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ASSISTED REPRODUCTION TECHNOLOGIES

Poor-responder patients do not benefit from intracytoplasmic morphologically selected sperm injection

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Abstract
Purpose To compare the outcomes of ICSI and IMSI in women presenting with poor ovarian response.
Methods Data of IMSI cycles performed from January 2011 to December 2013 were included in this retrospective cohort study. Patients were divided into two groups: normoresponder patients (NR group; patients with>4 oocytes retrieved) and poor-responder patients (PR group; patients with≤4 oocytes retrieved). Patients who underwent IMSI were matched with patients who underwent ICSI in the same period. The ICSI and IMSI outcomes were compared in the NR and PR groups.
Results A total of 414 matched cycles were included in this study. The NR group comprised 324 cycles (164 ICSI and 160 IMSI cycles), and the PR group comprised 90 cycles (43 ICSI and 47 IMSI cycles). In the NR group, no significant differences were observed between the ICSI- and IMSI-treated couples regarding cycle outcomes. In the PR group, fertilisation rate was significantly lower in IMSI-treated couples (53.9 %± 36.7 % vs. 79.8 %±29.3 %). The proportion of cycles with embryo transfer (57.4 vs. 79.1 %) and the number of transferred embryos (1.5±0.8 vs. 1.9±0.7) were significantly lower in IMSI compared with ICSI. Implantation, pregnancy and miscarriage rates were similar when ICSI or IMSI were performed.
Conclusions Our results suggest that unselected couples undergoing ICSI that present with poor ovarian response to controlled ovarian stimulation do not benefit from sperm selection under high magnification prior to ICSI.

Keywords Embryo · ICSI · IMSI · Oocyte · Poor responder

Capsule Unselected couples undergoing ICSI that present with poor ovarian response to controlled ovarian stimulation do not benefit from sperm selection under high magnification.

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Introduction

The incorporation of the motile sperm organelle morphology examination (MSOME) together with a micromanipulation system has led to the development of a modified intracytoplasmic sperm injection (ICSI) procedure, named 'intracytoplasmic morphologically selected sperm injection (IMSI) [1].

Several studies have reported that the IMSI procedure is positively associated with implantation and pregnancy rates [2–9]. In addition, recent meta-analysis showed that the IMSI procedure is associated with improved embryo quality [10], implantation [10, 11] and pregnancy rates [10–12], as well as lower miscarriage rates [10, 11] in couples with male factor [10–12] and previous ICSI failures [10, 11].

Recently, Setti et al. [13] hypothesised that women with advanced maternal age (AMA) could benefit from the injection of spermatozoa selected under high magnification. The results demonstrated that the outcomes of IMSI in women with AMA are significantly improved compared with ICSI. However, despite the advanced reproductive age, the patients in that study presented with a normal mean number of retrieved oocytes.

One previous study [14] has prospectively analysed the outcomes of IMSI in patients with poor and normal ovarian response. The results demonstrated that IMSI can be beneficial in terms of implantation and clinical pregnancy in cases with fewer than 5 oocytes retrieved. It is known that the duration of IMSI is significantly longer than ICSI [7, 15, 16]. The effects of postponing the injection are still to be elucidated. We hypothesised that, in women presenting with poor ovarian response, the extra time needed for the selection of a morphologically normal spermatozoon added to a poor oocyte quality would thus compromise the outcome of IMSI.

The aim of this study was to compare the outcomes of ICSI and IMSI in women presenting with poor ovarian response.

Materials and methods

Experimental design, patients and inclusion criteria

Data of IMSI cycles, performed from January 2011 to December 2013, in an unselected infertile population, were included in this retrospective cohort study. Patients were divided into two groups according to the number of retrieved oocytes: Poor-responder patients (PR group; patients with≤4 oocytes retrieved) and normoresponder patients (NR group; patients with>4 oocytes retrieved). This criterion has been used in our centre for the characterisation of poor and normal response to controlled ovarian stimulation (COS), and has also been adopted in several published studies [17–24]. Patients who underwent IMSI were matched with patients who underwent ICSI in the same period. The matching process was based on four features relevant for IMSI outcomes: cause of infertility, female age (±1 year), number of previous ICSI cycles (±1 cycle), and number of oocytes retrieved (±2 oocytes). If more than one cycle was eligible, the best match was chosen by random selection. The matching procedure was conducted blinded, without any information regarding the cycle outcome. The ICSI and IMSI outcomes (fertilisation rate, high-quality embryo rates on days 3 and 5 of development, number of transferred embryos, pregnancy rate, implantation rate and miscarriage rate) were compared in the PR and NR groups.

Written informed consent was obtained in which patients agreed to share their outcomes for research purposes, and the study was approved by the local institutional review board.

Controlled ovarian stimulation

Ovarian stimulation was achieved by the administration of recombinant follicle-stimulating hormone (r-FSH, Gonal-F®, Serono, Geneve, Switzerland) on a daily basis until the visualisation of at least one follicle≥14 mm, at which time we began the administration of gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide; Serono Laboratories, Geneva, Switzerland).

The ovulation trigger was given by the injection of recombinant human chorionic gonadotrophin (hCG, Ovidrel™, Serono, Geneve, Switzerland) when at least three follicles≥ 17 mm were observed. Oocyte retrieval was performed 35 h after the administration of hCG, through transvaginal ultrasonography.

Semen sample collection and preparation

Semen samples were collected in the laboratory and were evaluated according to the values established by the World Health Organization (WHO) in 2010 [25]. Sperm samples were prepared using a two-layered density gradient centrifugation technique (50 and 90 %; ISolate, Irvine Scientific, Santa Ana, CA, USA).

IMSI

For couples that underwent IMSI, the MSOME was performed immediately before the injection. An aliquot of the sperm cell suspension was transferred to a microdroplet of modified human tubal fluid medium containing 8 % polyvinyl pyrrolidone (PVP; Irvine Scientific, Santa Ana, CA) in a sterile glass dish (FluoroDish; World Precision Instruments, Sarasota, FL). The dish was placed on a microscope stage above an Uplan Apo 100x oil/1.35 objective lens covered by a droplet of immersion oil. The total calculated magnification was 6, 600x. A sperm cell exhibiting a normal nucleus (normal shape and normal chromatin content), as well as a normal acrosome, postacrosomal lamina, neck, tail and mitochondria and lacking cytoplasmic droplets or cytoplasm surrounding the head, was considered to be morphologically normal [5] and was selected for injection.

ICSI

ICSI was performed according to Palermo et al. [26]. Sperm selection was analysed at 400x magnification using an inverted Nikon Eclipse TE 300 microscope. The injection was performed in a micro-injection dish prepared with 4-μL droplets of buffered medium (Global® w/HEPES, LifeGlobal, Connecticut, USA) and covered with paraffin oil on a heated stage at 37.0 °C±0.5 °C in an inverted microscope.

Fertilisation, embryo quality and embryo transfer

Fertilisation was confirmed by the presence of two pronuclei (PN) and the extrusion of the second polar body approximately 16 h after ICSI. High-quality embryos were defined as those possessing the following: four blastomeres and 8–10 blastomeres on days 2 and 3 of development, respectively; less than

15 % fragmentation; and symmetrical and mononucleated blastomeres.

High-quality blastocysts were those that presented a tightly packed ICM containing a large number of cells and a trophectoderm (TE) in which many cells formed a cohesive epithelium; any blastocyst lacking one of these characteristics was considered to be of low quality [27].

Embryos were placed in a 50-μL drop of culture medium (Global®, LifeGlobal, CT, USA) supplemented with 10 % protein supplement, and were covered with paraffin oil in a humidified atmosphere under 7.5 % CO_2 at 37 °C for 5 days.

Embryo transfer was performed on day 5 of development using a soft catheter with transabdominal ultrasound guidance. One to four embryos were transferred per patient, depending on embryo quality and maternal age.

Clinical follow-up

A pregnancy test was performed 10 days after embryo transfer. All women with a positive test received a transvaginal ultrasound scan after 2 weeks. A clinical pregnancy was diagnosed when the foetal heartbeat was detected. Implantation rates were calculated per patient. Pregnancy rates were calculated per transfer. Miscarriage was defined as pregnancy loss before 20 weeks.

Data analysis and statistics

Data are expressed as the mean±standard deviation for continuous variables, while percentages are used for categorical variables. Mean values were compared by Student's t parametric test or Mann–Whitney non-parametric test. Percentages were compared by the Chi-squared or Fisher exact test when the expected frequency was five or lower. A $p<0.05$ was considered to be statistically significant. Data analyses were carried out using the Minitab® version 16 statistical program.

Results

A total of 414 matched cycles were included in this study (207 ICSI and 207 IMSI cycles, Table 1). The NR group comprised 324 cycles (164 ICSI and 160 IMSI cycles, Table 2), and the PR group comprised 90 cycles (43 ICSI and 47 IMSI cycles, Table 3 and Fig. 1).

In the NR group, there were no significant differences between the ICSI- and IMSI-treated couples regarding demographic characteristics or the cycle outcomes (Table 4).

In the PR group, similar mean female age, BMI, number of previous ICSI cycles, follicles, retrieved oocytes and mature oocytes were observed in couples that underwent ICSI or IMSI (Table 5). However, fertilisation rate was significantly lower in IMSI-treated couples compared with ICSI (53.9 %± 36.7 % vs. 79.8 %±29.3 %, respectively; $p<0.001$). Despite a similar rate of high-quality embryos on day 3 and similar blastocyst formation rates, the proportion of cycles with embryo transfer (57.4 vs. 79.1 %; $p=0.027$) and the number of transferred embryos (1.5±0.8 vs. 1.9±0.7; $p=0.042$) were

Table 1 Demographic characteristics and outcomes of ICSI and IMSI in the study group ($n=414$)

Variable	Mean	SD	Min	Max
Female age (years)	38.1	4.2	22	52
Paternal age (years)	39.6	5.9	25	61
Female BMI	24.7	3.9	17.3	39.1
Previous IVF cycles	1.8	1.5	1	9
Follicles	13.3	8.8	1	58
Retrieved oocytes	9.6	6.4	1	31
Mature oocytes	7.4	4.9	1	25
Fertilization rate (%)	72.4	24.5	0	100
High-quality embryos on D3 (%)	45.3	31.4	0	100
Blastocyst formation rate (%)	17.7	26.5	0	100
Transferred embryos	2.1	0.8	0	4
Implantation rate	20.7	32.9	0	100
	n/N			
Cycles with embryo transfer (%)	338/414 (81.6)			
Pregnancy rate (%)	112/338 (33.1)			
Miscarriage rate (%)	10/112 (8.9)			

SD Standard deviation, *BMI* body mass index

Table 2 Demographic characteristics and outcomes of ICSI and IMSI in the NR-group ($n=324$)

Variable	Mean	SD	Min	Max
Female age (years)	37.6	4.3	22	46
Paternal age (years)	39.4	5.9	25	61
Female BMI	24.4	3.9	17.3	39.1
Previous IVF cycles	1.8	1.5	1	9
Follicles	15.9	8.2	5	58
Retrieved oocytes	11.5	5.9	5	31
Mature oocytes	8.7	4.6	1	25
Fertilization rate (%)	74.1	20.1	0.0	100
High-quality embryos on D3 (%)	43.5	27.5	0.0	100
Blastocyst formation rate (%)	20.6	27.6	0.0	100
Transferred embryos	2.1	0.8	0	4
Implantation rate	22.9	33.7	0.0	100
	n/N			
Cycles with embryo transfer (%)	277/324 (85.5)			
Pregnancy rate (%)	102/277 (36.8)			
Miscarriage rate (%)	8/102 (7.8)			

NR-group Normoresponder group, *SD* Standard deviation, *BMI* body mass index

Table 3 Demographic characteristics and outcomes of ICSI and IMSI in the PR-group (n=90)

Variable	Mean	SD	Min	Max
Female age (years)	40.0	3.4	35	52
Paternal age (years)	40.6	5.6	31	54
Female BMI	25.5	3.9	20.2	34.2
Previous IVF cycles	1.7	1.6	1	8
Follicles	4.2	2.2	1	10
Retrieved oocytes	2.7	1.1	1	4
Mature oocytes	2.3	1.1	1	4
Fertilization rate (%)	66.3	35.7	0.00	100
High-quality embryos on D3 (%)	52.9	43.6	0.00	100
Blastocyst formation rate (%)	6.04	16.8	0.00	75.0
Transferred embryos	1.8	0.8	0	3
Implantation rate	10.9	27.4	0.00	100
	n/N			
Cycles with embryo transfer (%)	61/90 (67.8)			
Pregnancy rate (%)	10/61 (16.4)			
Miscarriage rate (%)	2/10 (20.0)			

PR-group Poorresponder group, *SD* Standard deviation, *BMI* body mass index

Table 4 Comparison between ICSI and IMSI outcomes in the NR-group (n=324)

Variable	ICSI (n=164)	IMSI (n=160)	p-value
Female age (years)	37.6±4.2	37.5±4.3	0.738
Paternal age (years)	39.8±6.5	38.9±5.3	0.214
Female BMI	25.1±4.3	24.9±3.4	0.709
Previous IVF cycles	1.8±1.3	1.8±1.6	0.609
Follicles	15.9±9.0	15.8±7.4	0.932
Retrieved oocytes	11.4±5.9	11.6±5.8	0.747
Mature oocytes	8.7±4.5	8.7±4.6	0.912
Fertilization rate (%)	75.9±18.9	72.3±21.2	0.107
High-quality embryos on D3 (%)	45.8±25.4	41.1±29.3	0.122
Blastocyst formation (%)	30.9±28.1	29.3±25.7	0.501
Transferred embryos	2.2±0.7	2.1±0.8	0.808
Cycles with embryo transfer (%)	142/164 (86.6)	135/160 (84.4)	0.572
Implantation rate	25.9±36.1	19.6±30.7	0.118
Pregnancy rate (%)	56/142 (39.4)	46/135 (34.1)	0.355
Miscarriage rate (%)	4/56 (7.1)	4/46 (8.7)	>0.999

NR-group Normoresponder group, *BMI* Body mass index

significantly lower in IMSI than ICSI. Implantation rates, pregnancy rates and miscarriage rates were similar when ICSI or IMSI were performed (Table 5).

Discussion

The results from this study suggest that unselected couples undergoing ICSI that present with poor ovarian response to controlled ovarian stimulation (COS) do not benefit from sperm selection under high magnification. We observed reduced fertilisation rates, cycles with embryo transfer and transferred embryos in IMSI-treated couples compared with ICSI.

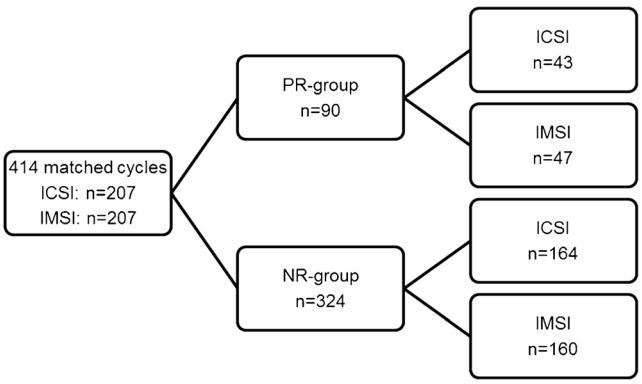

Fig. 1 Distribution of matched cycles into PR-group and NR-group, and into ICSI and IMSI group

Our results are in disagreement with those of De Vos et al. [28]. The study analysed 3004 ICSI cycles and 2413 IMSI cycles. For each arm, cycles were divided in sub-groups according to the number oocytes injected: 1–5 oocytes, 6–10 oocytes, 11–15 oocytes and≥16 oocytes. IMSI resulted in significantly higher implantation and clinical pregnancy rates

Table 5 Comparison between ICSI and IMSI outcomes in the PR-group (n=90)

Variable	ICSI (n=43)	IMSI (n=47)	p-value
Female age (years)	40.0±3.4	40.0±3.3	>0.999
Paternal age (years)	39.7±5.0	41.5±6.0	0.114
Female BMI	26.4±3.7	24.9±4.0	0.105
Previous ICSI cycles	1.5±1.5	1.9±1.6	0.291
Follicles	4.1±2.2	4.4±2.2	0.570
Retrieved oocytes	2.8±1.0	2.6±1.1	0.288
Mature oocytes	2.4±1.2	2.2±1.0	0.533
Fertilization rate (%)	79.8±29.3	53.9±36.7	<0.001
High-quality embryos on D3 (%)	48.7±44.3	57.4±43.0	0.392
Blastocyst formation (%)	9.8±21.1	13.9±33.0	0.532
Transferred embryos	1.9±0.7	1.5±0.8	0.042
Cycles with embryo transfer (%)	34/43 (79.1)	27/47 (57.4)	0.027
Implantation rate	8.8±26.0	13.6±29.3	0.511
Pregnancy rate (%)	4/34 (11.8)	6/27 (22.2)	0.314
Miscarriage rate (%)	0/4 (0.0)	2/6 (33.3)	0.466

PR-group Poor responder group, *BMI* Body mass index

in patients with 5 or fewer retrieved oocytes. Despite the large casuistic, analysis of the data according to other parameters such as female age, number of failed cycles and type of infertility were not performed. In addition, four sub-groups of retrieved oocytes were analysed separately, what could explain the discrepancy with our results.

A poor response to COS is often an indication of a decrease in oocyte quantity. The oocyte quality and quantity both decrease as a result of ovarian ageing, and a relationship between quantitative ovarian reserve and oocyte quality has also been demonstrated [29]. It is believed that both poor oocyte quality and quantity may affect the outcomes of ICSI by two different mechanisms: (i) compromised viability of the oocyte itself; and (ii) limited possibility of performing embryo selection. In addition, the viability of oocytes in poor responders is especially poor when associated with advanced maternal age.

The process of finding spermatozoa without vacuoles is difficult and time-consuming [7, 8, 15, 16, 30–33]. In addition, switching between the glass-bottomed dish that is appropriate for Nomarski microscopy and the plastic-bottomed dish that is appropriate for Hoffman modulation contrast requires additional time, delaying the injection procedure [16]. Balaban et al. [15] demonstrated that the duration of the procedure was significantly longer in the IMSI group than the ICSI group. Recently, it has been suggested that sperm injection should not be delayed in order to avoid oocyte ageing [28]. We could suggest that oocyte quality of poor-responder patients deteriorates along with the extra time required for sperm selection under high magnification, resulting in a reduced fertilisation rate. Nevertheless, once fertilised, the oocytes demonstrated a normal ability to sustain embryonic development until day 5.

In this study, it was clear that a cascade of events started with the reduced fertilisation rate and, therefore, a reduced number of zygotes that led to a lower number of embryos and blastocysts, affecting the proportion of cycles with embryo transfer and the possibility of embryo selection for transfer. However, no impact on implantation and pregnancy rates was observed.

It is important to highlight that in the PR group, we observed a high mean paternal age (40.6±5.6 years), which has been previously demonstrated to be associated with a decline in semen quality [34], the presence of sperm vacuoles [35], lower proportion of sperm with normal morphologies and higher DNA fragmentation rate. Therefore, the paternal age influence on the findings must not be disregarded.

In the NR group, no significant differences were observed between ICSI- and IMSI-treated couples in terms of demographics, cycle characteristics and outcomes. This is in agreement with previous studies that reported similar clinical outcomes with ICSI and IMSI in an unselected infertile population [15, 36].

The main limitations of this study are its retrospective design and the low number of cycles included, especially in the PR group. Further prospective randomised studies with larger samples are necessary to confirm our findings.

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