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GAMETE BIOLOGY

Intracytoplasmic morphologically selected sperm injection (IMSI) does not improve outcome in patients with two successive IVF-ICSI failures

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Abstract
Purpose Assessment of sperm morphology has been reconsidered since 2001 with the development of motile sperm organelle morphology examination (MSOME). This observation technique that combines high magnification microscopy and the Nomarski interference contrast makes it possible to select spermatozoa with as few vacuoles as possible before microinjection into the oocyte (intracytoplasmic morphologically selected sperm injection, IMSI). More than 10 years after the development of IMSI, the indications of the IMSI technique and its ability to increase pregnancy and/or birthrates (compared with conventional ICSI) are still subject to debate. We aimed to better define the interest of IMSI in the third attempt.
Methods We assessed the benefit of IMSI by carrying out a retrospective comparative study between IMSI and conventional ICSI during a third ART attempt. Two hundred sixteen couples with two previous ICSI failures were studied between February 2010 and June 2014.
Results IMSI did not significantly improve the clinical outcomes compared with ICSI, either for implantation (12 vs 10 %), clinical pregnancy (23 vs 21 %), or live birth rates (20 vs 19 %).
Conclusion This study provides supplementary arguments for not achieving IMSI procedure in the third attempt after two previous ICSI failures.

Keywords IMSI · ICSI failure · Vacuoles · MSOME · Sperm selection

Capsule This study provides supplementary arguments for not achieving IMSI procedure in the third attempt after two previous ICSI failures.

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Introduction

Over the last decade, higher resolution microscopy techniques have led to improved sperm observation. Since 2001 [1], a new technique called motile sperm organelle morphology examination (MSOME) involves the use of the Nomarski interference contrast microscopy at high magnification (at least ×6000). This technique revealed a new morphological criterion in human spermatozoa: the presence of nuclear vacuoles. Several studies have found increased levels of fragmented DNA in spermatozoa with large vacuoles [2–5], whereas others [6, 7] have shown abnormalities of chromatin condensation in such spermatozoa. Although the origin of these vacuoles raises many questions, some strong correlations have been established between the morphology of the spermatozoon, in particular the presence of large vacuoles, and its nuclear quality (degree of chromatin condensation and/or DNA integrity, chromosomal content) [8]. However, the high frequency of sperm head vacuoles in fertile men highlights the physiological nature of some of these vacuoles [9, 10]. The observation of sperm head vacuoles is used to select spermatozoa before microinjection: intracytoplasmic morphologically selected sperm injection (IMSI). A decade after the introduction of IMSI, the indications of the technique and its ability to increase pregnancy and/or birthrates (compared with conventional ICSI) are still subject to debate. A recent meta-analysis found no evidence of the value of IMSI in terms of either live

birth or miscarriage rates [11], and so these findings did not support the use of IMSI in clinical practice. According to these authors, the use of IMSI for sperm selection was associated with an improvement in the clinical pregnancy rate. However, in view of the risk of bias in the studies included, imprecision and strong suspicion of publication bias, the authors concluded that this evidence was of low quality and therefore the estimated benefit was very uncertain [11]. Initial reports showed that IMSI was associated with higher pregnancy rates in couples with repeated ICSI failures [12, 13]. In the first prospective randomized trial [14], the clinical benefit was more significant in patients with two or more previous failed treatment attempts: IMSI resulted in significantly higher clinical pregnancy rates (29.9 vs 12.9 %; $P<0.05$). If large vacuoles are linked to chromatin condensation defects, as has been demonstrated by many authors [6, 7, 15], more rigorous selection of the sperm cell (as few vacuoles as possible) could improve the quality of the sperm injected into the oocyte. In a previous randomized controlled trial, we reported that IMSI yielded no benefit during the first assisted reproductive technique (ART) attempt. However, we postulated that after two ICSI failures, the proportion of cases with a poor prognosis linked to sperm nuclear damage might be higher, and so we aimed to assess the value of IMSI in such cases. This retrospective study of 216 ART cycles aimed to provide supplementary data to better define the true indications of IMSI.

Materials and methods

Study design

This retrospective study included couples making their third ART attempt (after two previous ICSI failures) in the ART center of Hôpital Paule de Viguier, Toulouse University Hospital, France, between February 2010 and June 2014.

The initial inclusion criteria for the first ICSI attempt were the following:

1. Male infertility with use of fresh ejaculated spermatozoa, whatever the sperm morphology and whatever the female status.
2. Total failure to achieve fertilization with conventional IVF.

Only one microscope in our laboratory is equipped with an IMSI system (Nomarski interference contrast, total magnification ×6000) and only two of the five embryologists are trained in the IMSI technique. Patients undergoing a third attempt were therefore included in the IMSI group or in the ICSI group depending on the availability of the IMSI microscope and of a trained operator. The fertilization, implantation, and lysis rates of all the embryologists performing the ICSI technique are regularly assessed in the laboratory (every 2 months for fertilization and lysis rates). We found no significant difference in these rates throughout the duration of our study.

Conventional sperm parameter assessment before an ART attempt

Semen evaluation was performed according to standard WHO guidelines [16]. Sperm morphology was examined using the Kruger criteria [17]. Smears were air-dried for 10 min and then stained using the Diff-Quick procedure (Dade, Düdingen, Switzerland). An HTM-IVOS analyzer version 12.3 (Hamilton-Thorne Biosciences, Beverly, MA, USA) was used. The spermatozoon was identified and then analyzed by computerized software according to strict criteria [17, 18].

Semen preparation

Semen samples were collected by masturbation after 2–5 days of sexual abstinence and were processed for IVF after liquefaction for 15 to 60 min.

IMSI procedure

Preparation and selection of sperm for IMSI have previously been described [19]. An aliquot of the sperm preparation was placed in a glass-bottomed dish (WillCo-dish, WillCo Wells BV, Amsterdam, The Netherlands) and examined by the Nomarski interference contrast microscopy with a Leica DFC-280 camera (Leica Microsystems, Nanterre, France) mounted on a Leica DMI 6000 microscope with an immersion objective lens ×100 and camera magnification ×1. Spermatozoa with the smallest relative vacuole area were preferentially selected [6]. If available, spermatozoa without vacuoles or with only small vacuoles were preferentially injected.

Conventional ICSI procedure

Sperm selection for microinjection was performed at a magnification of ×400. Spermatozoa seen to have severe head defects at this magnification were excluded. The procedure of oocyte injection was the same in both the ICSI and the IMSI group and was performed at ×200 magnification using the Hoffman contrast.

Embryo culture

The injected oocytes were transferred to a four-well dish containing 50 μL of culture medium (G1Plus, Vitrolife, Göteborg, Sweden) overlaid with mineral oil (FertiCult Mineral Oil, Fertipro Belgium). The fertilization of the oocytes was checked the next day, 16–20 h after microinjection. Embryo quality was assessed on day 2 according to the Giorgetti

classification system [20]. Embryos with a score of 3 or 4 have good morphology.

Statistical analysis

Implantation rate was defined as the ratio of the number of gestational sacs with fetal heart beat to the number of transferred embryos. Statistical analysis was performed using StatView software (Abacus Concepts Inc., Berkeley, CA). Percentages were compared by the chi-square test. Means were compared using the Student's *t* test or the Mann–Whitney test according to the normality of data distribution. A *P* value lower than 0.05 was considered as statistically significant.

Results

Patient characteristics were similar in both groups in terms of female and male age, duration of infertility, and semen parameters (Table 1). Cycle parameters differed, with a greater number of metaphase II oocytes being retrieved in the IMSI group. As shown in Table 2, no statistically significant difference was observed between the two groups with regard to implantation rate (IMSI 12 %, ICSI 10 %, NS), ongoing pregnancy (IMSI 23 %, ICSI 21 %, NS), or delivery rate (IMSI 20 %, ICSI 19 %, NS). However, the fertilization rate was significantly lower in the IMSI group (54 ± 24 vs. 61 ± 26, $P < 0.05$) with no difference in the number of total embryos obtained, since there was a significantly higher number of mature oocytes in the IMSI group (IMSI 8.1 ± 3.6 %, ICSI 6.9 ± 3.1, $P < 0.01$). We assumed that there was no operator bias because the fertilization rate between the various operators (data not shown) did not differ significantly (see Materials and methods).

The data of the previous ICSI attempts in the two groups are summarized in Table 3. Except for the total number of mature oocytes, which was significantly higher in the IMSI group (15.1 ± 6.2 vs 13.4 ± 5.0, $P < 0.05$), we found no other statistically significant differences between the two groups.

Discussion

Although we are well aware of the limitations of our design (non-randomized trial), our study showed that the IMSI technique provided no real benefit over conventional ICSI in the case of a third ART attempt. We found no significant differences regarding live births (19 % with ICSI, 20 % with IMSI). Similarly, no statistical difference was demonstrated for implantation, miscarriage, or clinical pregnancy rate. It should be noted that previous studies evaluating IMSI differed significantly in terms of sperm classification and definition of normal spermatozoa. For some authors, it appeared clear that IMSI was not better than ICSI when used in the first treatment attempt [19, 21, 22]. However, in one randomized study, the benefit of IMSI was especially marked after at least two ICSI failures [14]. This first randomized controlled trial, comparing 227 IMSI attempts with 219 ICSI attempts (at an unspecified magnification) revealed a significantly higher clinical pregnancy rate in the IMSI than in the ICSI group (39 vs 27 %, $P = 0.004$) and also in the group with two previous ICSI failures (29.9 % in the IMSI vs 12.9 % in the ICSI group,

Table 1 Demographic and clinical data of the study population

	ICSI group	IMSI group	Statistical comparison
Number of cycles	127	89	
Female age	35.2 ± 3.7	34.8 ± 4.0	NS
Male age	37.9 ± 5.3	38.4 ± 5.5	NS
Infertility duration (months)	64 ± 27	65 ± 27	NS
Sperm parameters			
Volume (mL)	3.7 ± 1.7	3.5 ± 1.5	NS
Sperm count (10^6/mL)	35.5 ± 64.2	42.7 ± 73.4	NS
Progressive motility (%)	22 ± 15	26 ± 17	NS
Vitality (%)	64 ± 16	67 ± 17	NS
Normal morphology (Kruger) (%)	7 ± 6	6 ± 5	NS
Number of motile spermatozoa ($\times 10^6$) recovered after preparation	4.3 ± 9.0	8.9 ± 27.3	NS
	Median 0.92	Median 1.46	
	Range [0.002–72]	Range [0.005–76]	

Values are expressed as mean +/− SD. $P < 0.05$ is considered significant

ICSI intracytoplasmic sperm injection, *IMSI* intracytoplasmic morphologically selected sperm injection, *NS* not significant

Table 2 Results of ICSI and IMSI cycles after two previous ICSI failures

	ICSI group	IMSI group	Statistical comparison
Number of cycles	127	89	
Ovarian stimulation protocol			
Long agonist	31 (24 %)	22 (25 %)	NS
Antagonist	96 (76 %)	67 (75 %)	
Total injected FSH units	2085 ± 1021	2010 ± 833	NS
No. follicles ≥15 mm (at last US monitoring)	7.3 ± 2.5	7.5 ± 2.9	NS
No. metaphase II ovocytes	6.9 ± 3.1	8.1 ± 3.6	$P < 0.01$
Fertilization rate (%)	61 ± 26	54 ± 24	$P < 0.05$
No. embryos obtained	4.3 ± 2.6	4.5 ± 2.8	NS
% of good morphology embryos (score 3 and 4, Giorgetti classification)	32 ± 30	36 ± 35	NS
No. embryo transfers	119 (94 %)	86 (97 %)	NS
No. transferred embryos	2.3 ± 0.8	2.3 ± 0.8	NS
Clinical pregnancy rate per oocyte retrieval	28 % (35/119)	27 % (24/89)	NS
Implantation rate	10 % (28/270)	12 % (23/194)	NS
Ongoing pregnancy rate	21 % (25/119)	23 % (20/86)	NS
Delivery rate per embryo transfer	19 % (23/119)	20 % (17/86)	NS
Cycles with frozen embryo (% per transfer)	19 % (23/119)	22 % (19/86)	NS
Number of frozen embryos per freezing	2.3 ± 1.0	1.9 ± 0.7	NS

$P = 0.017$). The advantage of IMSI after repeated IVF-ICSI failure has been investigated in several studies (Table 4). Our results are in agreement with a prospective but non-randomized study which compared IMSI and ICSI outcomes in patients with more than two ICSI failures [25]. These authors found no statistical difference in terms of clinical pregnancy rate and live birth rate per cycle, although it must be borne in mind that the mean number of previous ICSI attempts was significantly greater in the IMSI group than in the ICSI group.

As in a previous study [19], the fertilization rate obtained using the IMSI technique was significantly lower than with conventional ICSI. Even if we checked that this result was not explained by any difference in skill between the two embryologists who performed IMSI compared with the three other embryologists (data not shown), a limitation of our study is that only two of the five embryologists are trained in the IMSI technique. To explain the difference in the fertilization rate, we had previously hypothesized the importance of the duration of gamete handling. It is important to note that we use two dishes for microinjection: one for sperm selection at ×100 immersion objective and one for oocyte injection at ×20 dry objective. This procedure could thus increase the duration of gamete handling, and the time required for the technique could possibly have been decreased by using a ×63 dry objective or a ×20 immersion objective. Considering the fertilization rates, none of the published data revealed an advantage of IMSI over ICSI.

Table 3 Results of the two previous ICSI cycles

	ICSI group	IMSI group	Statistical comparison
Total number of mature oocytes	13.4 ± 5.0	15.1 ± 6.2	$P < 0.05$
Fertilization rate (%)	53 ± 21	50 ± 22	NS
No. total embryos	7.2 ± 4.0	7.9 ± 4.8	NS
% of good morphology embryos (score 3 and 4, Giorgetti classification)	35 ± 30	38 ± 32	NS
No. embryo transfers	224	148	
No. transferred embryos	3.6 ± 1.6	3.9 ± 1.8	NS
Clinical pregnancy rate	4 (2 %)	11 (6 %)	NS
Implantation rate	1 % (4/441)	3 % (11/330)	NS
No. of freezing cycles in each group	45	37	
Total number of frozen embryos per cycle	0.8 ± 1.4	1.1 ± 1.9	NS

Table 4 Characteristics of studies assessing the results of IMSI after several ICSI failures

Authors	Study design	Study population	Number of previous ICSI failures	Implantation rate (%)	Clinical pregnancy rate (%)	Miscarriage rate (%)	Live birth rate (%)
Bartoov et al. [13]	Retrospective study	62 couples with altered semen analysis, at least two ICSI failures: comparison with 50 couples paired according to number of previous ICSI failures	4.1	IMSI 27.9 ICSI 9.5 $P<0.01$	IMSI 66 ICSI 30 $P<0.01$	IMSI 9 ICSI 30 $P<0.01$	IMSI 60 ICSI 20 $P<0.01$
Berkovitz et al. [23]	Retrospective study	80 couples with at least 2 ICSI failures	3.9	IMSI 31.3 ICSI 9.4 $P<0.05$	IMSI 60 ICSI 25 $P<0.05$	IMSI 14 ICSI 40 $P<0.05$	–
Antinori et al. [14] (subgroup C)	RCT	OAT 139 couples (62 ICSI, 77 IMSI)	≥2 (in subgroup C)	_	IMSI 29.9 ICSI 12.9 $P<0.05$	IMSI 17.4 ICSI 37.5 NS	–
Knez et al. 2011 [24]	RCT	57 couples (37 ICSI, 20 IMSI) male infertility with altered sperm parameters and arrested embryos after prolonged 5-day embryo culture in previous ICSI cycles	Not specified	IMSI 17.1 ICSI 6.8 NS (low number of couples)	IMSI 25 ICSI 8 NS	–	–
El Khattabi et al. [25]	Prospective non- randomized observational study	220 couples (90 IMSI, 130 ICSI)	2 or more previous ICSI failures	IMSI 16.7 ICSI 16.1 NS	IMSI 24 ICSI 26 NS		IMSI 21 ICSI 22 NS
Klement et al. 2013 [26]	Prospective non-randomized observational study	449 couples male infertility factor (127 IMSI, 322 ICSI)	One ICSI failure		IMSI 56 ICSI 38 $P=0.002$		IMSI 28 ICSI 18 $P=0.04$

OAT oligoasthenoteratozoospermia

We found no difference in embryo morphology. Except for two studies [23, 28], IMSI does not improve the morphology of early embryos [14, 19, 21, 22, 29]. However, an increase of development at the blastocyst stage using IMSI has been demonstrated by some authors [28, 30, 31], although randomization at the oocyte level did not confirm this finding [22].

Studies comparing IMSI with ICSI lead to contradictory conclusions. These discrepancies could be explained mainly by two reasons:

1. The way in which conventional ICSI is performed: accuracy of sperm selection and particularly the magnification used: ×200 or ×400 (some abnormalities that are not visible at ×200 might be detected at magnification ×400), and so the lack of superiority of IMSI over ICSI should not discredit the sperm selection.
2. The characteristics of the male population studied: [21, 32] demonstrated a significant increase in implantation rates using IMSI in the male infertility subgroup. Moreover, the benefit of IMSI was enhanced in the case of severe morphological alterations [8, 25, 33]. However, in our previous study we found no benefit of IMSI even in cases of severe teratozoospermia [19].

In conclusion, our results suggest that IMSI does not improve clinical outcomes in couples with two previous ICSI failures. More prospective randomized studies should be performed in order to confirm these results.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Comité d'Ethique de la Recherche des Hôpitaux de Toulouse. For this type of study formal consent is not required.

Conflict of interest The authors declare that they have no competing interests..

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