

Intracytoplasmic morphologically selected sperm injection benefits for patients with oligoasthenozoospermia according to the 2010 World Health Organization reference values

The comparison between the outcomes of intracytoplasmic morphologically selected sperm injection performed in couples with male factor infertility according to the World Health Organization guidelines from 1999 and 2010 was the objective of this study. Our results suggest that the sperm selection under high magnification results in improved treatment outcomes in patients with oligoasthenozoospermia, according to the new World Health Organization guidelines. (*Fertil Steril*® 2011;95:2711–4. ©2011 by American Society for Reproductive Medicine.)

Key Words: ICSI, male factor infertility, IMSI, oligoasthenozoospermia, sperm morphology

World Health Organization (WHO) reference values for human semen parameters are used widely to investigate male reproductive potential. Sperm morphology evaluation plays a crucial role in the diagnosis of male fertility potential, and it has demonstrated a predictive value for IVF (1–3).

Since its introduction, studies have been demonstrating that intracytoplasmic morphologically selected injection, based on motile sperm organelle morphology examination (4, 5), showed a positive association with pregnancy outcomes in couples with previous and repeated implantation failures (6, 7) and in patients with an elevated degree of DNA fragmented spermatozoa (8).

Although morphologically normal spermatozoa from patients with normozoospermia show a significant higher biologic competence, morphologically normal spermatozoa of patients with oligoasthenozoospermia have alterations in physiologic status similar to that found in spermatozoa with head abnormalities

(9). The factors that cause these sperm parameter alterations seem to affect, at the same time, the molecular mechanisms regarding DNA integrity, chromosome status, and chromosome segregation in all germ cells. Therefore, we could suggest that normal morphology in conventional intracytoplasmic sperm injection (ICSI) cycles does not indicate the selection of competent spermatozoa in patients with oligoasthenozoospermia. The benefits of a high-magnification approach according to the patient's alterations of reference values for semen parameters, such as sperm concentration and motility, are still a matter of debate and, therefore, were the aim of this study.

This prospective study included 500 couples who underwent their first IVF treatment. Inclusion criteria were presence of isolated male factor infertility (abnormal semen parameters according to WHO) and at least six oocytes available on retrieval. Exclusion criteria were presence of female factor infertility either isolated or associated, patients with azoospermia, and use of a cryopreserved semen sample for ICSI.

Written informed consent was obtained in which the patients agreed to share the outcomes of their cycles for research purposes. The study was approved by the local Institutional Review Board.

In the first analysis, couples were divided randomly into two groups according to a computer-generated randomization list: 250 couples were assigned to ICSI and 250 to intracytoplasmic morphologically selected sperm injection. The cycles' outcomes were compared between the groups.

In the second analysis, only the couples resorting to IVF as a result of oligoasthenozoospermia (OAT) ($n = 244$) were enrolled and split into two groups: [1] patients with OAT according to the WHO reference values from 1999 (OAT-1999 group, $n = 244$) and [2] patients with OAT according to the reference values of 2010 (OAT-2010 group, $n = 165$). For this, semen analysis results were reevaluated according to the new WHO cutoff points (concentration $\geq 15 \times 10^6/\text{mL}$, total count $\geq 39 \times 10^6$, progressive motility $>42\%$, and typical morphology $\geq 4\%$). The

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influence of intracytoplasmic morphologically selected sperm injection on treatment outcomes was assessed.

Stimulation protocol and standard ICSI procedure are given elsewhere (10). Sperm selection in the intracytoplasmic morphologically selected sperm injection group was examined at high magnification with an inverted microscope (Eclipse TE 300; Nikon, Tokyo, Japan) equipped with high-power differential interference contrast optics (DIC/Nomarski). The total calculated magnification was $\times 6,600$.

All semen samples were collected in the laboratory after 2 to 7 days of ejaculatory abstinence and evaluated according to the threshold values established by the WHO (concentration $\geq 20 \times 10^6/\text{mL}$, total count $\geq 40 \times 10^6$, and progressive motility $>50\%$). Typical morphology was evaluated according to Kruger criteria (2).

Semen samples were prepared by a gradient technique with two layers (90%, 70%) of Isolate (Irvine Scientific, Santa Ana, CA). The suspension after the first gradient centrifugation (20 minutes, $330 \times g$) was rewashed (7 minutes, $330 \times g$) with a HEPES-buffered human tubal fluid medium (mHTF; Irvine Scientific). Preparation of the final sperm cell suspension for further motile sperm organelle morphology examination and the ICSI procedure was performed as previously described (11).

The sperm cells exhibiting normally shaped nuclei (smooth, symmetric, and oval configuration) and [4] normal nuclear chromatin content (if it contained no more than one vacuole, which occupies $<4\%$ of the nuclear area) were selected for injection (8). Normal fertilization, high-quality embryos, clinical pregnancy, and miscarriage are defined elsewhere (10). Embryo transfer was performed on the third day of development.

The data are presented as odds ratios (OR) with 95% confidence interval (CI) and *P* value. The results were considered to be statistically significant at the 5% critical level ($P < .05$).

In this study, couples with male factor infertility were enrolled. Out of the 500 male patients, 244 (48.8%) had OAT, 158 (31.6%) had teratozoospermia, and 98 (19.6%) had asthenozoospermia.

There were no significant differences between the general characteristics of the ICSI and intracytoplasmic morphologically selected sperm injection cycles (Table 1). Fertilization rate was significantly higher in the intracytoplasmic morphologically selected sperm injection group (68.0% vs. 73.0%, $P = .013$). No significant differences were observed between ICSI and intracytoplasmic morphologically selected sperm injection groups for the percentage of high-quality embryos, pregnancy, implantation, and miscarriage rates (Table 1).

In a further analysis, only patients with OAT were included ($n = 244$) and divided into the OAT-1999 group ($n = 244$) and the OAT-2010 group ($n = 165$). In the OAT-1999 group, 128 underwent ICSI and 116 underwent intracytoplasmic morphologically selected sperm injection. In the OAT-2010 group, 88 underwent ICSI and 77 underwent intracytoplasmic morphologically selected sperm injection.

A positive influence of intracytoplasmic morphologically selected sperm injection on the fertilization was observed for the OAT-1999 group (OR 1.3, CI 1.1–2.1, $P = .043$). No impact of intracytoplasmic morphologically selected sperm injection on high-quality embryos (OR 0.9, CI 0.8–1.4, $P = .321$), pregnancy

(OR 1.1, CI 0.9–1.5, $P = .456$), implantation (OR 0.9, CI 0.7–1.6, $P = .657$), or miscarriage (OR 1.3, CI 0.8–2.1, $P = .259$) was observed for these patients.

Similarly, a close relationship between the intracytoplasmic morphologically selected sperm injection and fertilization was noted for the OAT-2010 group (OR 4.3, CI 2.2–6.4, $P = .004$). Furthermore, the intracytoplasmic morphologically selected sperm injection was determinant to the increased likelihood of implantation (OR 2.6, CI 1.2–5.7, $P = .013$) and pregnancy (OR 1.6, CI 1.1–3.0, $P = .045$) in these patients. No impact of intracytoplasmic morphologically selected sperm injection on high-quality embryos (OR 0.8, CI 0.6–1.8, $P = .784$) or miscarriage (OR 1.2, CI 0.6–1.6, $P = .487$) was observed.

This prospective randomized study was designed to investigate future applications of intracytoplasmic morphologically selected sperm injection. In the first analysis 500 couples undergoing IVF as result of male factor infertility were split into ICSI and intracytoplasmic morphologically selected sperm injection groups. Our results showed that, except for the significantly higher fertilization rate in the intracytoplasmic morphologically selected sperm injection group, the outcomes were similar between the groups.

In our second analysis, patients with OAT were split into two groups according to the WHO guidelines from 1999 and 2010, and the efficacy of intracytoplasmic morphologically selected sperm injection was assessed in both groups. Our results demonstrated that intracytoplasmic morphologically selected sperm injection was determinant to the increased likelihood of fertilization in patients with oligoasthenozoospermia according to 1999 and 2010 guidelines.

However, in oligoasthenozoospermia according to 2010 guidelines, intracytoplasmic morphologically selected sperm injection was also determinant to the increased likelihood of implantation, and pregnancy was 2.5 times as likely to occur in the intracytoplasmic morphologically selected sperm injection group as compared with the ICSI group.

Indeed, Burrello et al. (9) described that sperm morphology criteria applied with use of conventional ICSI are able to differentiate competent sperm cells with success only in cases of patients with normozoospermia. Furthermore, Balaban et al. (12), in a prospective randomized study, observed that intracytoplasmic morphologically selected sperm injection and ICSI provided comparable outcomes when used in an unselected infertile population. However, a subgroup analysis demonstrated that patients with severe male factor significantly benefited from intracytoplasmic morphologically selected sperm injection, especially those who had sperm concentrations $<1 \times 10^6/\text{mL}$ in the basal ejaculate.

In a recent meta-analysis, the selection of spermatozoa under high magnification has been proved to improve embryo development, implantation, pregnancy, and miscarriage rates (13). Biochemical mechanisms behind this phenomenon are not clear and may reflect some underlying chromosomal or DNA damage of spermatozoa with abnormal nuclei (14, 15).

Deoxyribonucleic acid damage cannot be evaluated directly in spermatozoa selected for intracytoplasmic morphologically selected sperm injection. Therefore, Wilding et al. (16) used the deoxyuride-5'-triphosphate biotin nick end labeling (TUNEL) assay to test whether motile sperm organelle morphology examination could deselect physiologically abnormal spermatozoa. Their

TABLE 1

Demographics data and treatment outcomes of ICSI and intracytoplasmic morphologically selected sperm injection groups.

Characteristics, laboratory values, and clinical outcomes of the study groups

	ICSI (n = 250)	IMSI (n = 250)	P value
Woman's age (y)	35.1 ± 3.4	35.3 ± 3.2	.7865
Man's age (y)	37.6 ± 5.0	37.9 ± 4.9	.6984
Total gonadotropin dose (IU)	2,301 ± 452	2,425 ± 358	.6897
Total aspirated follicles	12.9 ± 7.4	13.3 ± 9.7	.3593
Total retrieved oocytes	8.5 ± 4.2	9.9 ± 6.7	.2876
No. of retrieved oocytes/no. follicles (%)	74.3	76.2	.3885
Metaphase II oocytes/total no. of retrieved oocytes (%)	78.1	79.2	.6748
Normal fertilization rate (%)	78.9	79.2	.6897
High-quality embryo rate (%)	37.3	44.4	.4456
Embryos transferred	2.5 ± 0.3	2.7 ± 0.2	.2705
Clinical pregnancy (%)	92/250 (36.8)	93/250 (37.2)	.5476
Implantation rate (%)	156/613 (25.4)	158/664 (23.8)	.6487
Miscarriage rate (%)	28/156 (17.9)	29/158 (18.4)	.5984

Note: IMSI = intracytoplasmic morphologically selected sperm injection.

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results demonstrated that 64.8% of spermatozoa, otherwise selectable for ICSI, were characterized by abnormalities after computer-assisted sperm analysis. These sperm also were characterized by an increase in the level of DNA fragmentation.

It is accepted generally that semen parameters and chromatin remodeling influence fertilization or early events after fertilization (17). It has been shown that sperm with protamine deficiency and increased histone remnants lead to premature chromatin condensation that is the cause of failures in fertilization and embryo development (18, 19). Therefore, we could hypothesize that the improved fertilization in intracytoplasmic morphologically selected sperm injection cycles performed in cases of male factor infertility and in the OAT-1999 group can be explained by the selection of a better spermatozoon under high magnification associated with an efficient sperm chromatin packaging.

Immature human sperm show diminished plasma membrane remodeling and zona-binding ability, increased rate of aneuploidies,

and increased rate of lipid peroxidation and consequential DNA fragmentation, and, collectively, these factors cause reduced fertilization rates and adversely affect the early and late paternal contributions to the zygote (20). Therefore, in the OAT-2010 group, which enrolled patients with a higher degree of semen parameter alteration as compared with the OAT-1999 group, it could be hypothesized that the selection of a morphologically normal spermatozoon under high magnification did overcome both early and late paternal effects, resulting in higher fertilization, implantation, and pregnancy rates.

In conclusion, the investigation of new reference values for semen parameters has proved that the previous guidelines had limitations and provided too-high cutoff points concerning sperm morphology, concentration, and motility. Our results suggest that the sperm selection under high magnification results in improved treatment outcomes in patients with OAT, according to the new WHO guidelines.

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