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REVIEW

# Twelve years of MSOME and IMSI: a review

Amanda Souza Setti [a,b], Daniela Paes de Almeida Ferreira Braga [a,b], Assumpto Iaconelli Jr [a,b], Tsutomu Aoki [c], Edson Borges Jr [a,b,*]

[a] *Sapientiae Institute — Centro de Estudos e Pesquisa em Reprodução Humana Assistida, Rua Vieira Maciel, 62, São Paulo, SP 04503-040, Brazil;* [b] *Fertility — Centro de Fertilização Assistida, Av. Brigadeiro Luis Antonio, 4545, São Paulo, SP 01401-002, Brazil;* [c] *Faculdade de Ciências Médicas da Santa Casa de São Paulo, Rua Dr. Cesário Motta Junior, 61, São Paulo, SP 01221-020, Brazil*

* Corresponding author. *E-mail address:* edson@fertility.com.br (E Borges).

Amanda S. Setti obtained her BSc degree in 2005 at the University of Santo Amaro (UNISA) and specialized in human assisted reproduction in 2007 at the Sapientiae Institute. At present, she is a scientific researcher of Fertility — Centro de Fertilização Assistida and Instituto Sapientiae and a Master's degree student at Faculdade de Ciências Médicas da Santa Casa de São Paulo in São Paulo, Brazil.

**Abstract** A promising method for observing spermatozoa, motile sperm organelle morphology examination (MSOME) enables the evaluation of the nuclear morphology of motile spermatozoa in real time at high magnification and has allowed the introduction of a modified microinjection procedure, intracytoplasmic morphologically selected sperm injection (IMSI). Since its development, several studies have intensively investigated the efficacy of MSOME and IMSI. The objective of the present study is to review the current literature on the MSOME and IMSI techniques.



**KEYWORDS:** ICSI, IMSI, MSOME, sperm morphology

## Introduction

Since its introduction in 1992 (Palermo et al., 1992), intracytoplasmic sperm injection (ICSI) has become the treatment of choice in the presence of abnormal sperm parameters. In preparation for ICSI, the embryologist selects a spermatozoon presenting both motility and normal morphology, based on evaluation of its tail, neck and head. ICSI is usually performed under a magnification of ×400, which only enables the observation of major morphological defects. As a consequence, the selection of the 'best-looking' spermatozoon may not represent the selection of a spermatozoon free of morphological abnormalities.

In the last decade, a new approach involving real-time high-magnification observation of unstained spermatozoa, motile sperm organelle morphology examination (MSOME), has been introduced (Bartoov et al., 2001). The incorporation of this technique together with a micromanipulation system has allowed the introduction of a modified ICSI procedure, intracytoplasmic morphologically selected sperm injection (IMSI). This system of real-time detailed morphological sperm examination at high magnification, ranging from ×6600 to ×13,000 with Nomarski optics

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(Garolla et al., 2008), enables the selection of the best available motile spermatozoa before oocyte injection (Bartoov et al., 2001, 2002, 2003; Berkovitz et al., 2006a,b).

Several publications have reported that IMSI is positively associated with implantation and pregnancy rates (Bartoov et al., 2002, 2003; Berkovitz et al., 1999, 2005, 2006a,b; Hazout et al., 2006; Setti et al., 2011). However, the exact indications for IMSI are under debate. The objective of this review is to summarize the current literature on MSOME and IMSI.

## MSOME

A physicist and optics theoretician, Georges Nomarski, is credited with many inventions and patents, including a major contribution to the renowned differential interference contrast microscopy technique in the mid-1950s. Also referred to as Nomarski interference contrast, the method allows transparent objects to be seen by using the difference in the refraction of light when transmitted through the varying thicknesses of a specimen and provides a greater depth of focus allowing thicker specimens to be observed under higher magnification (Ruzin, 1999).

Sperm morphological examination is performed under an inverted microscope equipped with high-power differential interference contrast. An aliquot of the prepared motile sperm fraction is transferred to a microdroplet of modified human tubal fluid medium containing polyvinyl pyrrolidone in a sterile glass-bottomed dish. The dish is placed on the microscope stage above an Uplan Apo ×100 oil/×1.35 objective lens that is previously covered by a droplet of immersion oil. The images are captured by a video camera, which has a 3-chip power charge-coupled device containing several effective picture elements (pixels) for high-quality image production, and a video monitor. The morphological assessment is conducted on the monitor screen which, under the above configuration, reaches a magnification that is calculated based on four parameters: (i) objective magnification; (ii) magnification selector; (iii) video coupler magnification; and (iv) a calculated video magnification (Bartoov et al., 2001). In general, the total calculated magnification is ×6600 (objective magnification (×100) × magnification selector (×1.5) × video coupler magnification (×0.99) × calculated video magnification (×355.6MM/8MM).

The MSOME assesses six sperm organelles: the acrosome, post-acrosomal lamina, neck, tail, mitochondria and nucleus. The acrosome and post-acrosomal lamina are considered abnormal if absent, partial or vesiculated. The mitochondria should not be absent, partial or disorganized. The neck must not be abaxial nor should it contain disorders or cytoplasmic droplets, and the tail should not be coiled, broken, short or double (Bartoov et al., 2002, 2003). Among the six organelles, the sperm nucleus seems to be the most important. According to the description given by Bartoov et al. (2002) the morphological normalcy of the sperm nucleus is evaluated in terms of shape (smooth, symmetric and oval) and chromatin content (homogeneous chromatin containing no more than one vacuole that occupies <4% of the nuclear area).

## Sperm vacuoles: pathological or physiological events?

Conventional light microscopic analysis of spermatozoa has limitations in evaluating the fine structures, such as the acrosome and nucleus (Baccetti et al., 1996). The early ultrastructural studies of human spermatozoa demonstrated that the sperm nucleus often present at least one vacuole (Schultz-Larsen, 1958). The vacuole is a concavity extending from the surface of the sperm head to the nucleus through the acrosome (Tanaka et al., 2012) that can be visualized only at a high magnification.

Recently, De Vos et al. (2013) aimed at documenting the prevalence of vacuoles in spermatozoa within a general ICSI population. The study analysed 330 semen samples under high magnification and showed that approximately 18.1% of the spermatozoa were normally shaped and free of vacuoles, 15.2% presented less than two small vacuoles, 12.3% displayed more than two small or at least one large vacuole and 54.4% were grade IV, mainly because of being amorphous in shape and/or presenting with large vacuoles. The prevalence of vacuoles in normally shaped spermatozoa was as low as 27.5%. Nevertheless, it is important to emphasize that the magnification obtained in this study yielded a much lower resolution than that obtained in other MSOME studies.

The origin of sperm vacuoles is disappointingly unknown and even after several investigations the question remains: are sperm vacuoles degenerative structures with no physiological importance or common physiologic features of the sperm head?

Some studies suggested that sperm vacuoles should be regarded as a normal feature of the sperm head (Chrzanowski, 1966; Fawcett, 1958; Pedersen, 1969; Tanaka et al., 2012), while others suggested that it is related to male subfertility (Mundy et al., 1994), lower mitochondrial membrane potential (Garolla et al., 2008), higher incidence of chromosomal abnormalities (Garolla et al., 2008; Perdrix et al., 2011) and sperm chromatin packaging/DNA abnormalities (Bartoov et al., 2001, 2002, 2003; Berkovitz et al., 2006a; Boitrelle et al., 2011; Cassuto et al., 2012; Franco et al., 2008, 2012; Oliveira et al., 2010a; Watanabe et al., 2011). It has also been suggested that sperm vacuoles reflect non-reacted acrosome (Kacem et al., 2011; Montjean et al., 2012) and therefore, the spermatozoa devoid from vacuoles selected through MSOME have undergone acrosome reaction and are likely to induce oocyte activation. Indeed, it has been demonstrated, in animal models, that the injection of spermatozoa with an intact acrosome is potentially hazardous to embryo development (Morozumi and Yanagimachi, 2005).

### Vacuoles and sperm maturation process

Recently, Tanaka et al. (2012) suggested that sperm vacuoles are cavities in the nucleus that occur naturally during the process of sperm maturation, even in early stage spermatids, and should not be considered as degenerative structures. In addition, the authors pointed out that the incidence of vacuoles increased, but the size tended to become smaller, during the spermiogenesis and epididymal transit. However, the authors highlighted that the size of



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the vacuoles is of importance and suggested that spermatozoa with large vacuoles are not used for injection.

## Vacuoles and acrosome reaction

A recent study investigated the nature of the nuclear vacuoles (Kacem et al., 2011). As these vacuoles are localized at the front of the sperm head the authors postulated that they might be of acrosomal origin. More than 3200 sperm cells obtained from 30 semen samples from infertile patients were evaluated regarding their acrosomal status using *Pisum sativum* agglutinin staining and MSOME. A significant difference in the proportion of sperm cells containing vacuoles was observed between spermatozoa presenting acrosomal material or intact acrosomes and acrosome-reacted spermatozoa (61.0% versus 29.0%). In addition, induction of the acrosomal reaction by ionophore A23587 significantly increased the percentage of vacuole-free spermatozoa from 41.2% to 63.8% and the percentage of acrosome-reacted spermatozoa significantly increased from 17.4% to 36.1% (Kacem et al., 2011).

Montjean et al. (2012) evaluated 35 sperm samples that were incubated with the follicular fluid and with hyaluronic acid and analysed for sperm DNA condensation and morphology through MSOME, in order to determine if there was a correlation between the presence of vacuoles and acrosome reaction. In accordance with the findings from Kacem et al. (2011), the results showed that the presence of sperm vacuoles negatively influences sperm capacity to undergo acrosome reaction. The authors concluded that sperm vacuoles are a reflection of sperm physiology rather than an expression of abnormalities in the nucleus.

## Vacuoles and sperm DNA damage and chromosomal status

The human spermatozoon is crucial for contributing three components: (i) the paternal genome; (ii) the signal to initiate oocyte activation; and (iii) the centriole; which participates in the initial development of the zygote (Barroso et al., 2009). In addition, the human spermatozoon plays an essential role in embryogenesis that goes beyond the fertilization process. The activation of the embryonic genome at the stage of 4–8 cells depends on the expression of the paternal genome (Braude et al., 1988). Studies suggest that the injection of DNA-damaged spermatozoa is related to blockage of embryonic development during/after the implantation of embryos, which reflects a late paternal effect (Borini et al., 2006; Tesarik et al., 2004).

Sperm DNA integrity and chromosomal constitution cannot be assessed in the sperm cell used for ICSI, therefore several studies have investigated the relationship between sperm morphology by MSOME and DNA fragmentation and/or sperm chromosomal status. The following studies evaluating the relationship between the presence of sperm vacuoles and chromatin and/or DNA and/or chromosomal abnormalities are summarized in **Table 1**.

Garolla et al. (2008) evaluated the correlation between DNA fragmentation and sperm morphology under high magnification (×13,000) in 10 patients with severe testicular impairment. A total of 20 single immotile sperm cells per patient were retrieved and classified on the basis of normal morphology and absence (group A, 10 cells) or presence of vacuoles (group B, 10 cells). The same cells were further characterized as normal or pathological for DNA fragmentation (terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling assay; TUNEL). The authors found that group A contained a lower percentage of DNA-fragmented spermatozoa than group B. In addition, fluorescent in-situ hybridization (FISH) analysis showed that no chromosomal alteration was present in normal sperm cells (group A).

Franco et al. (2008) evaluated the extent of DNA fragmentation (TUNEL assay) and the presence of denatured single-stranded or normal double-stranded DNA (acridine orange fluorescence method) in spermatozoa with large nuclear vacuoles (LNV) selected by high magnification from 30 patients. Spermatozoa with a normal nucleus (NN) and LNV were selected and placed on different slides. DNA fragmentation in spermatozoa with LNV (29.1%) was significantly higher ($P < 0.001$) than in spermatozoa with NN (15.9%). Similarly, the percentage of denatured-stranded DNA in spermatozoa with LNV (67.9%) was significantly higher ($P < 0.001$) than in spermatozoa with NN (33.1%).

In a study by de Almeida Ferreira Braga et al. (2011), MSOME, sperm DNA fragmentation (TUNEL assay) and sperm (FISH) evaluations were performed in 200 sperm cells from each of 50 patients undergoing ICSI as a result of male infertility. The results showed that the presence of vacuoles and abnormal nuclear cell size observed via MSOME was positively correlated with the incidence of sperm DNA fragmentation; however, the presence of sperm aneuploidy was not correlated with MSOME.

Wilding et al. (2011) assessed the correlation between sperm morphology according to MSOME and DNA fragmentation in 860 spermatozoa derived from eight separate analyses. The authors showed that only 331 of these spermatozoa were considered morphologically normal after MSOME. Of these, 4.2% were characterized as having fragmented DNA after TUNEL assay. The study suggested a link between abnormal morphology after MSOME and the presence of fragmented DNA, since only 14.4% of the spermatozoa presenting vacuoles after MSOME were found to contain fragmented DNA, a significantly higher proportion of spermatozoa than MSOME normal spermatozoa ($P = 0.031$).

Perdrix et al. (2011) assessed spermatozoa from neat semen samples and spermatozoa presenting a vacuole occupying ≥13.0% total head area (spermatozoa with large vacuole; SLV), isolated under high magnification (×6600) from 20 patients with teratozoospermia. Both the neat samples and SLV were evaluated for DNA fragmentation (TUNEL assay), chromatin condensation (aniline blue staining) and sperm aneuploidy (FISH). The results showed that complete DNA fragmentation was significantly more frequent in native spermatozoa than SLV, while chromatin condensation was significantly altered in SLV. In addition, aneuploidy and diploidy rates were significantly increased in SLV.

A recent study suggested that sperm head vacuoles are not pathological or an indication of DNA damage and should be considered as an ordinary characteristic in normal spermatozoa (Watanabe et al., 2011). The study showed that the frequency of chromosomal alterations, which are derived from DNA fragmentation after fertilization, did not differ significantly between motile normally shaped spermatozoa with a large vacuole and those without large vacuoles (9.1% versus 4.1%). In addition, the frequency of chromosomal alterations was similar to that obtained for



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**Table 1** Studies evaluating the relationship between the presence of sperm vacuoles and chromatin and/or DNA and/or chromosomal abnormalities.

| *Reference* | *Patients (n)* | *Cells (n)* | *Method* | *DNA integrity* | *Susceptibility to DNA denaturation* | *Euploidy* | *Chromosomal structure* | *Chromatin packaging* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *TUNEL* | *Acridine orange* | *FISH* | *Giemsa C-banding* | *Aniline blue* | *CMA3* |
| Garolla et al. (2008) | 10 | 200 | Groups A (vacuole-free spermatozoa) and B (vacuolated spermatozoa) were analysed and compared | Group A showed a lower percentage of DNA-fragmented spermatozoa than group B (9.3% versus 40.1%) | – | No chromosomal alteration was present in normal sperm cells (group A) | – | – | – |
| Franco et al. (2008) | 30 | 792 | Spermatozoa with a normal nucleus (NN) and LNV were selected and placed on different slides and compared | DNA fragmentation in spermatozoa with LNV (29.1%) was significantly higher than in spermatozoa with NN (15.9%) | Denatured-stranded DNA in spermatozoa with LNV (67.9%) was significantly higher than in spermatozoa with NN (33.1%) | – | – | – | – |
| de Almeida Ferreira Braga et al. (2011) | 50 | 10,000 | Semen samples were evaluated for sperm DNA fragmentation, presence of vacuoles and incidence of aneuploidy | Presence of vacuoles was positively correlated with the incidence of sperm DNA fragmentation | – | Presence of vacuoles was not correlated with the incidence of aneuploidy | – | – | – |
| Wilding et al. (2011) | 8 | 860 | Assessment of the correlation between sperm morphology according to MSOME and DNA fragmentation | A significantly higher proportion of spermatozoa with fragmented DNA was observed in cells presenting vacuoles after MSOME as compared with normal spermatozoa (14.4% versus 4.2%) | – | – | – | – | – |
| Perdrix et al. (2011) | 20 | 519–580 per patient for TUNEL, 499–531 per patient for aniline blue and a mean of 1087 per patient for FISH | Spermatozoa from the native sample and vacuolated spermatozoa (≥13.0% head area), isolated under high magnification, were assessed | Complete DNA fragmentation was significantly higher in native spermatozoa than SLV | – | Aneuploidy and diploidy rates were significantly increased in SLV than native spermatozoa (7.8% versus 1.3%) | – | Chromatin condensation was significantly altered in SLV (50.4% versus 26.5%) than native spermatozoa | – |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Watanabe et al. (2011) | 20 | 33 for chromosomal analysis and 2877 for TUNEL | Sperm heads were analysed for the presence of vacuoles under ×1000, structural chromosomal damage and DNA damage in spermatozoa exhibiting large vacuoles | No significant difference in frequency of TUNEL-positive cells was found between normal spermatozoa with large vacuoles and those without vacuoles (3.3% versus 3.5%) | — | — | No differences in the incidence of aberrations between spermatozoa exhibiting large vacuoles and those without vacuoles were observed (9.1% versus 4.1%) | — | — |
| Boitrelle et al. (2011) | 15 | 900 | For each sperm sample, 30 normal spermatozoa and 30 spermatozoa with a LNV (≥25% head area) were selected | Vacuole-free and vacuolated spermatozoa did not differ significantly in terms of DNA fragmentation (0.7% versus 1.3%) | — | Vacuole-free and vacuolated spermatozoa did not differ in terms of aneuploidy rates (1.1% versus 2.2%) | — | Condensed chromatin was significantly higher for vacuolated spermatozoa than for normal spermatozoa (36.2% versus 7.6%) | — |
| Cassuto et al. (2012) | 26 | 10,400 | Spermatozoa with normal and abnormal sperm head were compared | DNA fragmentation rate was comparable between normal and abnormal sperm head groups (3.7% versus 4.2%) | — | — | — | Sperm chromatin decondensation rate of abnormal spermatozoa was twice as high as the controls (19.5% versus 10.1%) | — |
| Franco et al. (2012) | 66 | 2186 | Numbers of cells with normal and abnormal chromatin packaging were determined on slides with normal and LNV spermatozoa | — | — | — | — | — | Presence of abnormal chromatin packaging was significantly higher in spermatozoa with LNV than in normal spermatozoa (53.2%% versus 40.3%) |
| Hammoud et al. (2012) | 8 | 8000 | Vacuole-free and vacuolated spermatozoa from semen samples presenting high sperm DNA fragmentation were compared | Motile normal vacuole-free spermatozoa had a significantly lower mean DNA fragmentation rate (4.1%) than all other types of spermatozoa | — | — | — | — | — |

— = Not performed; CMA3 = chromomycin A3; LNV = large nuclear vacuole; NN = normal nucleus; SLV = spermatozoa with large nuclear vacuole; TUNEL = terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling assay.



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spermatozoa examined under ×400 magnification, suggesting that normal spermatozoa with DNA damage are not efficiently excluded by sperm selection under ×1000 magnification (Watanabe et al., 2011). Nevertheless, it is noteworthy that sperm morphology was examined under a magnification of ×1000 in this study, while in the majority of studies a magnification of at least ×6000 was applied.

Boitrelle et al. (2011) used the MSOME (×10,000) to select 450 normal spermatozoa and 450 spermatozoa with a large vacuole (≥25% of the nuclear area) from semen samples of 15 infertile patients and analysed chromatin condensation (aniline blue staining), DNA fragmentation (TUNEL) and chromosomal status (FISH X, Y, 18). The results showed that the rate of non-condensed chromatin was significantly higher for vacuolated spermatozoa than for normal spermatozoa (36.2 ± 1.9% versus 7.6 ± 1.3%). The authors concluded that large vacuole appears to be a nuclear 'thumbprint' linked to failure of chromatin condensation.

Cassuto et al. (2012) investigated whether chromatin damage (TUNEL and aniline blue assays) of 26 infertile man with oligoasthenoteratospermia and IVF failures was linked with sperm-head abnormalities identified at high magnification. The analysis of 10,400 spermatozoa showed that the sperm chromatin-decondensation rate of abnormal spermatozoa (presenting abnormal head, presenting one or several vacuoles and an abnormal base) was twice as high as the controls (19.5% versus 10.1%; $P < 0.0001$).

Franco et al. (2012) investigated the presence of abnormal sperm chromatin packaging in spermatozoa with LNV by analysing the pattern of chromomycin A3 (CMA3) staining in 66 men undergoing infertility diagnosis and treatment. The authors showed that the presence of CMA3-positive (abnormal) staining was significantly higher in spermatozoa with LNV than in CMA3-negative (normal) spermatozoa (53.2% versus 40.3%; $P < 0.001$, respectively).

Finally, Hammoud et al. (2012) analysed different types of spermatozoa in eight patients with high degree of sperm DNA fragmentation in terms of incidence of DNA fragmentation. Vacuole-free spermatozoa showed a significantly lower incidence of DNA fragmentation (4.1 ± 1.1%) than all other types of spermatozoa.

## MSOME and conventional semen analysis

World Health Organization (WHO) reference values for human semen parameters are widely used to investigate male reproductive potential. Evaluation of sperm morphology plays a crucial role in the diagnosis of male fertility potential and has demonstrated a predictive value for IVF–ICSI treatments (Kruger et al., 1986, 1987, 1988). However, other authors found no relationship between sperm morphology and the success of ICSI (Host et al., 2001; Nagy et al., 1998; Oehninger et al., 1998).

MSOME provides an accurate description of spermatozoa abnormalities, particularly the presence of head vacuoles (Bartoov et al., 2002). However, no consensus has been established concerning normal or abnormal MSOME criteria, despite being essential to transposing MSOME analysis into routine evaluation of male infertility (Perdrix et al., 2012). Some studies have analysed the relationship between sperm normalcy according to the WHO or Tygerberg criteria and MSOME.

Bartoov et al. (2002) investigated the relationship between normal spermatozoa according to the WHO reference values (WHO, 1999) and MSOME in 20 patients. The authors found no significant correlation between the percentage of morphologically normal spermatozoa as defined by the WHO and the percentage of morphologically normal spermatozoa as defined by MSOME, since the incidence of sperm normalcy by routine sperm analysis was significantly higher than that by MSOME (26.1 ± 7.2% and 2.9 ± 0.5%, respectively).

Oliveira et al. (2009) evaluated the correlation between MSOME classification and sperm morphology classification according to the Tygerberg criteria (Kruger et al., 1986) in 97 semen samples from an unselected group of couples undergoing infertility investigation. The study showed a strong positive correlation between the percentage of normal sperm forms according to the Tygerberg criteria and MSOME ($r = 0.83$; $P < 0.001$). However, MSOME was shown to be much more restrictive, presenting significantly lower normality percentages for the semen samples in comparison to those observed after analysis according to the Tygerberg criteria (3 ± 3.2% versus 9.4 ± 4.8% respectively; $P < 0.001$).

In a previously mentioned study, Cassuto et al. (2012) observed significant correlations between the incidence of score-0 spermatozoa (presenting an abnormal head, one or several vacuoles and an abnormal base) and sperm concentration ($r = -0.41$), motility ($r = -0.42$) and morphology ($r = -0.63$).

Finally, Perdrix et al. (2012) analysed semen samples from 440 males, aged between 24 and 66 years, consulting for infertility investigation. One sample was obtained from each man and conventional semen analysis (WHO, 1999)and MSOME evaluation were performed simultaneously on the same sample. A total of 109 men (24.8%) had normal semen parameters (normal group) and 331 men (75.2%) had at least one abnormal semen parameter (abnormal group). MSOME analysis was performed on 10,975 spermatozoa. Sperm head vacuoles were significantly larger in abnormal semen samples ($P < 0.001$). Relative vacuolar area (RVA), defined as vacuole area ($\mu m^2$)/head area ($\mu m^2$) ×100, was the most discriminative MSOME criterion between normal and abnormal semen samples, and was negatively correlated with poor sperm morphology ($r = 0.53$; $P < 0.001$).

It is noteworthy that routine morphological examination is applied to the entire semen sample, whereas the most remarkable feature of MSOME is the focus on motile sperm fractions, providing information about the sample fraction referred for ICSI treatment. In addition, a recent study demonstrated that MSOME is a reliable technique for analysing semen and supported the future use of MSOME as a routine method for semen analysis (Oliveira et al., 2010b).

## MSOME and sperm preparation and manipulation

Given the importance of selecting a sperm preparation technique that minimizes possible paternal effects on embryo development by enhancing the sample with sperm cells with few vacuoles, Monqaut et al. (2011) analysed sperm samples from 53 patients undergoing fertility treatment. Samples were analysed by high-magnification microscopy before and after two preparation methods (swim-up and density gradient centrifugation) and classified

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according to the degree of vacuolization. Although both methods showed a positive effect on sperm quality, the swim-up method produced significantly higher increments of morphologically normal spermatozoa than gradient centrifugation (59.3% versus 15.7%; $P < 0.001$).

It has previously been demonstrated that prolonged in-vitro incubation at 37°C may reduce sperm viability (Calamera et al., 2001). Since the morphological evaluation of spermatozoa under high magnification is a time-consuming procedure (Berkovitz et al., 2005) that should be conducted at 37°C, the inventors of MSOME investigated the impact of incubation at 37°C on the morphological normalcy of the sperm nucleus (Peer et al., 2007).The study showed that after 2 h of incubation at 37°C, there was a significant increase in the frequency of vacuolated nuclei (80.8 ± 7.2% versus 75.0 ± 7.6%; $P < 0.01$). No significant morphological changes in sperm nuclei were observed upon prolonged incubation at 21°C. Finally, after 2 h of incubation, the incidence of spermatozoa with vacuolated nuclei was significantly higher at 37°C compared with 21°C (56.5 ± 10.8% versus 45.5 ± 10.0%; $P < 0.01$).

## MSOME and male age

A recent study investigated the influence of paternal age on sperm quality by MSOME. Two hundred sperm cells from 975 patients were analysed at ×8400 magnification (Silva et al., 2012) and the percentage of normal and LNV spermatozoa was determined. The subjects were divided into three groups according to paternal age. The study demonstrated a significantly lower percentage of normal spermatozoa in the older group (≥41 years) compared with the younger groups (≤35 years and 36–40 years). In addition, the proportion of LNV spermatozoa was significantly higher in the older group, while regression analysis demonstrated that a 1-year increment in paternal age increased the incidence of spermatozoa with LNV by 10%. This correlation was corroborated by findings obtained by de Almeida Ferreira Braga et al. (2011).

## MSOME and cryopreservation

It has previously been demonstrated that human sperm cryopreservation is associated with alterations in sperm motility, viability and morphology (O'Connell et al., 2002). Boitrelle et al. (2012) investigated the potential value of IMSI for frozen–thawed spermatozoa, and the current study group used MSOME, chromatin condensation assessment (aniline blue staining) and viability assessment (eosin permeability) before and after freezing–thawing to assess the relationship between cryopreservation and potential nuclear alterations in spermatozoa. The results showed that cryopreservation decreases the percentage of morphologically normal spermatozoa and viability rate and increases the proportion of spermatozoa with non-condensed chromatin.

## Conclusions

The reason for the occurrence of vacuoles in the sperm head is yet to be elucidated and requires further studies. Several studies have investigated the origin of this feature and the results are controversial. Nonetheless, only two studies showed that there is no relationship between sperm nuclear vacuoles and sperm function; however, it is important to emphasize that one of these studies was an unpowered investigation (Tanaka et al., 2012) and the other evaluated sperm cells at ×1000 (Watanabe et al., 2011). The remaining studies agreed that sperm nuclear vacuoles are either related to acrosome reaction, chromosomal status, chromatin condensation or DNA fragmentation.

Both studies that investigated the relation between sperm vacuoles and acrosome reaction agreed that there is a negative relation between the presence of vacuoles and the sperm capacity to undergo acrosome reaction. Therefore, the MSOME selection could be a tool for the elimination of the acrosome reaction-resistant spermatozoa. Regarding sperm DNA fragmentation, from nine studies, six reported that vacuole-free spermatozoa yields lower rates of DNA fragmentation as compared with vacuolated spermatozoa. It is important to emphasize that the TUNEL assay was the method of choice in all these studies, which could have reduced the occurrence of bias. As for chromatin status, a negative correlation between the incidence of vacuoles and chromatin condensation was observed in all the conducted studies (one study used CMA3 and three used aniline blue staining). Finally, it seems that sperm aneuploidy is not related to the presence of vacuoles, nevertheless, one study observed increased rates of aneuploidy and diploidy in SLV.

According to De Vos et al. (2013), the prevalence of vacuoles in normally shaped spermatozoa seems to be low. In addition, the use of 'second-best' spermatozoa appears to have no implications on fertilization and embryo development. Nevertheless, it has been reported that up to 65% spermatozoa deemed suitable for ICSI by conventional methods were subsequently deselected after high-magnification analysis (Wilding et al., 2011)

Although the process of finding spermatozoa without vacuoles is difficult and time consuming and requires highly skilled laboratory personnel, so far, the majority of the studies suggest that there is a link between the presence of vacuoles and sperm function, either with the acrosome reaction, chromatin condensation or DNA integrity. More importantly, the SLV seems to be the most compromised spermatozoa and should not be used for injection.

## IMSI

MSOME followed by ICSI is a novel technique that involves prolonged sperm manipulation (Berkovitz et al., 2005) and special instrumentation with considerable costs. In addition, the technique requires a high level of technical expertise and inter-observer reproducibility (Said and Land, 2011). A meta-analysis comparing ICSI versus IMSI outcomes concluded that IMSI not only significantly improves the percentage of top-quality embryos, implantation and pregnancy rates, but also significantly reduces miscarriage rates as compared with ICSI (Souza Setti et al., 2010). These findings can be explained by the fact that during ICSI morphological assessment of the sperm nucleus takes place at ×400. Wilding et al. (2011) performed a mock ICSI trial to determine the proportion of spermatozoa, otherwise



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**Table 2** Studies comparing ICSI and IMSI outcomes.

| Reference | Cycles (n) | Indication | Inclusion criteria | Method | Fertilization (%) | Top-quality embryo (%) | Implantation (%) | Pregnancy (%) | Miscarriage (%) |
|---|---|---|---|---|---|---|---|---|---|
| Bartoov et al. (2001) | 24 | Previous ICSI failures | Female age ≤37 years, >3 retrieved ova, male infertility, previous failure of ≥5 cycles | IMSI outcomes were assessed in couples with previous ICSI failures | ICSI 60.1; IMSI 66.8[a] | – | ICSI 0.0; IMSI 46.9[b] | ICSI 0.0; IMSI 58.0[b] | ICSI 0.0; IMSI 5.0[b] |
| Bartoov et al. (2003) | 50 | Previous ICSI failures | Female age ≤37 years, >3 retrieved ova, male infertility, previous failure of ≥2 ICSI cycles | IMSI outcomes were matched with ICSI outcomes from similar couples | ICSI 65.5; IMSI 64.5[a] | ICSI 31.0; IMSI 45.2[c] | ICSI 9.5; IMSI 27.9[c] | ICSI 30.0; IMSI 66.0[c] | ICSI 33.0; IMSI 9.0[c] |
| Hazout et al. (2006) | 125 | Previous ICSI failures | Female age <38 years, previous failure of ≥2 ICSI cycles | IMSI outcomes were assessed in couples with previous ICSI failures | ICSI 65.0; IMSI 68.0[a] | ICSI 52.5; IMSI 63.5[a] | ICSI 0.8; IMSI 20.3[c] | ICSI 2.4; IMSI 37.6[c] | ICSI 100; IMSI 13.2[c] |
| Gonzalez-Ortega et al. (2010) | 60 | Previous ICSI failures | Female age <38 years, previous failure of ≥2 ICSI cycles | IMSI outcomes were matched with ICSI outcomes from similar couples | ICSI 89.0; IMSI 91.2[a] | ICSI 43.3; IMSI 45.7[a] | ICSI 29.7; IMSI 44.8[c] | ICSI 50.0; IMSI 63.0[a] | ICSI 26.6; IMSI 15.7[a] |
| Wilding et al. (2011) | 8 | Previous ICSI failures | Couples with 1 previous ICSI failure | IMSI outcomes was compared with the previous ICSI cycle | ICSI 79.4; IMSI 70.1[a] | ICSI 60.3; IMSI 83.6[c] | ICSI 0.0; IMSI 20.8[b] | ICSI 0.0; IMSI 37.5[b] | – |
| Oliveira et al. (2011) | 200 | Previous ICSI failures | Female age ≤39 years, ≥4 retrieved ova in previous cycles, previous failure of ≥2 ICSI cycles with good quality embryos | Couples were divided into ICSI and IMSI groups | ICSI 62.0; IMSI 65.4[a] | – | ICSI 9.8; IMSI 13.6[a] | ICSI 19.0; IMSI 26.0[a] | ICSI 31.6; IMSI 15.4[a] |
| Antinori et al. (2008) | 446 | Male factor | Female age ≤35 years, severe oligoasthenoteratozoospermia | Couples were randomized to ICSI and IMSI | ICSI 94.4; IMSI 94.7[a] | – | ICSI 11.3; IMSI 17.3[c] | ICSI 26.5; IMSI 39.2[c] | ICSI 24.1; IMSI 16.9[a] |
| Mauri et al. (2010) | 30 | Male factor | Male factor infertility and/or ≥2 previous failures of implantation or previous miscarriages after IVF–ICSI | Sibling oocytes of each patient were randomly assigned to ICSI or IMSI | ICSI 70.9; IMSI 70.4[a] | ICSI 57.8; IMSI 52.2[a,*] | – | – | – |
| Knez et al. (2011) | 57 | Male factor | Poor semen quality and all arrested embryos following a prolonged 5-day culture in previous ICSI cycles | Couples were randomized to ICSI and IMSI | ICSI 52.7; IMSI 51.2[a] | – | ICSI 6.8; IMSI 17.1[a] | ICSI 8.1; IMSI 25.0[a] | – |
| Setti et al. (2011) | 500 | Male factor | Isolated male factor infertility, ≥6 oocytes available on retrieval | Couples were randomized to ICSI and IMSI | ICSI 78.9; IMSI 79.2[a] | ICSI 37.3; IMSI 44.4[a] | ICSI 25.4; IMSI 23.8[a] | ICSI 36.8; IMSI 37.2[a] | ICSI 17.9; IMSI 18.4[a] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Wilding et al. (2011) | 232 | Male factor | Sperm concentration between $1 \times 10^6$/ml and $20 \times 10^6$/ml | Patients were randomized to ICSI or IMSI | ICSI 65.9; IMSI 68.0[a] | – | ICSI 14.8; IMSI 24.2[c] | ICSI 40.0; IMSI 65.6[c] | – |
| Knez et al. (2012) | 122 | Male factor | Isolated teratozoospermia, ≥6 mature oocytes available on retrieval | Patients were randomized to ICSI or IMSI | ICSI 64.0; IMSI 60.0[a] | – | – | – | ICSI 24.0; IMSI 48.0[c] |
| Balaban et al. (2011) | 168 | Unselected patients | Unselected infertile population | Patients were randomized to ICSI or IMSI | ICSI 81.0; IMSI 81.6[a] | ICSI 64.0; IMSI 66.4[a] | ICSI 19.5; IMSI 28.9[a] | ICSI 44.4; IMSI 54.0[a] | – |
| Hazout et al. (2006) | 72 | High incidence of sperm DNA fragmentation | Female age <38 years, previous failure of ≥2 ICSI cycles | DNA fragmentation rate was evaluated by TUNEL and patients were divided into three groups: (A) <30%, (B) 30–40% and (C) >40% fragmented spermatozoa before comparing ICSI and IMSI | – | – | Group A: ICSI 0.; IMSI: 23.6[c]<br>Group B: ICSI 0.0; IMSI: 17.4[c]<br>Group C: ICSI 0.7; IMSI: 33.3[c] | – | – |
| Braga et al. (2011) | 50 | High incidence of sperm DNA fragmentation | Cycles with male patients showing a high incidence of DNA fragmentation (>30%) | Oocytes were split into ICSI ($n = 82$) and IMSI groups ($n = 79$) | ICSI 80.2; IMSI 82.6[a] | ICSI 60.0; IMSI 61.6[a] | – | – | – |

–: Not evaluated.
[a]Not significantly different. [b]Not applicable. [c]Significantly different.
*Day 2 of embryo development.



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selected for ICSI, that had morphological abnormalities. The results showed that 64.8% of the analysed spermatozoa were deselected after digital analysis. Reasons for rejection of spermatozoa included poor morphology, the presence of multiple vacuoles, the presence of vacuoles that occupied >4% of the nuclear area and poor morphology of the mid-piece. The study suggested that selection of spermatozoa under high magnification reveals morphological features not visible using the conventional ICSI procedure and deselects spermatozoa otherwise selected for ICSI.

Cassuto et al. (2009) retrospectively evaluated 27 couples with male factor infertility referred for ICSI treatment in order to establish a classification score for the spermatozoon with the highest predictive fertilizing potential in real time under a magnification of ×6100. The authors suggested the following formula for a morphologically 'normal top' spermatozoon: (normal head score = 2) + (lack of vacuole score = 3) + (normal base score = 1). In women aged ≥30 years, the authors recommended using spermatozoa with a score of 4–6 for injection. In younger patients, scoring is not as critical, at least with regards to fertilization.

Several studies have investigated the benefits of IMSI by comparing the results obtained using this technique with those obtained via ICSI. The results are controversial and are described below according to the type of infertility (**Table 2**).

## IMSI indications

### IMSI in cases of previous IVF–ICSI failure

In a preliminary study, Bartoov et al. (2001) assessed 24 couples in which the woman was <37 years old, with previous failure of at least five consecutive cycles of IVF and ICSI, who had undergone a single cycle of IMSI, with at least three oocytes retrieved, as a result of male infertility. The study showed that after IMSI, the pregnancy rate was 58%, the implantation rate was 47% and the miscarriage rate was 5%.

In a continuation of the aforementioned study, Bartoov et al. (2003) investigated whether microinjection of motile spermatozoa with morphologically normal nuclei improved the pregnancy rate in 50 couples with repeated ICSI failures. After a single IMSI procedure, the couples were matched with couples who had undergone a routine ICSI procedure and experienced the same number of previous ICSI failures. The study revealed that fertilization and the proportion of top-quality embryos were similar, but that the pregnancy rate in the IMSI group was significantly higher than that in the ICSI group (66.0% versus 30.0%; $P < 0.01$) and the miscarriage rate was significantly lower in the IMSI group (33.0% versus 9.0%; $P < 0.01$).

Hazout et al. (2006) evaluated the efficacy of IMSI in 125 couples with at least two repeated ICSI failures in which the woman was <38 years old. The results showed that fertilization, cleavage and top-quality embryo rates were similar in the two previous ICSI attempts and sequential IMSI attempt. However, improved clinical outcomes such as pregnancy (37.6% versus 2.4%), implantation (20.3% versus 0.8%), delivery (33.6% versus 0.0%) and birth rates (17.6% versus 0.0%) were observed in IMSI attempts compared with ICSI ($P < 0.001$), respectively.

Gonzalez-Ortega et al. (2010) compared the results of 30 IMSI cycles performed in couples with at least two previous ICSI failures and female age <38 years, and 30 ICSI cycles performed in couples with similar characteristics. The data showed a significant difference in implantation rate in favour of IMSI (44.8% versus 29.7%). Although not significantly different, the pregnancy rate tended to be higher in IMSI cycles (63% versus 50%), demonstrating a trend in favour of IMSI.

Wilding et al. (2011) compared embryo quality obtained after IMSI in eight couples with their previous ICSI cycle. The results showed a significant difference in embryo quality between the ICSI and IMSI cycles (60.3% versus 83.6%, respectively). The authors extended the study into a prospective randomized trial involving couples undergoing ICSI ($n = 110$) and IMSI ($n = 122$). The authors noted a significantly higher percentage of high-quality embryos transferred (66.0% versus 98.6%) and higher rates of implantation (14.8% versus 24.2%) and pregnancy (40.0% versus 65.6%) after IMSI.

Oliveira et al. (2011) compared the outcomes of ICSI and IMSI in 200 couples with at least two repeated ICSI failures. The study revealed trends toward lower rates of miscarriage (15.4% versus 31.6%), higher rates of ongoing pregnancy (22.0% versus 13.0%) and live birth (21.0% versus 12.0%) in the IMSI group compared with the ICSI group. The study also analysed subpopulations with or without male factors, and similar results to those obtained with the whole population were observed.

### IMSI in cases of male factor infertility

Antinori et al. (2008) assessed the advantages of IMSI over conventional ICSI in the treatment of 446 couples with severe oligoasthenoteratozoospermia and female age under 35 years in a prospective randomized trial. The couples were randomized into ICSI ($n = 219$) and IMSI ($n = 227$) groups. The results showed that IMSI resulted in a higher pregnancy rate (39.2% versus 26.5%; $P = 0.004$) compared with ICSI. In addition, the study demonstrated that patients with two or more previous ICSI failures benefited the most from IMSI in terms of pregnancy rate (29.8% versus 12.9%; $P = 0.017$).

Mauri et al. (2010) evaluated whether IMSI could influence early paternal effects by observing embryo quality at day 2 in 30 couples with male factor infertility and/or at least two previous failures of implantation and/or previous miscarriages after IVF–ICSI. The results obtained using sibling oocytes showed that ICSI and IMSI provided a similar proportion of top-quality embryos.

Knez et al. (2011) compared the results obtained with ICSI (37 couples) and IMSI (20 couples) in couples with poor semen quality and all embryos arrested after culture to the blastocyst stage in their previous ICSI attempts in a prospective randomized study. The outcomes of current cycles were compared with the outcomes of the previous ICSI cycles. The IMSI group showed a higher number of blastocysts (0.80 versus 0.65) and lower number of cycles without embryo transfer (0% versus 27.0%, $P = 0.048$) compared with the ICSI group. A trend toward higher implantation (17.1% versus 6.8%) and pregnancy rates (25.0% versus 8.1%) was observed in the IMSI group.

Setti et al. (2011), in a prospective randomized study, compared ICSI and IMSI outcomes in 500 couples with male factor infertility and at least six retrieved oocytes. The

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results showed that the outcomes were similar between the groups. In a further analysis, only 244 oligoasthenozoospermic patients were included and a positive influence of IMSI on fertilization (OR 4.3, 95% CI 2.2−6.4), implantation (OR 2.6, 95% CI 1.2−5.7) and pregnancy (OR 1.6, 95% CI 1.1−3.0) was observed.

Wilding et al. (2011) randomized 232 couples undergoing ICSI as a result of male factor infertility into ICSI and IMSI groups, and the outcomes were compared. The results showed that implantation and pregnancy rates were significantly higher in IMSI group (14.8% versus 24.2% and 40.0% versus 65.6%).

In a recent prospective randomized trial, Knez et al. (2012) compared the outcomes obtained with ICSI ($n = 70$) and IMSI ($n = 52$) in couples with isolated teratozoospermia. The study showed a significantly higher rate of morulae development (21.0% versus 13.0%) and a lower number of embryos arrested at low-cell developmental stages (44.0% versus 62.0%) after IMSI. A significantly higher clinical pregnancy rate was observed in the IMSI group compared with the ICSI group (48.0% versus 24.0%; $P < 0.05$). In addition, the authors investigated the influence of individual sperm morphology on embryo development in 30 patients undergoing IMSI. Oocytes were injected with different classes of spermatozoa and the results showed that fertilization with spermatozoa without head vacuoles yielded a higher number of morphologically normal zygotes, a higher blastocyst formation rate and a smaller proportion of arrested embryos than spermatozoa with vacuoles and other head defects.

### IMSI in unselected infertile patients

Balaban et al. (2011) compared the outcomes of 87 IMSI cycles with 81 ICSI cycles in an unselected infertile population in a prospective randomized study. The results showed trends for higher rates of implantation (28.9% versus 19.5%), pregnancy (54.0% versus 44.4%) and live birth (43.7% versus 38.3%) in the IMSI group. When only couples presenting severe male factor were analysed, the IMSI procedure resulted in significantly higher implantation rates compared with the ICSI group (29.6% versus 15.2%, $P = 0.01$).

### IMSI in patients with a high rate of sperm DNA fragmentation

Hazout et al. (2006), in a previously mentioned study, assessed sperm DNA integrity in 72 patients. Improved implantation and birth rates were observed not only in patients with an elevated degree of sperm DNA fragmentation but also in those with normal sperm DNA status.

In a previously mentioned study, de Almeida Ferreira Braga et al. (2011) showed that fertilization and high quality embryo rates in patients with a high incidence of sperm DNA fragmentation were similar between sibling oocytes split into ICSI and IMSI groups.

All the aforementioned studies comparing ICSI and IMSI outcomes are summarized in **Table 2**.

### IMSI in patients with poor blastocyst development

Because early paternal effects on embryo development are not expressed up to day 3, it has been suggested that the presence of nuclear vacuoles, detected under high magnification may influence the development to the blastocyst stage.

Vanderzwalmen et al. (2008) investigated the association between the presence of vacuoles in sperm nuclei and the ability of embryos to develop to blastocyst stage on day 5 of development in couples in which the woman was <40 years old and at least eight oocytes were retrieved. The authors graded spermatozoa from 25 patients as follows: grade I, no vacuoles; grade II, ≤2 small vacuoles; grade III, ≥1 large vacuole; and grade IV, large vacuoles with other morphological abnormalities. The study showed that after sibling oocyte injection, no differences were observed in embryo quality on day 3 of development in the four different grades of spermatozoa. However, blastocyst formation occurred in 56.3% and 61.4% with grade I and II spermatozoa, respectively, compared with 5.1% and 0% with grade III and IV spermatozoa, respectively ($P < 0.001$). Similarly, Cassuto et al. (2009) showed that only one embryo (5.3%) developed to blastocyst stage after the injection of 19 oocytes with score 0 spermatozoa (spermatozoa presenting several head abnormalities). In addition, Knez et al. (2011) observed a higher number of blastocysts with IMSI as compared with ICSI. In addition, with IMSI 26.0% of embryos developed to the blastocyst stage after IMSI, whereas in the previous ICSI cycles all embryos were arrested at earlier developmental stages.

De Vos et al. (2013) conducted a prospective randomized sibling-oocyte study, enrolling 340 couples undergoing ICSI as a result of oligoasthenoteratozoospermia, in order to evaluate the influence of high-magnification sperm analysis on embryo development. No significant differences were observed between ICSI and IMSI for embryo development on day 3 and 5. Despite the low number of grade III and IV spermatozoa used for injection, it seems that blastocyst formation is not excluded when using these grades of spermatozoa.

### IMSI with vacuolated versus non-vacuolated spermatozoa

Berkovitz et al. (2005) investigated whether the higher rate of pregnancy was attributable to the fine nuclear morphology of the injected spermatozoa by comparing two matched IMSI groups in which the woman was <40 years old and at least three oocytes were retrieved. In one group, no spermatozoa with intact nuclei were available for microinjection, and in the other, only spermatozoa with strictly defined morphologically normal nuclei were injected. The results showed that the fertilization rate (71.3% versus 50.3%), percentage of top-quality embryos (34.9% versus 19.4%), implantation (25.0% versus 5.9%) and pregnancy rates (52.6% versus 18.4%) were significantly higher, and abortion rates (10.0% versus 57.1%) significantly lower, in the group in which only spermatozoa with morphologically normal nuclei were injected.

In a retrospective study dealing with spermatozoa and spermatids from 11 normozoospermic, 10 oligozoospermic or asthenozoospermic, four obstructive azoospermic and three nonobstructive azoospermic men, Tanaka et al. (2012) evaluated whether sperm vacuoles affected ICSI outcomes. The results demonstrated that >85% of the cells possessed vacuoles of various sizes and that this frequency was significantly higher in ejaculated cells. In addition, removal of the acrosome did not influence sperm vacuolization. There was no difference in the fertilization rate when spermatozoa with large or small vacuoles and spermatozoa



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with no vacuoles were injected. However, a significantly lower rate of development to the blastocyst stage was observed when spermatozoa with no vacuoles was injected.

### IMSI and preimplantation genetic screening (PGS)

Figueira Rde et al. (2011) examined the effect of sperm morphology under high magnification on embryo chromosomal status in 30 ICSI–PGS and 30 IMSI–PGS cycles performed in couples of advanced maternal age in a prospective randomized trial. Biological and clinical outcomes were compared. The data showed a significantly increased incidence of sex chromosome aneuploidy in ICSI embryos compared with IMSI embryos (23.5% versus 15.0%, respectively), and the incidence of chaotic embryos was also significantly higher under the ICSI procedure (27.5% versus 18.8%). In addition, the proportion of cycles without embryo transfer was significantly higher in ICSI–PGS cycles (11.8% versus 2.5%). Furthermore, the authors reported an unexpected significant difference in gender incidence rates of euploid embryos. 'Best looking' spermatozoa seemed to carry a higher proportion of the X chromosome. In a recent study performed by the same group, Setti et al. (2012) confirmed the aforementioned finding, showing a significantly higher incidence of XX embryos derived from IMSI compared with ICSI cycles (66.9% versus 52.5%, respectively). It is noteworthy that the study evaluated the gender of all embryos that were biopsied and did not consider which of these embryos were transferred, implanted and resulted in live births.

### IMSI with testicular spermatozoa

Ai et al. (2010) investigated whether IMSI with testicular spermatozoa improves the clinical outcome in patients with azoospermia. A total of 66 azoospermic patients were provided with conventional ICSI and 39 with IMSI. The results showed no difference between groups regarding pregnancy rates; however, the rate of early abortion was significantly lower in the IMSI group compared with the ICSI group (4.5% and 11.8%, respectively).

### IMSI in patients with globozoospermia and macrocephalic sperm head syndrome

The high magnification approach is also of particular benefit when used in situations in which the identification of specific sperm organelles is required, such as the acrosomal components in cases of globozoospermia. Sermondade et al. (2011) reported a successful pregnancy and healthy childbirth in a case of total globozoospermia after IMSI.

Chelli et al. (2010) studied the chromosomal content of spermatozoa selected by IMSI in two cases of macrocephalic sperm head syndrome. FISH was performed in selected spermatozoa with normal-sized heads after IMSI selection. However, of the six spermatozoa that could be selected, all were aneuploid.

### IMSI drawbacks

Sperm selection under high magnification is performed using a glass-bottomed dish that is appropriate for Nomarski microscopy. On the other hand, the ICSI procedure is performed with a plastic-bottomed dish that works with Hoffman modulation contrast. Therefore, it is important to emphasize that switching between the two systems requires additional time, delaying the injection procedure.

In addition, high magnification requires the use of an appropriate video camera and software system, which is able to provide digital zoom, an aspect that make MSOME and IMSI very expensive approaches. It is noteworthy that for IMSI, the optical magnification uses ranges from ×1000 to ×1500 and the additional magnification (×6600 and so on) involves digital magnification with no further gain in resolution.

Berkovitz et al. (2005) mentioned that the selection process has an average range of duration between 1.5–5 h. Indeed, Balaban et al. (2011) demonstrated that the duration of the procedure was significantly longer in the IMSI group as compared with the ICSI group (13.6 min versus 20.5 min; $P < 0.001$). Having said that, the extra time necessary for sperm selection and the elevated equipment costs are a limitation to a more widespread use of IMSI.

To date, a single study reported a potential harmful impact of IMSI on the outcomes (Junca et al., 2010). A significantly higher incidence of low birthweights for IMSI infants was observed as compared with ICSI (29.1% versus 23.1%).

## Conclusions

Sperm selection methods are an important challenge in assisted reproduction because most sperm characteristics cannot be tested, either in real time or in single cells referred to the ICSI procedure. Sperm selection under a magnification of ×400, in preparation for ICSI, allows the identification of major sperm morphological defects but does not provide information regarding the nuclear status of the sperm cell.

An interesting solution was introduced with the advent of MSOME, which is performed prior to the IMSI procedure, under an overall optical magnification of at least ×6000, enabling the selection of spermatozoa free of nuclear vacuoles, which are related to blockage of embryonic development during and/or after implantation. Therefore, IMSI has been proposed as an alternative to routine ICSI, initially for couples with repeated ICSI failures and subsequently for couples with increased rates of DNA-fragmented spermatozoa.

The efficiency of IMSI with regard to subsequent fertilization, embryo development, implantation, pregnancy and miscarriage rates has been the focus of several studies; however, the results are controversial. These conflicting results might have occurred due to differences in inclusion criteria, stimulation protocols, seminal and oocyte qualities and many other confounding variables within the IVF cycles.

In general, studies have not observed significant differences in fertilization rate following ICSI and IMSI. It has been suggested that IMSI is not beneficial at improving the early paternal effects (Mauri et al., 2010). Clinical evidence from assisted reproduction suggests that failure to complete the fertilization process, syngamy or early cleavage might be the result of an early paternal effect (Barroso et al., 2009).



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This phenomenon may also account for the similar results obtained with embryo quality after ICSI and IMSI. Another important fact that could have influenced this outcome is the day of development at which the top-quality embryo rate was calculated.

On the other hand, the late paternal effect is characterized by poor embryo development to blastocyst stage, implantation failure and pregnancy loss and is associated with sperm abnormalities at the level of DNA chromatin. Despite some controversies, several studies observed tendencies or significantly better outcomes, mainly in blastocyst formation, pregnancy and implantation rates, following the utilization of IMSI compared with conventional ICSI. Therefore, it seems that IMSI is effective in overcoming the late paternal effects.

In the light of these findings, MSOME seems to be a surrogate tool for the selection of strictly morphologically normal spermatozoa prior to oocyte injection, resulting in higher rates of embryonic development, blastocyst formation, implantation and pregnancy. It is noteworthy that more prospective randomized trials are required to confirm the superiority of IMSI over conventional ICSI and to identify the causes of infertility that could benefit from the IMSI procedure. Nevertheless, as Vanderzwalmen and Fallet (2010) proposed: 'Are there any indications to not select the best spermatozoa? Of course not.'

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