its morphology [3]. The identification and the use of objective criteria able to predict oocyte quality would permit improved results of assisted reproduction techniques by assisting in oocyte and embryo selection, as well as minimizing the ethical problems related to embryo freezing because fewer oocytes would be inseminated and therefore fewer embryos of inadequate quality would be produced [4,5]. However, the influence of the morphological characteristics of oocytes on fertilization, implantation and pregnancy rates, and on embryo quality is still a matter of controversy [6–8].

ICSI is performed when the oocytes are classified as mature, i.e., when they reach metaphase II (MII), which is characterized by the observation of the first PB under the microscope [9]. The absence of the first PB, in turn, may indicate that the oocyte is still immature or that it is already post mature and inadequate for insemination in either case. The morphology of the first PB was initially thought to indicate the postovulatory age of the oocyte [8]. However, some studies have shown that the transfer of embryos selected on the basis of first PB morphology resulted in a higher fertilization rate and in embryos of better quality, with higher implantation and pregnancy rates [6,10–13]. On the other hand, these data are controversial, with contradictory results reported in the literature [14–16].

The establishment of simple criteria for use with light microscopy to help embryologists choose the embryo(s) for transfer is fundamental for improving pregnancy rate and reducing the incidence of multiple pregnancies. In view of the high clinical applicability of potential morphologic criteria that could predict oocyte quality—one of the most important determinants of embryo quality [17]—and considering the controversies existing in the literature about the influence of first PB morphology [9] on the rates of success of assisted reproduction techniques, the aim of the present study was to assess the effect of first PB morphology on fertilization and cleavage rates, and on embryo quality.

2. Materials and methods

The study consisted of a retrospective longitudinal analysis of 3177 metaphase II oocytes obtained from 582 consecutive ICSI cycles of

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patients under 40 years at the Assisted Reproduction Laboratory of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, from July 2003 to July 2005. Only severe male factor infertility (sperm count <100 000/mL, sperm morphology <4% according to Kruger strict criteria, and/or azoospermic patients) was excluded from the study.

The menstrual period was programmed with the use of combined oral contraceptives. For pituitary suppression, 10 IU/day subcutaneously of GnRH agonist leuprolide acetate (Lupron, Abbott Laboratories, Abbott Park, USA; or Reliser, Serono, Sao Paulo, Brazil), or 400 μg/day of nafarelin (Synarel; Pharmacia, Brazil) by the nasal route, was administered starting 10 days before the programmed menstruation. Transvaginal ultrasound (TVUS) was performed from the 1st to the 3rd day of the menstrual cycle to confirm ovarian suppression, and controlled ovarian stimulation was then started using recombinant follicle-stimulating hormone (Puregon, Organon, Brazil; or Gonal-F, Serono, Brazil) at a dose of 200–400 IU/day during the first 5 days and adjusted according to ovarian response starting on the 6th day of stimulation. When 2 or more follicles ≥18 mm in mean diameter were present, 10 000 IU of urinary hCG (Profasi; Serono, Brazil) was administered intramuscularly, or 250 μg of recombinant hCG (Ovidrel; Serono, Brazil) was administered subcutaneously. Oocyte retrieval was performed 34–36 hours after hCG administration. Follicles were aspirated with a negative pressure of 110 mm Hg with transvaginal ultrasound guidance. The cumulus–oocyte complexes (COC) were separated from the follicular fluid under a stereomicroscope (Nikon SMZ645) and washed in human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA, USA) culture medium modified with HEPES (Irvine Scientific) supplemented with 10% synthetic serum substitute (SSS; Irvine Scientific) before being transferred to culture plates previously incubated at 37 °C in the presence of 5% CO_2 in air, containing HTF culture medium supplemented with 10% SSS. After 2–5 hours of incubation in this medium, cumulus cells were removed by COC exposure to hyaluronidase (H-4272; Sigma-Aldrich, St Louis, MO, USA), followed by mechanical removal with glass pipettes.

Semen samples were obtained soon after oocyte retrieval and manipulated and analyzed for ICSI according to previous recommendations [7].

Before ICSI, the denuded oocytes were placed in 5-μL microdroplets of HTF-HEPES supplemented with 10% SSS on Falcon plates and observed under the inverted microscope (400× magnification) with Hoffman optical contrast. The morphology of the first PB was assessed immediately before ICSI and categorized as: (1) intact first PB of normal size with an ovoid shape and a smooth surface; (2) fragmented first PB with an irregular shape and a rugose surface; and (3) first PB of enlarged size much larger than those of the previous two groups and without fragmentation (Fig. 1). A single observer (MCPMA) characterized the first PB according to the criteria of Ebner et al. [11].

ICSI was performed as previously described [18], 36–41 hours after hCG administration. After ICSI, the oocytes were incubated separately in 25-μL microdroplets of HTF plus 10% SSS, at 37 °C in the presence of 5% CO_2 in air. Fertilization was assessed 18–20 hours after ICSI, with the visualization of 2 pronuclei and 2 PBs being considered to represent fertilization. Oocytes presenting 3 or more pronuclei were classified as abnormal and were discarded. Cleavage was verified at about 24 hours after fertilization by the observation of cell division. Embryo quality was assessed according to variables such as percent cytoplasm fragmentation, symmetry, and number of blastomeres. On the second day after ICSI, embryos with 4 cells, symmetrical blastomeres, and no fragmentation were considered to be of good quality.

For statistical analysis, the rates of fertilization, cleavage, and formation of good quality embryos originating from the injection of oocytes with an intact first PB of normal size were compared with a fragmented first PB and an enlarged first PB using the Fisher exact test and GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA). The level of significance was set at 5% ($P<0.05$).

Table 1
Distribution of the rates of fertilization, cleavage, and good quality embryos according to first polar body morphology

Parameters	First polar body morphology		
	Normal	Fragmented	Enlarged
Inseminated mature oocytes	1366	1448	363
No. of fertilized oocytes	967	1008	75
Fertilization rate, % [g]	70.8[a]	69.7[a]	20.7[b]
Cleaved embryos	854	876	68
Cleavage rate, % [h]	62.5[c]	60.5[c]	18.7[d]
Good quality embryos	260	248	18
Proportion of good quality embryos, %	19.0[e]	17.1[e]	5.0[f]

[a,c,e] Same superscript letters on the same line indicate the absence of significant differences ($P>0.05$).
[a,b,c,d,e,f] Different superscript letters on the same line indicate a significant difference ($P<0.05$ using Fisher exact test).
[g] Fertilization rate: number of fertilized oocytes × 100 divided by the number of oocytes injected.
[h] Cleavage rate: number of cleaved embryos × 100 divided by the number of fertilized oocytes.
[i] Proportion of good quality embryos: number of embryos with 4 symmetrical cells without fragmentation on the second day after ICSI × 100 divided by the total number of embryos produced.

3. Results

A total of 3177 metaphase II oocytes obtained from 582 consecutive cycles for ICSI were analyzed and divided into 3 categories according to first PB morphology: (1) intact first PB of normal size (n=1366); (2) fragmented first PB (n=1448); and (3) enlarged first PB (n=363) (Table 1).

Of the 1366 oocytes with a normal first PB that were injected, 967 were normally fertilized (70.8%), 854 cleaved (62.5%), and 260

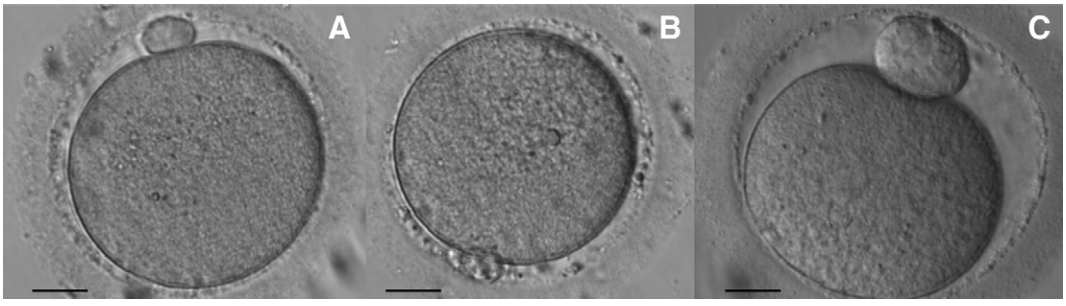

Fig. 1. Morphology of the first polar body (PB): (A) intact polar body of normal size; (B) fragmented polar body; (C) enlarged polar body. Images obtained by inverted light microscopy at 400× magnification. Scale bar=20 μm.