Correlation of Oocyte Morphology with Fertilization Rate and Embryo Quality After Intracytoplasmic Sperm Injection

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Abstract

To determine whether the morphology of the oocyte assessed under light microscopy is related to the results of intracytoplasmic sperm injection (ICSI). 135 ICSI oocytes from 15 patients were analyzed. Transvaginal ultrasound - guided oocytes retrieval with oocyte morphology evaluation based on shape of the first polar body and perivitelline space followed by ICSI. After 48 hours, embryo quality was evaluated and compared to each pre-injected oocyte morphology. Normal fertilization was achieved in 81.5 per cent of the oocytes (110/135). Abnormal fertilization (3 pronuclei) was 1.5 per cent (2/135). Fertilization rate of oocytes with good morphology was higher than those with poor morphology, but there was no statistical significance (82.4% *vs* 79.5%; $p > 0.05$). Oocytes with good morphology were significantly fertilized to be embryos with good quality ($p < 0.001$). This study suggests that oocyte morphology correlates with embryo quality after ICSI.

Key word : Oocyte Morphology, Fertilization, Embryo Quality, Intracytoplasmic Sperm Injection

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Intracytoplasmic sperm injection (ICSI) has been widely used after Palermo performed the first ICSI in 1991[1]. Indication for performing ICSI was male factor problems such as low sperm count, poor morphology and low motility. Other indications were failure of fertilization with conventional *in vitro* fertilization (IVF) or subzonal insemination (SUZI)[2,3]. It is well known that the overall fertilization rate of *in vitro* fertilization (IVF) program does not exceed 60-70 per cent[4].

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About one-third of inseminated oocytes do not become fertilized and this is mostly due to the absence of sperm penetration, as has been shown by the analysis of unfertilized oocytes[5]. When ICSI is employed, the need for the spermatozoon to penetrate the oocyte is rendered obsolete, but some morphological defects of the oocytes are the strong predictor of pregnancy for ICSI[6,7].

Since ICSI requires cleaning of the oocytes from their surrounding cumulus cells within a few hours after retrieval, this creates an opportunity to study in detail some morphological deviations from what is expected to be an ideal metaphase II oocyte. There are a number of variations with oocyte morphology, such as shape of first polar body, size of the perivitelline space, characteristics of cytoplasm, etc[8]. Pregnancy rate after embryo transfer is related to embryo quality, therefore, criteria for evaluating oocyte morphology is very important for the application of ICSI and the selection of good quality embryos for transfer. In this report, we studied the relationship between morphological variations in injected oocytes after ICSI and results of fertilization rates and embryo quality.

MATERIAL AND METHOD

A total of 15 patients with 135 injected oocytes from ICSI process were carried out from January to May 1998. All patients had severe sperm defects, such as total motile sperm count was < 500,000, the number of abnormal forms was > 85 per cent, total motility was < 20 per cent or previous fertilization failure with IVF.

Ovarian stimulation

The long protocol was used to stimulate follicular development with the combination of gonadotropin releasing hormone agonist (GnRHa) and human menopausal gonadotropin (hMG). Follicular development was monitored by transvaginal ultrasound. When at least two follicles reached 18 mm in diameter, 10,000 IU of hCG was given and oocyte retrieval was scheduled for 34-36 hours after hCG injection.

Oocyte retrieval

Oocyte retrieval was performed by transvaginal aspiration under ultrasound guidance 34-36 hours after hCG administration using 18 G, single lumen ovum pick up needle.

Oocyte preparation

Immediately after retrieval, all oocytes were trimmed of excess cumulus cells using 27 G needles, then treated with 80 IU/ml hyaluronidase for 30-60 seconds to complete removal of cumulus cells. The adhering corona radiata was removed by aspiration of the oocytes in and out of a glass pipette with a diameter ranging from 180-220 micron. The oocytes were washed 4 times and incubated in culture medium under oil until further processing.

Sperm preparation

All semen samples were obtained by masturbation just after oocyte retrieval. The morphology parameters were evaluated and classified according to the strict criteria described by Kruger et al[9]. Active spermatozoa were separated by discontinuous Percoll gradient centrifugation method.

Evaluation of oocyte morphology

All oocytes were examined under an inverted microscope and those with a first polar body present were selected for ICSI. The preinjected oocytes were graded on the morphology of the first polar body and perivitelline space and divided into 4 groups:

Grade I fragmented first polar body with large perivitelline space

Grade II intact first polar body with large perivitelline space

Grade III fragmented first polar body with normal perivitelline space

Grade IV intact first polar body with normal perivitelline space

Intracytoplasmic sperm injection

ICSI was carried out by commercial injection and holding pipettes. Active sperm were immobilized by touching the midpiece with the injection pipette, then loaded into the injection pipette and injected into the center of the oocyte after aspirating some ooplasm. The injected oocytes were washed four times and incubated in culture medium at 37°C in an atmosphere of 5 per cent CO_2.

Assessment of fertilization and embryo quality

At 18 hours after ICSI procedure, the oocytes were checked for the presence of one, two

or more pronuclei. Normal fertilization was defined by the presence of two pronuclei. All normal two pronuclei stages were further cultured for another 48 hours, then embryo quality was graded using the following criteria[10]:

Grade I any size blastomeres with > 50 per cent fragmentation
II equal or unequal size blastomeres with 10-50 per cent fragmentation
III unequal size blastomeres with 0 per cent fragmentation
IV equal size blastomeres with < 10 per cent fragmentation
V equal size blastomeres with 0 per cent fragmentation

Statistical analysis

Statistical comparisons were performed with Chi-Square test, Kruskal-Wallis test and Mann-Whitney- U test. A 95 per cent confidence level was used to determine statistical significance.

RESULTS

The patients' background and fertilization outcome are shown in Table 1 and Table 2. A total of 160 oocytes from 15 patients were collected. Of these, 135 oocytes (84.4%) were mature in metaphase II and could be injected. 15 Oocytes (11.1%) were damaged following ICSI procedure, 8 oocytes (5.9%) were non-fertilized and 2 oocytes (1.5%) were abnormally fertilized (3PN). All of these were excluded from further analysis. The remaining 110 oocytes were fertilized normally and assessed in this study. There were 91 good morphological oocytes (grade III, IV) and 44 poor morphological oocytes (grade I, II) which produced 75 and 35 normal fertilized oocytes respectively. Fertilization rate of good morphological oocytes was higher than poor morphological oocytes but showed no statistical significance (82.4% *vs* 79.6%; $p > 0.05$). Table 3 shows the relationship between oocyte grading and embryo quality. The oocyte morphology significantly correlated with embryo quality ($p < 0.001$) and good oocyte morphology (grade III, IV) also significantly ($p < 0.001$) correlated with good embryo quality (grade IV, V).

Table 1. Background details.

	Mean ± SD.	Range
Patient's age (years)	32.9 ± 4.6	24-38
Husband's age (years)	38.0 ± 4.5	30-47
Infertility period (years)	7.4 ± 3.6	2-13
Dose of hMG (ampoules)	25.7 ± 8.4	15-42
Number of oocytes retrieval	9.0 ± 4.3	4-18

Table 2. Fertilization outcome.

	n	%
Total oocytes retrieved	160	100
Metaphase II oocytes	135	84.4
Damaged oocytes	15	11.1
Non-fertilized oocytes	8	5.9
Abnormal fertilized oocytes	2	1.5
Normal fertilized oocytes	110	81.5
- Good morphological oocytes	75/91	82.4 *
- Poor morphological oocytes	35/44	79.6 *

* $p > 0.05$

Table 3. Relationship between oocyte grading and embryo quality*.

Oocyte grading	Embryo grading					Total
	I	II	III	IV	V	
I	2	12	1	0	0	15
II	2	8	2	5	3	20
III	0	10	4	8	6	28
IV	0	17	0	18	12	47
Total	4	47	7	31	21	110

* $p < 0.001$

DISCUSSION

In the ICSI process, complete removal of cumulus cells from oocytes was important and allowed clear assessment of oocyte morphology and evaluated fertilization. Because implantation rate and pregnancy rate were related to embryo quality[11], criteria for assessment of gross oocyte morphology prior to fertilization was important in selection of embryos for transfer. Although De Sutter et al[12,13] reported that oocyte morphology did not correlate with fertilization rate and embryo quality after ICSI, in this study we found that oocyte morphology significantly correlated with embryo quality, but there was no significant correlation with fertilization rate. Oocyte maturation was divided into two situations, nuclear maturation and cytoplasmic maturation. The cause of cytoplasmic morphological abnormalities was multifactorial including ovarian stimulation, hormonal environment and handling procedures immediately after aspiration. Studies in mice suggested that oocytes could acquire competence to undergo cytoplasmic maturation independently of competence to complete nuclear maturation[14]. Ovarian stimulation with GnRH analogue allowed more synchronous follicles but some oocytes retrieved might be from slower developing follicles. The oocyte cytoplasm in these follicles would be at a different maturation stage when exposed to hCG. Therefore, asynchrony of nuclear and cytoplasmic maturation could occur in metaphase II oocyte stage. A recent study has demonstrated a high degree of morphological and nuclear anomalies in unfertilized eggs in patients undergoing ovarian stimulation with pure FSH following pituitary desensitization[15]. Hormonal environment affecting cytoplasmic abnormalities were estrogen and progesterone that were involved in the initiation of cytoplasmic maturation and the final stage of nuclear maturation of the oocytes[16]. Oocytes with abnormal cytoplasm morphology had a high frequency of aneuploidly[17] and developmental failure[18]. Previous studies showed that metaphase II oocytes with atypical first polar body (fragmented, very small, very large) demonstrated a slight decrease in normal fertilization with increased abnormal fertilization[19]. Oocytes with fragmented polar bodies and large perivitelline spaces (grade I) may have over mature cytoplasms at the time of administration of hCG. Oocytes with intact polar bodies and normal perivitelline spaces (grade IV) were the most synchronous group with respect to nuclear and cytoplasmic maturation. It has been postulated that a brief period of time is required after extrusion of the first polar body for the oocyte to gain full cytoplasmic competence before insemination[20]. Poor outcomes from oocytes with an immature first polar body could be due to the short period of time between first polar body extrusion and full cytoplasmic competence. Such oocytes should probably be cultured for a longer period of time before injecting with spermatozoa.

In conclusion, our study suggests that oocyte morphology at the light microscopic level based on shape of first polar body and perivitelline space significantly correlates with embryo quality but has no significant correlation with fertilization rate.

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ความสัมพันธ์ระหว่างรูปร่างลักษณะไข่และอัตราการปฏิสนธิและคุณภาพตัวอ่อนที่เกิดจากการฉีดอสุจิเข้าภายในไข่

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ทำการศึกษาเพื่อหาความความสัมพันธ์ระหว่างรูปร่างลักษณะไข่จากการดูด้วยกล้องจุลทัศน์และผลของการฉีดอสุจิเข้าภายในไข่ (ICSI) โดยศึกษาในไข่ 135 ฟองจากคู่สมรสที่มีบุตรยาก 15 ราย ประเมินรูปร่างลักษณะของไข่ก่อนการฉีดอสุจิโดยบันทึกลักษณะของ first polar body และ perivitelline space ประเมินคุณภาพตัวอ่อนภายหลังการฉีดอสุจิ 48 ชั่วโมงโดยเปรียบเทียบกับลักษณะของไข่ก่อนการฉีดอสุจิ พบว่าอัตราการปฏิสนธิปกติ 81.5%, อัตราการปฏิสนธิผิดปกติ (3PN) 1.5% ไข่ซึ่งมีลักษณะดีจะพบอัตราการปฏิสนธิมากกว่าไข่ซึ่งมีลักษณะด้อยแต่ไม่มีความแตกต่างอย่างมีนัยสำคัญ (82.4% และ 79.5%; $p > 0.05$) ไข่ซึ่งมีลักษณะดีจะสัมพันธ์กับตัวอ่อนที่มีคุณภาพดีภายหลังการฉีดอสุจิอย่างมีนัยสำคัญ ($p < 0.001$)

คำสำคัญ : รูปร่างลักษณะไข่, การปฏิสนธิ, คุณภาพตัวอ่อน, การฉีดอสุจิเข้าภายในไข่

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