

Intracytoplasmic Morphologically selected Sperm Injection vs Intracytoplasmic Sperm Injection: A Retrospective Analysis

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ABSTRACT

This study was conducted to determine the efficacy of using the technique of intracytoplasm morphologically selected sperm injection (IMSI) as compared to the conventionally used intracytoplasmic sperm injection (ICSI) procedure. IMSI, as the name suggests, is the technique of selecting the most normal looking sperm by magnifying it to about 7200 times. A total of 192 patients who underwent the treatment of *in vitro* fertilization (IVF) with us by either one of the two procedures of IMSI or ICSI over a period of 18 months were included in the study. Out of these 92 were included in the IMSI group while 100 others in the ICSI group according to our inclusion and exclusion criteria. The pregnancy rate among patients who underwent IMSI was found to be significantly higher (52.1%) as compared to those with ICSI (36%). Furthermore, even the implantation rates were higher in the IMSI group (30.12%) than the ICSI (19.93%) group.

Keywords: High magnification, ICSI, IMSI, Male infertility, MSOME, Sperm selection, OAT syndrome.

INTRODUCTION

A new method of high magnification ($\times 7200$) motile sperm organellar morphology examination (MSOME) published by Bartoov et al¹ in 2002 showed that the fertilization rate is directly proportional to the incidence of morphologically normal spermatozoa. This was found to be statistically significant.

The main purpose of our retrospective study was to determine the efficacy of the intracytoplasmic morphologically selected sperm injection (IMSI) procedure compared to the conventional intracytoplasmic sperm injection (ICSI) procedure in the treatment of patients with severe oligoasthenoteratozoospermia (OAT) and/or at least two previous failed IVF attempts.

MATERIALS AND METHODS

Patient Selection

From January 2009 up to August 2010, 192 couples were observed. Observation criteria included: (1) Patients with severe oligoasthenoteratozoospermia and/or at least two previous failed pregnancy outcomes. (2) An undetected female factor or no obvious pathology in the female partner. (3) Woman's age 35 years or younger. (4) Couples with more than or equal to

two years of infertility. Exclusion criteria: (1) Patients with unexplained infertility with less than two previous *in vitro* fertilization (IVF) attempt failures. (2) Any detected pathology in the wife/female. (3) Patients with established azoospermia even with testicular biopsy. (4) Patients with two or more previous consecutive miscarriages.

Patients were divided into following groups as follows: group 1 (IMSI) (n = 92), group 2 (ICSI) (n = 100). These two groups were further subdivided into subgroups as: (a) With no previous IVF attempt failure (b) with one previous IVF attempt failure and (c) with two or more IVF attempt failures. All the patients underwent the same mid-luteal phase down-regulation protocol with GnRH agonist ('long' protocol) and started stimulation with recombinant FSH from day 2 of cycle.

Semen Evaluation and Preparation

Semen analysis was done in the laboratory just prior to preparation for injection. The diagnosis of severe oligoasthenoteratozoospermia was confirmed by the World Health Organization (WHO) criteria for sperm concentration ($< 5 \times 10^6$ /ml) and motility ($< 20\%$ progressive) (WHO, 1992), and strict Kruger's criteria ($< 4\%$ normal forms) for sperm morphology evaluation.²

We use only freshly ejaculated semen for sperm preparation for performing ICSI/IMSI. The preparation of the semen was performed using pellet-swim up technique for normal samples (WHO criteria) and pellet reconstitution technique for severe

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oligozoospermic samples. The washing of semen was done with MediCult HEPES buffered flushing medium MediCult (Catalog no) and the pellet was overlaid/reconstituted with embryo culture medium (Vitrolife catalog no...).

IMSI Sperm Selection

The glass Petri dish (Willco-dish; Willco wells BV, Amsterdam, The Netherlands) for IMSI was prepared as follows: (i) In the center, three observation droplets of polyvinylpyrrolidone medium (MediCult medium) were made (ii) surrounding the PVP drops were 7 μ l drops of flushing medium to host the oocytes that were to be injected with the selected sperms.

The droplets were overlaid with sterile liquid paraffin (MediCult).

The sperm cell suspension obtained after semen preparation was used for real-time high magnification MSOME³ that was performed on the observation droplets by means of a Narishige micromanipulator, with attached inverted microscope (Nikon Eclipse, TE2000S, Tokyo, Japan). The images were captured by a high definition USB2.0 camera 3 MPx and visualized on a monitor screen with diagonal dimension of 42 cm.

Sperms with obvious defects like pin head, amorphous head, large mid-piece, double tail/head were not evaluated. In this study, it is assumed that the sperms exhibiting normal morphology according to MSOME criteria have normal nuclear DNA content and organization, which have been reported to exert a significant effect on ICSI fertilization rate and embryo development.

To perform the evaluation, the following steps were taken:

1. First, the seemingly normal sperms were immobilized.
2. Five to six of them were placed in a row one below the other in the microscopic field.
3. They were magnified real time ($\times 6000$) and then an image was clicked.
4. With the help of a computer program, the measurement of the image of the sperm was taken.
5. The strict descriptive criteria for normally shaped nuclei were based on those defined by scanning electron microscopy, i.e. smooth, symmetric and oval configuration.^{1,3} Only motile spermatozoa with normal head dimensions (length 4.5 to 5.0 μ m, width 3.0 to 3.5 μ m) were selected (Fig. 1).
6. The sperms thus selected were further magnified 7200 times and observed for vacuoles. Those sperms with no or a maximum of one vacuole ($<1.0 \mu$ m) were selected for injected into the oocytes. The nuclear chromatin content was considered normal if it contained no more than one vacuole, which occupies $<4\%$ of the nuclear area (Fig. 2).

Two embryologists worked together for analyzing the sperms to minimize the chances of human error.

Microinjection

The transferred, retrieved, cumulus-free ova were placed into drops of MediCult buffered medium prepared in the same glass

dish with the recipient droplet. Sperm cells morphologically selected for IMSI were then finally used for injection into the oocytes by classical ICSI.⁴ This procedure was performed using Narishige Micromanipulation System using Injection Pipette (COOK'S MPIP catalog no....) and holding pipette (COOK'S HPIP catalog no.....) at $400 \times$ magnification.

Embryo Culture

The injected oocytes were immediately transferred to a center well dish (Falcon 3037), incubated in 0.8 ml of IVF medium (Vitrolife) covered with 0.4 ml of mineral oil (MediCult) at 37°C with an atmosphere of 5% CO₂. Three to four oocytes were cultured per dish.

Embryo transfer was done on day 2 or 3 after visual grading and selection of best embryos (when more than three embryos were formed). Usually three embryos per patient were transferred except in cases when only one or two embryos were formed, in which case whichever embryos obtained were transferred. The pregnancy rate was calculated per transfer attempt.

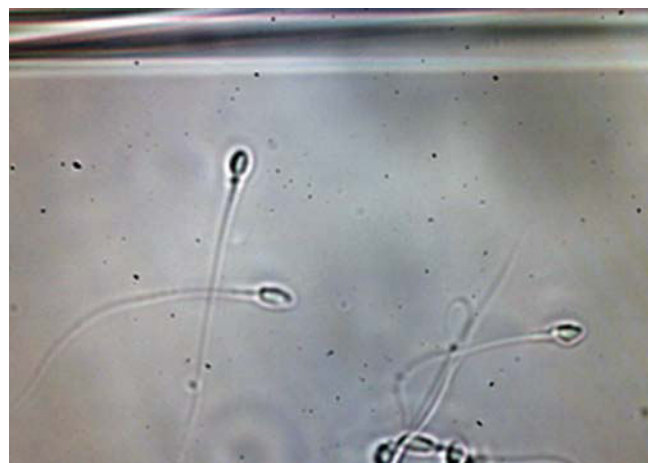


Fig. 1: Appearance of sperms with IMSI procedure ($\times 7200$ magnification)



Fig. 2: Sperms magnified 7200 times to identify vacuoles

STATISTICAL METHODS

Data are presented as mean \pm SD. Statistical evaluation was performed with the Student's t-test to compare continuous variables while a Chi-square test was used to compare discrete variables. $p < 0.05$ was considered statistically significant.

RESULT

There were no statistical differences between the main groups in terms of mean age, number of previous failed ICSI attempts, number of recovered oocytes and transferred embryos (Table 1).

A total of 24 IMSI and 18 ICSI patients attained positive β -hCG followed by fetal heart beat detection by the sixth week. Up to now, the IMSI procedure has resulted in seven deliveries of a total of 11 healthy babies (four twins), 13 ongoing pregnancies and four miscarriages. As for the ICSI group, six healthy babies were born (2 twins), four miscarriages

occurred and 10 pregnancies are still ongoing. All miscarriages took place during the first trimester.

By comparing groups 1 and 2, IMSI pregnancy and implantation rates appear to be significantly higher than those for ICSI (pregnancy rate 52.17 vs 36%, and implantation rate 30.63% vs 19.83%).

PN = pronucleate. Continuous variables are presented as means \pm SD.

$$^a p < 0.05; ^b p < 0.05$$

Upon comparison of the two techniques by subgroups with different previous failed attempts (Table 2), the following pregnancy rate results were obtained: (i) Subgroup 1C vs subgroup 2C: 25% vs 51.35%, $p < 0.05$; (ii) no statistical difference was observed between subgroup 1A and subgroup 2A (40% vs 50%), and between subgroup 1B and subgroup 2B (44.12% vs 54.84%) (Table 3), although the clinical outcome was clearly in favor of the IMSI method.

Table 1: Comparison of fertilization, pregnancy and implantation rates arising from intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) groups

	Group 1 ICSI (n = 100)	Group 2 IMSI (n = 92)	Unpaired t-test applied		
			t-value	p-value	Difference
Mean age (years)	32.91 \pm 3.30	32.65 \pm 3.23	- 0.530	0.5963	NS
Number of previous ICSI failures	1.65 \pm 1.57	1.69 \pm 1.69	0.17	0.8652	NS
Number of oocytes recovered	10.39 \pm 3.04	9.58 \pm 3.34	- 1.759	0.0802	NS
Number of injected oocytes	8.92 \pm 0.26	8.85 \pm 0.29	- 1.763	0.0794	NS
Number of 2 PN zygotes	6.66 \pm 0.42	7.23 \pm 0.45	9.078	< 0.0001	NS
Number of transferred embryos/patient	2.81 \pm 0.67	2.82 \pm 0.68	0.103	0.9184	NS
Clinical pregnancy rate (%)	36/100 (36) ^a	48/92 (52.17) ^a	4.457 ^c	0.0347	S
Implantation rate (%)	56/281(19.93) ^b	78/259 (30.12) ^b 2 twins	6.960 ^c	0.0083	S 4 twins

^aPregnancy detected by observing a viable pregnancy (with heart beat) on ultrasound examination

^bImplantation rate calculated per embryo transferred

^cChi-square test applied with continuity correction; NS: Nonsignificance; S: Significance

Table 2: Comparison of pregnancy arising from intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) subgroups with a different number of previous IVF failures

Subgroup	Rate	Group 1, IMSI	Group 2, IMSI
Subgroup A (0 ICSI failures)	Pregnancy	12	12
	No pregnancy	18	12
	Total	30	24
	Percentage of pregnancy rate	40%	50%
Subgroup B (1 ICSI failure)	Pregnancy	15	17
	No pregnancy	19	14
	Total	34	31
	Percentage of pregnancy rate	44.12%	54.84%
Subgroup C (2 ICSI failures)	Pregnancy	9	19
	No pregnancy	27	18
	Total	36	37
	Percentage of pregnancy rate	25%	51.35%

Table 3: Statistical analysis of the above-mentioned subgroups in terms of pregnancy rate

Chi-square tests	Value	df	p-value	Association
Subgroup A (0 ICSI failures)	0.211	1	0.646 ^a	NS
Subgroup B (1 ICSI failure)	0.378	1	0.538 ^a	NS
Subgroup C (2 ICSI failures)	4.302	1	0.038 ^a	S

^aChi-square test applied with continuity correction; NS: Nonsignificance; S: Significance

DISCUSSION

Evolutionary biologists have developed several hypotheses that link sperm selection to the inheritance of superior fitness traits (i.e. disease resistance, offspring survival and fecundity). It is believed that the healthiest sperm is naturally selected by the ovum for fertilization. Hence, since the advent of IVF, semen parameters have played an important role in determining the outcome of treatment.⁵⁻⁹ However, with the introduction of microinsemination techniques^{4,10-12} sperm morphology evaluation has lost its importance as a routine diagnostic criteria in infertility management. Sperm selection is based on the judgment of an embryologist who selects the most normal-looking spermatozoon available under $\times 200/400$ magnification. Though some researchers feel that the outcome of ICSI is not related to basic sperm parameters.¹³⁻¹⁶ Numerous studies have established that the correct selection of a morphologically normal sperm greatly enhances the outcome of ICSI.¹⁷⁻¹⁹

However, the dependence on the expertise of the embryologist for the selection of a morphologically normal sperm is very subjective and not completely devoid of errors. To overcome this hurdle a number of advanced microscopes have been recently developed to magnify sperms thousands of times to clearly observe the morphology.

Benjamin Bartoov in Israel was the first to use the technique of IMSI in 2003. He selected patients with at least two previous ICSI failures. He used the MSOME criteria to select morphologically normal sperms and injected them into the eggs. They suggest that ICSI pregnancy rates may be affected by subtle morphological malformations of the sperm nucleus, which embryologists may not detect during routine ICSI sperm selection. The study compared 50 couples in each group. They found that the couples who underwent IMSI had a significantly higher pregnancy rate (66%) than those who underwent conventional ICSI (33%). However, this was based on a small sample size.³

Since then numerous studies have been performed with encouraging results in favor of the IMSI procedure.²⁰⁻²²

The concept of IMSI can be better appreciated if we understand the fact that after all in cases of natural conception, the most healthy and morphologically and physiologically normal sperm is selected out of the millions to attain fertilization of a single egg. This can be extrapolated into saying that if we want to achieve successful *in vitro* fertilization, it will do us good to be able to select the sperm which is the most normal in all aspects. However, there is no denying the complexity of natural sperm selection mechanisms which is supported by the fact that despite the normally large numbers of spermatozoa in an ejaculate, only a minority are able to meet the stringent requirements needed to fertilize an egg. This leads to the obvious conclusion that sperm quality assessment methods in the laboratory would improve if they could incorporate aspects of these selective processes. However, one has to consider that there are a number of physiological, morphological and genetic aspects that come in the natural selection of sperms that the right mix still remains elusive despite years of research.

The best method of selection of the best sperm can be via the process of elimination. In case when a sperm is being singled out for injection into an egg, selection can be done based on the assumption that the sperms that pass the MSOME test are closer to normal than the ones that do not.¹ The morphology of the sperm organelles defined by this method was found to have the highest predictive ability for male fertility potential.³

Our results demonstrate that the morphological state of the entire sperm cell, and especially of its nucleus, has predictive value for fertilization, pregnancy outcome, or both following ICSI. We applied the MSOME criteria prior to ICSI for 92 couples (group 1) as compared to the 100 couples (group 2) and found spermatozoa free of any specific morphological malformations exhibited the highest positive correlation with ICSI pregnancy rate. These present results are not in accordance with some authors who believe that ICSI outcome is not related to strict morphology of the spermatozoon used for microinjection.²³⁻²⁶

In reviewing the literature, there are conflicting studies. For example, few studies indicate that fertilization, embryo development and pregnancy is possible even if normal spermatozoa are not available (100% of teratozoospermia).^{13,27-29} Whereas a few other studies have found that fertilization, pregnancy and also implantation rates are adversely affected when sperms with severe abnormalities were used for ICSI.^{17,19,30} One more study in 2001 also concludes into saying that sperm morphology is significantly correlated to the percentage of embryos developing to the blastocyst stage (30.3% vs 51.9%) and to high quality blastocysts (13.6% vs 28.2%).¹⁸

CONCLUSION

This study is a unique retrospective study that proves the effectiveness of IMSI over ICSI to benefit patients who have severe oligoteratozoospermia and/or repeated IVF/ICSI failures. The patients with more than one failed attempt at IVF/ICSI seem to have increased their chances of pregnancy two-fold after undergoing IMSI. The implantation rate per embryo also significantly increased from 19.93 to 30.12%. However, one should bear in mind the small sample size. The authors opine that a controlled prospective study involving a large sample size is in order to be able to analyze the true statistical significance of the benefit of IMSI over ICSI.

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