Sperm retention site and its influence on cleavage rate and early development following intracytoplasmic sperm injection

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Background: Intracytoplasmic sperm injection (ICSI) has risen to the forefront of reproductive technology. In the present study, the location of the sperm injection was noted, and a prospective study was conducted to evaluate the effect of the sperm retention site on cleavage rates and embryo quality after ICSI.

Methods: This study involved 336 ICSI patients (age 27–44; average 37.4) where 1545 oocytes were observed. An oocyte was divided into nine sites and the sperm retention site was observed microscopically after injection. The polar body was placed at either the twelve or six o'clock position. The injection pipette was introduced at the three o'clock position and oolemma rupture was ascertained by mild suction. The main outcome measures were the relationship of sperm remaining in position in the oocyte to fertilization rate and embryo quality.

Results: When the injection pipette was introduced at the three o'clock position, about 80% of the sperm remained in the center or left of center. The fertilization rate was significantly lower ($p < 0.05$) when the sperm remained near the site of introduction. Embryo quality was not significantly affected by the sperm retention site.

Conclusions: About 12–14% of the spermatozoa remained near the introducing position, and in these cases the fertilization rate was low. However, once fertilization occurred, the sperm retention site had minimal impact on embryo quality. Injecting sperm near the spindle site may improve embryo quality.

KEY WORDS: Cleavage rate; early development; intracytoplasmic sperm injection; sperm position.

INTRODUCTION

Since the first pregnancy following ICSI in 1992 (1), ICSI quickly spread to become one of the most powerful reproductive medicine technologies. Furthermore, ICSI has developed into a fundamental method of reproductive technology. Many studies were performed in order to ascertain factors that af-

fect the fertilization rate, where the fertilization rate and embryo quality have been described in conjunction with polar body orientation or meiotic spindle position. The M-II spindle is located at the periphery of the ooplasm, subjacent to the first polar body (2). Therefore, during the ICSI procedure sperm injection at the M-II spindle area should be avoided, and a six or twelve o'clock position in relation to the polar body chosen (3,4). Blake *et al.* reported that injection at the seven or eleven o'clock position in relation to the polar body resulted in the greatest number of high-quality embryos (5). Moreover, a high degree of misalignment between the meiotic spindle and the first polar body results in an increased

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risk of fertilization abnormalities (6). The injection procedure is an extremely important step in instituting fertilization; however, it is technically difficult to precisely inject a sperm at a desired location during ICSI. This process is influenced by instrument calibration as well as the condition of the oocyte cytoplasm.

In this study, the polar body was placed at the six or twelve o'clock position, and the relationship of the sperm retention site to the fertilization rate and embryo quality after ICSI was examined.

MATERIALS AND METHODS

Patients

Informed consent was obtained from all patients who participated in this study. A total of 336 patients (age 27–44; average 37.4) who underwent ICSI in the Showa University Hospital were enrolled. All patients were stimulated with either the long protocol or clomiphene cycle. The indications for ICSI were either a previous fertilization failure or oligospermia. 1545 oocytes were aspirated 35 h after hCG injection, guided by transvaginal ultrasound.

ICSI Procedure

After follicular aspiration and oocyte collection with modified human tubal fluid (Irvine Scientific, Santa Ana, CA), the oocytes were cultured in P1 medium (Irvine Scientific, Santa Ana, CA) with human serum substitute supplement (Irvine Scientific, Santa Ana, CA) under mineral oil. The oocytes were exposed to 80 IU of hyaluronidase (SAGE In-Vitro Fertilization, Inc., Trumbull, CT) in order to remove cumulus cells. Only mature oocytes were selected for micromanipulation. Sperm were washed in P1 medium with human serum substitute supplement and collected by the swim-up method. The ICSI procedure was microscopically performed (Olympus America, Melville, NY) with an injection and hoarding needle (HUMAGEN Fertility Diagnostics, Inc, Charlottesville, VA). The procedure was performed by one physician.

The polar body was placed at either the twelve or six o'clock position. The injection pipette was introduced at the three o'clock position and oolemma rupture was ascertained by mild suction. The oocyte was divided into nine sites (Fig. 1). The sperm was released to the left of the oocyte center (site 1 when the polar body was at twelve o'clock and site 3 when

Fig. 1. An oocyte was divided into nine sites and the sperm injection site was observed microscopically.

the polar body was at six o'clock). The sperm retention site was microscopically noted after injection. Oocytes were cultured individually and fertilization was confirmed 19 h after injection. Fertilization was determined by the presence of two distinct pronuclei and two polar bodies. Embryo quality was evaluated three days after insemination (D3EQ). We divided embryo quality into three groups: G1, G2, and G3 (Table I). The χ^2 test was used to analyze differences. A $p < 0.05$ was considered to be significant.

RESULTS

A total of 1545 M-II oocytes from 336 patients were examined in this study.

Sperm Position

Nearly 80% of the sperm remained at the center or left of center (Fig. 2; site 1 or 2). Placing the polar body at either the six or twelve o'clock gave identical results.

Fertilization Rate

The fertilization rate was significantly lower ($p < 0.05$) when the sperm remained near the introductory position (site 1 when the polar body was at

Table I. Day 3 Embryo Quality				
	Cell	Fragmentation	Size	Vacuous
G1	<6	10% <	Unequal	(+)
G2	6 ≤ < 8	≤ 10%	Unequal	(+)
G3	8 ≤	≤ 5%	Equal	(-)

Fig. 2. Deposition of spermatozoa. When the injection pipette was introduced at the three o'clock position, about 80% of the sperm remained to the left of center. About 12–14% of the spermatozoa remained near the injection site.

twelve o'clock and site 3 when the polar body was at six o'clock). Placing the polar body at either six or twelve o'clock gave identical results. Other sites were found to have a fertilization rate between 70 and 80% (Fig. 3).

Embryo Quality

Embryo quality was almost identical in sites 1, 2, and 3, and independent of polar body position. In sites 4, 7, 8, and 9, differences in embryo quality were observed. G3 had a higher rate in sites 4 and 9 when the polar body was at six o'clock. Conversely, G3 had a higher rate in site 7 when the polar body was at twelve o'clock (Fig. 4). However, the differences were not significantly different due to the small sample size.

DISCUSSION

Many studies have attempted to ascertain factors that would increase the fertilization rate. Recently, the meiotic spindle location with regards to polar body position has been studied (6–8). In clinical practice, instrument calibration and the condition of the oocyte cytoplasm influence sperm injection. Although an appropriate injection site is fundamental to the procedure, there are no reports in the literature that presently address this issue. In this study, the polar body was positioned at either six or twelve o'clock and the relationship of the sperm retention site to fertilization rate and embryo quality after ICSI was examined. Nearly 80% of the sperm remained in the center or left of center in the oocyte cytoplasm. Between 12% and 14% of the sperm remained near

Fig. 3. Fertilization rate. The fertilization rate was significant lower ($p < 0.05$) when the sperm remained near the three o'clock injection site. There was no difference between the six and twelve o'clock polar body positions.

Fig. 4. Distribution of the embryo quality by site. Embryo quality was almost identical in sites 1, 2, and 3 independent of polar body position. In sites 4, 7, 8, and 9, differences in embryo quality were observed. G3 had a higher rate in sites 4 and 9 when the polar body was at six o'clock. Conversely, G3 had a higher rate in sites 7 and 8 when the polar body was at twelve o'clock.

the injection site (site 1 when the polar body was at twelve o'clock and site 3 when the polar body was at six o'clock). Placing the polar body at either six or twelve o'clock did not affect the results.

The fertilization rate was significantly lower when the sperm remained at that injection site. This may be explained by cases where the whole sperm remained visible after injection, and the sperm may be moved to the subzonal area of the oocyte during the reparative process after ICSI.

In this study, although there were no statistically significant differences, G3 had a higher rate in sites 4 and 9 when the polar body was at six o'clock, and a higher rate in site 7 when the polar body was at twelve o'clock. When the suction method was used for rupturing the oolemma, strength and elongation of oolemma played important roles in the institution of ICSI. Moreover, the direction of the injection needle tip may also be important. These results may differ with the oolemma's relationship to the polar body if the oolemma is ruptured by a technique other than mild suction.

Although a study by Domoulin *et al.* failed to show any differences (9), Blake *et al.* reported the highest number of high quality embryos at the seven o'clock injection site; theorizing that their finding was due to the shorter distance between the sperm and the spindle (5). It has been suggested that injecting sperm closer to the site of the spindle may improve outcome (8,10). Nagy *et al.* suggested that cleavage would be facilitated when the sperm was injected closer to the spindle (11). Although the sample size in this study was quite small, our results may support their findings.

The M-II spindle is believed to be located in the periphery of the ooplasm subjacent to the first polar body (2); therefore, it is essential to avoid direct injection into the spindle site. However, only 62% of the first polar bodies are located above the spindle, according to the report by Cooke *et al.* (7). Rienzi *et al.* suggested an explanation for displacement of the polar body during manipulations to remove cumulus cells and the corona (6). The utilization of three-dimensional observations, such as polarization microscopy, may clarify findings in this process.

Our study suggested that if the sperm remained near the injection site, fertilization rate was low. Spermatozoa should be injected at the center or left of center. Once fertilization occurs, fertilization rate and embryo quality are minimally impacted even if the sperm is injected into the spindle site. Injecting spermatozoa near the spindle site may improve embryo quality.

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