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Assessment of ICSI Vs IMSI Outcome

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Abstract: Rational sperm morphology assessment plays a pivotal role in the diagnosis of male fertility potential and it has considered a predictive value for fertilization and pregnancy outcomes in IVF treatments. Objective to evaluate whether intracytoplasmic morphologically selected sperm injection (IMSI) could influence early paternal effects by observing embryo quality and pregnancy rate. *Subjects and Methods:* A total of 255 couples with at least two previous diagnoses of severe oligo-astheno-teratozoospermia, 3 years of primary infertility, the woman aged 30 years or younger and an undetected female factor were randomized to IVF micro-insemination treatments: ICSI (n = 120; group 1) and IMSI (n = 135; group 2). A comparison between the two different techniques was made in terms of pregnancy, miscarriage and implantation rates. *Results*: Showed that IMSI resulted in a higher clinical pregnancy rate of 61.4% versus 45.0 in ICSI when applied to severe male infertility cases. Despite their initial poor reproductive prognosis, patients with two or more previous failed attempts benefited the most from IMSI in terms of pregnancy (19.2% versus 12.2%) and decrease in miscarriage rates in IMSI group (16.8% versus 25.9%). *Conclusion*: IMSI is significantly more beneficial than ICSI on all patients with severe oligo-astheno-teratozoospermia, regardless of the number of previous IVF failures. Thus, the authors recommended that IMSI could be used as a routine IVF technique to solve complicated male infertility cases from their first attempt.

Key words: IMSI · ICSI · Male Infertility · Implantation · Pregnancy · Miscarriage

INTRODUCTION

Sperm selection is a valuable factor that could help in solving of the problem of male infertility; however, it has been estimated that approximately 40% of infertility in couples can be attributed to male subfertility. Sperm morphology has been recognized as the best predictor of outcome for natural fertilization [1, 2]. In last decade, several authors have suggested that sperm morphology also plays a significant role in ICSI outcome. Recently, several studies demonstrated that fine morphological integrity of human sperm nuclei is positively associated with fertilization and pregnancy rates following IVF-ICSI, these results were obtained by the application of a new method of unstained, Real-time and high magnification motile sperm organelle morphology examination (MSOME) to the left over sperm fraction selected for micro injection in 100 random couples referred for ICSI [3-8]. The modified IVF procedure—intracytoplasmic morphologically selected sperm injection (IMSI)—based on microinjection into retrieved oocytes of selected spermatozoa with strictly defined morphologically normal nuclei. The modified IMSI treatment results in significantly higher pregnancy rates, compared with conventional IVF-ICSI, the modified IVF procedure (IMSI) method adapted some minor modifications in sperm

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preparation which were not conducted in the conventional IVF-ICSI process, i.e. use of a density gradient (Sill Select) in the routine preparation prior to selection, use of polyvinylpyrrolidine (PVP), low temperature and a glass-bottomed dish during selection, prolonged sperm manipulation post separation from the seminal fluid and sperm storage prior to micro injection [3, 5]. Several authors have shown how the correct selection of most normal- looking spermatozoon available under high magnification improves intracytoplasmic sperm injection outcome [9-11]. It also, resulted in a positive and significant incidence of morphologically normal spermatozoa and fertilization rate following ICSI. The morphologically integrated sperm should be with six sub cellular organelles examined (Neck, tail, mitochondria, acrosome, post-acrosomal lamina, nucleus), the morphological normalcy of the sperm nucleus (shape, chromatin content) was significantly and positively associated with both fertilization rate and pregnancy outcome [3]. In 2003, Bartoov used IMSI criteria to select single motile spermatozoa for ICSI in patients with at least two previous ICSI failures. The patients who underwent IMSI obtained significantly better results in terms of pregnancy and miscarriage rates [5]. Later, based on same IMSI criteria, Hazout [12] applied high magnification techniques (×6000) to the sperm selection process for the ICSI procedure, showing a significant improvement in clinical IVF outcome in patients with previous failed ICSI attempts. These encouraging results offer a new perspective for future improvements in assisted reproduction techniques. The main purpose of this study was to shed more light and assess the potential advantages of the IMSI procedure in the treatment of patients with severe oligoasthenoteratozoospermia regardless of their previous failed ICSI attempts.

MATERIALS AND METHODS

Patient Selection: A total of 255 cycles of 255 couples enrolled in the ICSI program in Centre for Human Reproduction Dr. Faris Medical Center were admitted to this study. Inclusion criteria were (i) male factor infertility; (ii) at least 2 previous failures of implantation or previous miscarriages after ICSI; (iii) the woman being 35 years or younger; (iv) The male factor infertility was defined according to WHO/Kruger criteria for sperm concentration, motility and morphology, which have previously been tested. If at least two of these three parameters were abnormal, the couple was considered for ICSI treatment. Written consent was taken from all the patients and the study was performed according to the norms of the Institutional Ethics Committee.

All study participants were blinded to treatment assignment for the duration of the study. The following groups were formed: ICSI (n = 120; group 1) and IMSI (n = 135; group 2).

After this randomization, based on the number of previous failed ICSI attempts, three subgroups were identified for each technique: group A, no previous attempts; group B, one previous failed attempt; group C, two or more previous failed attempts. Hence, patients were divided as follows: subgroup 1A (n = 20), subgroup 1B (n = 42), subgroup 1C (n = 58), subgroup 2A (n = 48), subgroup 2B (n = 48), subgroup 2C (n = 39).

Ovarian Stimulation, Oocyte Recovery and Culture Protocol: All patients were submitted to the same scheme of controlled ovarian stimulation. After pituitary down-regulation with **Decapeptyl (Triptorelin) - 0.1mg**/day (United Pharmacies-UK) started in the mid-luteal phase, recombinant human FSH (r-FSH/Gonal F1; Serono, SP, Brazil) was administered at a starting dose of 150–300 IU, depending on the age of the patient and recombinant LH (r-LH/Luveris1, Serono, SP, Brazil) was administered at a dose of 75 IU/day for a period of 7 days. On day 8 of stimulation, follicular development was monitored by 7 MHz transvaginal ultrasound only (Medison Digital Color MT, Medison Co. Ltd, Seoul, Korea) and the FSH dose was adapted according to ovarian response. The r-LH supplementation was increased to150 IU/day when one or more follicles measuring 10 mm in diameter were found. When at least three follicles measuring 17 mm in diameter were observed, recombinant chorionic gonadotropin (HCG/Ovidrel1 250 mg Serono, SP, Brazil) was administered. Transvaginal, ultrasound-guided oocyte retrieval was performed 36 h after HCG.

Semen Preparation: Only freshly ejaculated semen was used for this study.

Semen samples were collected in sterile containers by masturbation after a sexual abstinence period of 2 to 5 days. The liquefied fresh semen samples were prepared by Isolate Earl's salt solution NaHCo3 (Biochrom-Germany) discontinuous concentration gradient. The final pellet was resuspended in 0.2 ml modified HTF medium (Milieu BM1) supplemented with 10% HSA (Eurobio, France). Parts of each semen sample were immediately taken for ICSI and IMSI procedures. The remainder of the semen sample was analysed for standard semen quality parameters according to the World Health Organization.

ICSI Procedure: Conventional ICSI was performed utilizing a Leica inverted microscope DMI 3000 B (Germany) equipped with Eppendorf transfer man NK 2 micromanipulator (Germany). One manipulator is used for moving the holding capacity and a second for transferring the sperm. The oocyte is held in place using the manual injector CellTram Air. Sperm transfer is carried out using the hydraulic manual injector Cell Tram vario. Spermatozoa were selected at 400 magnification using Hoffman modulation contrast according to a set of previously published guidelines [13].

IMSI Procedure: One ml aliquot of sperm cell suspension was transferred to a 5 ml microdroplet of modified HTF medium containing 10% polyvinyl pyrrolidine solution containing 5% human serum albumin (PVP medium Irvine Scientific, USA). This microdroplet was placed in a sterile glass dish (FluoroDish wellcon BV Netherland-Amestrdam) under sterile paraffin oil (Light mineral oil, Irvine USA).The sperm cells, suspended in the micro droplet, were placed on a microscope stage above an Uplan Apo 100 oil/1.35 objective lens previously covered by a droplet of immersion oil. In this manner, suspended motile sperm cells in the observation droplet could be examined at high magnification. The spermatozoa used for IMSI were classified in to 5 groups. Grade I consisted of spermatozoa free of any morphological abnormality (Normal spermatozoa). A spermatozoon was classified as morphologically normal when it exhibited a normal Nucleus as well as acrosome, post-acrosomal lamina, neck, tail and mitochondria, besides not presenting a cytoplasmic droplet or cytoplasm around the head. For the nucleus, the morphological state was defined by the form and content of the chromatin. The criterion for normality of nuclear form was a smooth, symmetric and oval configuration. Normal means for length and width were estimated the nuclear form was considered abnormal if extrusion or invagination of the nuclear chromatin mass has been detected (regional malformation of nuclear form). Chromatin content was considered abnormal if one or more vacuoles were observed to occupy more than 4% of the nuclear area. A nucleus was considered normal if both nuclear form and chromatin content were normal. When no Grade I spermatozoa were available or the number was insufficient for injection, spermatozoa were classified by head forms as Grade II: large oval: (5.31 mm), small oval (4.19 mm), wide (>3.7 mm width) or narrow (<2.9 mm width); Grade III: presence of regional disorders; Grade IV: presence of large vacuoles on 5–50% of head surface and Grade V: presence of large vacuoles on >50% of head surface. The same technician, blinded to subject identity, performed all sperm selection. The time involved in the selection step was 30 min/sample. At least five spermatozoids were selected for each MII oocyte. After the sperm selection, the microinjections were carried out in the same manner as in ICSI. Spermatozoids were still motile when captured for final selection.

Oocyte and Embryo Culture, Embryo Grading and Transfer Sperm Injected Oocytes, Zygotes and Embryos from IMSI and ICSI Were Submitted to the Same Culture Conditions: Oocytes were examined after 17–20 h to assess fertilization; zygotes with two distinct equal-sized pronuclei were considered normal. 25 to 27 h after injection, on day 1 of culture, early cleavage was evaluated [14]. Embryos, graded on day 2 or day 3 or day 5 according to the day of embryos transferres, on day 2 were deem top quality if there were four identical blastomeres (44 h after the sperm injection) with no fragments or multinucleation [15, 16]. On day 3 good quality eight cells and compacting blastomers (68 h after the sperm injection) with no fragments or degenertaed blastomeres, on day 5 embryos are assisted using several markers to the blastocyst scoring criteria (have cavitated or expanded, have distinct inner cell mass, a well laid down trophoectoderm with sickle-shaped cell, thin zona pellucida and high total cell number) good quality late cavitating embryos and blastocsyt (early expanded).

The same technique performed to all oocyte/embryo evaluations, the best scoring embryos were transferred to the patient's uterus, independent of origin (ICSI or IMSI).

Statistical Analysis and Sample Size: Student's *t*-test was used to compare continuous variables, whereas a chi-squared test was applied to discrete variables. $P < 0.05$ was considered statistically significant.

RESULTS and DISCUSSION

According to some authors, ICSI outcome is not related to strict morphology of the spermatozoon used for microinjection [17-20]. No differences in terms of fertilization and clinical pregnancy rates have been shown when samples with poor morphology (<5% normal cells) were used as if the only requirement for achieving satisfactory results were the presence of a living, motile spermatozoon [21].

Furthermore, fertilization, embryo development and pregnancy seem to be achievable even if normal spermatozoa are not available (100% of terato-zoospermia) [22-24]. On the other hand, some trials have shown low

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

		ICSI n=120	IMSI n=135
Mean Age	Female	30.03±5.5	30.62±4.5
	Male	36.84±8.09	35.99±6.29
Type Of Infertility	Primary	102±0.35	115±0.36
	Secondary	18±0.35	20±0.36
Number of Previous	0	20±1.19	48±1.47
IVF	1	42±1.19	47±1.47
Failure	≥2	58±1.19	40±1.47
Number of cumulus recovery		12.63±7.73	14.14±6.85
Number of injected oocytes (MII)		10.5±6.91	11.05±6.13
Number of fertilized 2PN Zygotes		8.89±6.08	9.66±5.77
Number of top embryos		4.22±2.16	5.44±3.51
Number of embryos transferred/patients		3.141±0.91	3.22±0.91
Clinical Pregnancy%		54/120 (45)	83/135 (61.4)
Implantation%		41/335 (12.2)	75/389 (19.2)
Miscarriage%		14/54 (25.9)	14/83 (16.8)

MII = metaphase II; PN = pronucleate. Continuous variables are presented as means±SD.

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

Subgroups	Rate	Group ICSI	Group IMSI
Subgroup A	Pregnancy	13/20 (65%)	35/48 (72.9%)
(0 IVF Failure)	Miscarriage	1/13 (7.6%)	6/35 (17.1%)
Subgroup B	Pregnancy	19/42 (45.2%)	32/48 (66.6%)
(1 IVF Failure)	Miscarriage	8/19 (42.1%)	7/32 (21.8%)
Subgroup C	Pregnancy	22/58 (37.9%)	15/39 (38.4%)
(≥ 2 IVF Failure)	Miscarriage	4/22 (18.1%)	1/15 (6.6%)

Table 3: Comparison of pregnancy and miscarriage rates arising from intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) sub-groups with a different number of previous IVF failures.

	Subgroup A (0 IVF Failure)	Subgroup B (1 IVF Failure)	Subgroup C (≥2 IVF Failure)
Pregnancy Rate	48/68 (70.5%)	51/90 (56.6%)	37/97 (38.1%)
Miscarriage Rate	7/48 (14.5%)	15/51 (29.4%)	5/37 (13.5%)

fertilization, pregnancy and implantation rates when spermatozoa with severe anomalies were used for ICSI [9, 11].

Later studies reported new opportunities to treat severe male infertility by using micro-insemination procedures made under high magnification systems [5, 25]. Following injection of spermatozoa without nuclear alterations into oocytes from couples with at least two previous ICSI failures and an undetected female infertility factor, the pregnancy rate doubled and the miscarriage rate was reduced by 50% against similar cases treated by conventional ICSI [25-27].

In order to assess these results and investigate future applications of this method, a prospective randomized trial was designed that involved patients with severe oligoasthenoteratozoospermia regardless of previous ICSI failures. Based on a first overview of all 446 treated cases, a poor reproductive prognosis could be assigned to the subgroups with two or more failures having a pregnancy rate of 38.1%, which was significantly lower ($P = 0.005$) than 70.5% and 56.6% scored by patients with none and one previous failure, respectively (Table 3) as shown by other authors [28, 29].

As far as the reproductive outcome is concerned, IMSI resulted in a significantly higher pregnancy rate than ICSI in all treated cases ($P = 0.004$) and notably in patients with two or more failures for whom the success rate increased by over 100% ($P = 0.017$), which confirms the data already published in the literature [5, 25-27].

It is likely that in those couples the male factor could be the result of semen impairment, undetected by conventional diagnostic tools, thus reducing the effectiveness of previous ICSI treatments (Figures 1).

The above comparisons did not show any statistical difference in terms of miscarriages but the clinical trend seems to support the IMSI method with a remarkable 50% reduction in the miscarriage rate in sub-group 2C versus subgroup 1C (6.6% and 18.1%, respectively). In this respect, it should be added that at the time of writing, about half of ICSI and IMSI pregnancies are still ongoing; furthermore, the comparison between IMSI and ICSI in the three subgroups was clearly affected by the number of cases, since subgroup splitting reduced the sample size, preventing statistical significance from being reached. Thus, the better results in terms of pregnancy and miscarriage rates following IMSI in couples with no or one previous IVF failure are of interest, although further investigations are needed to enlarge the sample size.

In conclusion, this paper is so far the only prospective randomized study showing that IMSI is significantly more beneficial than ICSI on all patients with severe oligoasthenoteratozoospermia, regardless of the number of previous IVF failures. Those cases with two or more failed attempts, despite their poor reproductive prognosis, seem to benefit with statistically significant doubling of the pregnancy rate ($P = 0.017$) and remarkable halving of the miscarriage rate. It is the opinion of the authors that in the near future, after adequate standardization of the method in order to gain insight into

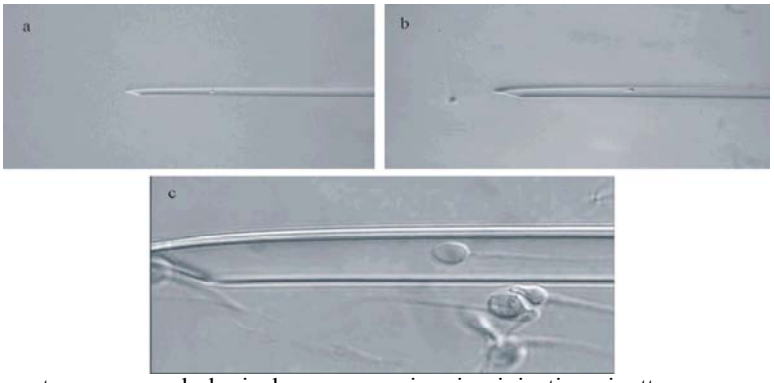

Fig. 1: A human spermatozoon: morphological appearance in microinjection pipette
(a) ×200 undetectable,
(b) ×400 undetectable,
(c) ×5880 normal morphology.

the selection criteria and overcome some of the above practical difficulties, IMSI could be recommended as a routine IVF technique to solve complicated male infertility cases from their first attempt.

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