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ARTICLE

Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study

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Abstract Recent evidence shows that the selection of spermatozoa based on the analysis of morphology under high magnification (×6000) may have a positive impact on embryo development in cases with severe male factor infertility and/or previous implantation failures. The objective of this prospective randomized study was to compare the clinical outcome of 87 intracytoplasmic morphologically selected sperm injection (IMSI) cycles with 81 conventional intracytoplasmic sperm injection (ICSI) cycles in an unselected infertile population. IMSI did not provide a significant improvement in the clinical outcome compared with ICSI although there were trends for higher implantation (28.9% versus 19.5%), clinical pregnancy (54.0% versus 44.4%) and live birth rates (43.7% versus 38.3%) in the IMSI group. However, severe male factor patients benefited from the IMSI procedure as shown by significantly higher implantation rates compared with their counterparts in the ICSI group (29.6% versus 15.2%, $P = 0.01$). These results suggest that IMSI may improve IVF success rates in a selected group of patients with male factor infertility.

KEYWORDS: ICSI, IMSI, sperm morphology, sperm selection

Introduction

The recent introduction of morphologically normal spermatozoa selection under high magnification (×6000) necessitates a reconsideration of the intracytoplasmic sperm injection (ICSI) procedure that has been conventionally performed by selecting spermatozoa under ×200 optical magnification. It is not clear whether sperm morphology correlates with embryo quality and implantation since the outcome of ICSI does not seem to change provided that spermatozoa of normal morphology can be identified and utilized. Abnormal morphology of the sperm head and

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presence of nuclear vacuoles have been associated with inferior laboratory and clinical outcomes following ICSI procedures (Berkovitz et al., 2006a; Cassuto et al., 2009; Vanderzwalmen et al., 2008).

Analysis of fine nuclear morphology of the spermatozoa under high magnification allows abnormal sperm heads with nuclear vacuoles to be identified, which would not be possible with conventional magnification by Hoffman contrast (Bartoov et al., 2001, 2002, 2003; Berkovitz et al., 1999, 2005, 2006b; Garolla et al., 2008). Several case–control studies show that intracytoplasmic morphologically selected sperm injection (IMSI) has a positive impact on fertilization rates and embryo development (Cassuto et al., 2009; Franco et al., 2008). Studies to date show a beneficial effect in cases with severe male factor infertility or previously failed ICSI attempts (Antinori et al., 2008; Bartoov et al., 2002; Berkovitz et al., 2006a). However, it has not as yet been demonstrated whether sperm selection under high magnification when used in an unselected patient population positively impacts the clinical outcome of ICSI.

This study was designed to compare in a prospective randomized manner the clinical outcome of ICSI in an unselected infertile patient population where the spermatozoa were selected using either conventional or high magnification.

Materials and methods

The study group consisted of 168 ICSI cycles which were randomly divided into two groups according to a computer-generated randomization list: 87 cycles were assigned to a conventional magnification technique (ICSI) and 81 cycles to high magnification (IMSI). Approval was obtained from the Institutional Review Board of the American Hospital and all patients gave consent to the study.

Stimulation protocols, oocyte recovery and embryo-transfer techniques are given elsewhere (Balaban and Urman, 2005; Urman et al., 2003). Motile sperm organelle morphology examination (MSOME) was performed as previously described by Bartoov et al. (2002). The procedure was performed in real time using an inverted microscope (Olympus IX-71; Japan) with actual digitally enhanced magnification, as determined by a 0.01 mm Olympus objective micrometer, at ×6300. Only fresh ejaculated semen was used in this study. Semen samples were washed by a gradient technique with two or three layers (90%, 70%, 50%) of PureSperm suspensions, which were prepared from PureSperm 100 with PureSperm Buffer being used as the dilution media (cat nos. PSB-100 and PS 100–100, respectively; Nidacon International, Mölndal, Sweden). The suspension after the first gradient centrifugation (20 min, 250*g*) was rewashed (10 min, 500*g*) with a bicarbonate-buffered medium (G-IVF, 10135; Vitrolife, Gothenburg Sweden) supplemented with human serum albumin (HSA, 10064; Vitrolife). Preparation of the final sperm cell suspension for further MSOME and the ICSI procedure was performed as previously described (Bartoov et al., 2003; Berkovitz et al., 2005). Normal-shaped nuclei were defined as smooth, symmetric, having an oval configuration, with average length and width limits of 4.75 ± 0.28 and 3.28 ± 0.20 μm, respectively with a homogeneous nuclear chromatin mass, with no regional nuclear disorders and containing no more than one small vacuole with a borderline diameter of 0.78 ± 0.18 μm.

Embryo culture was performed as described previously (Balaban and Urman, 2005). Sequential media system (G5 series; Vitrolife) designed for prolonged embryonic development was used. Cleavage-stage embryos were graded as previously described (Balaban and Urman, 2005) and embryo transfer was performed on day 3.

Duration of the ICSI procedure, fertilization, embryo quality and clinical outcome parameters following cleavage-stage embryo transfer were compared between the two groups. Subgroup analyses were conducted according to the causes of infertility and the presence or absence of a male factor.

Statistics

Normal distribution of data was verified prior to selecting statistical tests. Numerical variables were analysed using paired Student's *t*-test. Categorical variables were analysed using chi-squared and Fisher's exact tests when applicable. Implantation rates were compared according to Mann Whitney *U*-test. Continuous data were compared using ANOVA or Kruskal–Wallis test where appropriate. A P-value <0.05 was accepted as significant.

Results

Characteristics of 168 treatment cycles are shown in **Table 1**. The ICSI and IMSI groups were similar in terms of parental age and causes of infertility. The majority of couples had male factor infertility in both ICSI and IMSI groups (48.1% and 43.7%, respectively). Cycle characteristics did not show any statistical difference between the groups.

Table 2 presents gamete cell characteristics in the ICSI and IMSI groups. There was no statistically significant difference between the two groups in terms of sperm parameters or oocyte characteristics (**Table 2**).

Comparison of laboratory and clinical outcomes between the two groups is presented in **Table 3**. The duration of the ICSI procedure was significantly longer in the IMSI group (13.55 versus 20.54 min, $P < 0.001$). Both groups were

Table 1 Study group characteristics.

Characteristic	*ICSI*	*IMSI*
No. of couples	81	87
Female age (years)	28.80 ± 4.08	29.76 ± 4.03
Male age (years)	32.53 ± 4.87	33.97 ± 5.52
Aetiology of infertility		
Male factor	39 (48.1)	38 (43.7)
Ovulatory	1 (1.2)	2 (2.3)
Tubal	10 (12.3)	7 (8.0)
Unexplained	24 (29.6)	30 (34.5)
Multiple factors	7 (8.6)	10 (11.5)

Values are mean ± SD or *n* (%).
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection.

Table 2 Sperm parameters and oocyte characteristics.

Characteristic	*ICSI*	*IMSI*
Sperm parameters		
Sperm count (million/ml)	41.96 ± 39.42	38.30 ± 34.38
Ejaculate volume (ml)	2.83 ± 1.18	2.64 ± 1.34
Motility (% total count)	41.35 ± 16.68	40.74 ± 17.22
Morphologically normal spermatozoa (% total count)	2.89 ± 1.68	2.89 ± 1.59
Spermatozoa with a vacuolar nucleus (%)	32.72 ± 16.81	34.88 ± 18.45
Oocyte characteristics		
No. of oocytes collected	12.30 ± 4.75	11.47 ± 3.96
No. of metaphase-II oocytes	9.25 ± 3.43	8.71 ± 2.95

Values are mean ± SD.
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection.

Table 3 Comparison of laboratory and clinical outcome measures for both groups.

Outcome	*ICSI*	*IMSI*	*P-value*
Duration of ICSI procedure (min)	13.55 ± 5.43	20.54 ± 9.43	<0.001
2-pronuclei fertilization rate (%)	80.97 ± 15.06	81.60 ± 10.65	NS
Embryos with 4 blastomeres on day 2 post fertilization (%)	34.70 ± 21.88	30.43 ± 16.23	NS
Embryos with 8 blastomeres on day 3 post fertilization (%)	31.65 ± 17.21	33.61 ± 16.34	NS
Grade 1 and 2 embryos on transfer day (%)	4.84 (63.95)	5.01 (66.44)	NS
Mean no. of embryos transferred[a]	2.76 ± 0.46	2.72 ± 0.48	NS
Clinical pregnancy per initiated cycle (%)	36/81 (44.4)	47/87 (54.0)	NS
Live birth rate per initiated cycle (%)	31/81 (38.3)	38/87 (43.7)	NS
Implantation rate (%)	42/215 (19.5)	66/228 (28.9)	NS
Multiple pregnancy rate (%)	6/36 (16.7)	16/47 (34.0)	<0.001

Values are mean ± SD or *n* (%).
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection; NS = not significant.
[a]Six patients (three in each group) who did not undergo embryo transfer due to total fertilization failure (one in IMSI) or pending ovarian hyperstimulation syndrome (two in IMSI, three in conventional ICSI groups) are excluded from this analysis.

comparable in terms of fertilization rates, the number and quality of embryos transferred as well as clinical outcome parameters. Mean numbers of embryos transferred were 2.76 and 2.72 for ICSI and IMSI groups, respectively. Implantation and clinical pregnancy rates were slightly higher in the IMSI group compared with the ICSI group (28.9% versus 19.5% and 54.0% versus 44.4%, respectively; **Table 3**). There was also a trend for a higher live birth rate in the IMSI group compared with the ICSI group (43.7% versus 38.3%, $P = 0.1$). The multiple pregnancy rate was significantly higher in the IMSI group (34.0% versus 16.7%; $P < 0.001$).

When subgroup analyses were conducted, male factor infertility patients were seen to benefit more from the IMSI procedure as shown by significantly higher implantation rates compared with their counterparts in the ICSI group (29.6% versus 15.2%, $P = 0.01$; **Table 4**). There was also an improvement in the live birth rate (36.8% versus 28.2%); however, the difference did not reach statistical significance. The improvement in implantation rate was more prominent in patients who had sperm concentrations lower than 1 million/ml in the basal ejaculate.

Discussion

IMSI is a promising new technique that may help to improve laboratory and clinical performance in assisted reproduction. Despite the presence of detailed information regarding the effect of oocyte morphology on the clinical outcome of ICSI cycles, only limited data is available regarding the effect of sperm morphology (Balaban and Urman, 2006). Berkovitz et al. (1999) were the first to propose that selection of spermatozoa according to its ultrastructural morphology could improve the outcome of ICSI. The same group reported the real-time examination of the fine morphology of motile spermatozoa with an inverted light microscope equipped with high-power differential interference contrast optics (magnification ×150) enhanced by digital imaging (magnification ×44) to achieve a total magnification of over ×6000 (Bartoov et al., 2001). This method, known as MSOME, was able to identify motile spermatozoa with a normal nucleus and nuclear content and provided a positive impact on clinical results (Bartoov et al., 2002).

Table 4 Comparison of clinical pregnancy and implantation rates for ICSI and IMSI according to the presence and severity of male factor infertility.

	Live birth per initiated cycle (%)			*Implantation rate (%)*		
	ICSI	*IMSI*	*P-value*	*ICSI*	*IMSI*	*P-value*
No male factor	20/42 (47.6)	24/49 (49.0)	NS	26/110 (23.6)	34/120 (28.3)	NS
Male factor	11/39 (28.2)	14/38 (36.8)	NS	16/105 (15.2)	32/108 (29.6)	0.01
Sperm count						
<1 million/ml	4/16 (25.0)	4/11 (36.4)	NS	7/43 (16.3)	11/31 (35.5)	NS
1–20 million/ml	7/22 (31.8)	10/27 (37.0)	NS	9/59 (15.3)	21/77 (27.3)	NS

Values are *n*/total (%).
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection; NS = not significant.

Several reports supported this hypothesis suggesting that the morphological quality of spermatozoa used for ICSI plays an important role in fertilization, implantation and pregnancy (Chemes and Rawe, 2003; De Vos et al., 2003; Tesarik, 2005). Then, Berkovitz et al. (2005) introduced IMSI to infertility practice showing an increase in implantation (25% versus 5.9%) and pregnancy rates (20% versus 7%) over conventional ICSI for patients with previous failed ICSI attempts. Hazout et al. (2006) reported a case series of 125 patients with previous implantation failures. Laboratory and clinical performance of IMSI was compared with the previously failed ICSI cycles of the same patient. The authors suggested a favourable clinical outcome following IMSI despite comparable fertilization rates, embryo cleavage and quality.

Berkovitz et al. (2006a) reported a significant difference in clinical outcome parameters for IMSI over conventional ICSI using the 'best ultrastructural-morphology-sperm' and the 'second best' one. The presence of nuclear vacuoles in the injected spermatozoa was reported to be the most important morphological feature that was associated with the clinical results (Berkovitz et al., 2006b).

Vanderzwalmen et al. (2008) showed that, in 25 patients where sibling oocytes were injected with different grades of spermatozoa which had been graded under high magnification according to the extent and size of nuclear vacuoles, cleavage-stage embryo quality did not differ but blastocyst formation and quality were closely associated with the grade of the spermatozoa used. While more than half of embryos derived from injection of oocytes using spermatozoa with no or few small vacuoles developed into blastocysts, almost no blastocysts were obtained when spermatozoa with large vacuoles were used.

In a retrospective laboratory study, Cassuto et al. (2009) reported significant differences in fertilization rate as well as blastocyst development and expansion between embryos derived from the injection of spermatozoa scored according to their high-magnification characteristics.

In the only prospective clinical trial on this subject, which was reported from Italy where the number of oocytes fertilized and embryos transferred were limited and predefined, the authors were able to demonstrate higher pregnancy and implantation rates in severe male factor infertility cases following IMSI (Antinori et al., 2008). IMSI resulted in significantly higher implantation (17.3% versus 11.3%, $P = 0.007$) and clinical pregnancy rates (39.2% versus 26.5%, $P = 0.004$) compared with conventional ICSI. Clinical benefit was more significant in patients with two or more previously failed treatment attempts.

Studies to date have shown comparable laboratory and clinical performances for IMSI and ICSI from fertilization to cleavage-stage embryo development. However, blastocyst development and quality were reported to be better for IMSI-derived embryos. This can be explained by the effect of paternal genomic activation after day 3 of cleavage, which is reflected in blastocyst culture (Borini et al., 2006; Menezo, 2006; Tesarik, 2005).

This study showed that IMSI and conventional ICSI procedures provide comparable laboratory and clinical results when used in an unselected infertile population. However, subgroup analysis demonstrated that severe male factor patients significantly benefit from IMSI. At the present time, morphology is the major tool for the selection of spermatozoa for intracytoplasmic injection. None of the enzymatic or genetic tests can be performed on a spermatozoon that is to be injected into the oocyte. Since analysis has to rely basically on morphology, higher magnification may be theoretically beneficial as a selection tool. This is particularly true when the number of morphologically normal spermatozoa at conventional magnification (×200) is limited or absent.

It has been demonstrated that the injection of spermatozoa selected for their ability to bind to hyaluronic acid (Parmegiani et al., 2010a,b) or zona pellucida (Braga et al., 2009) may improve the clinical outcome in ICSI cycles. Spermatozoa bound to hyaluronic acid were shown to have significantly lower rates of DNA fragmentation and nuclear abnormalities compared with randomly selected and polyvinylpyrrolidone-treated spermatozoa (Parmegiani et al., 2010a,b). The authors suggest that hyaluronic acid binding may also help to speed up the time-consuming IMSI procedure by selecting a subpopulation of spermatozoa with a normal nucleus.

This data should be contemplated with further studies before the widespread adoption of this technique into routine laboratory practice. It is suggested that IMSI may improve IVF success rates in severe male factor infertility patients. Although untested in this study, couples with a history of failed treatment cycles may also benefit from this procedure.

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