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Review

MSOME and IMSI: reasonable rationale, selective clinical value

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Summary

Purpose: Motile sperm organelle morphology examination (MSOME) is a real-time high magnification motile sperm examination applied prior to intracytoplasmic morphologically selected sperm injection (IMSI) - intracytoplasmic injection of the selected spermatozoon. Over the past decade, there is an important shift concerning MSOME, focusing on the shape of the sperm head and presence of vacuoles, resulting with different selection criteria. The authors investigated the relevance of sperm vacuoles phenomenon. From a clinical perspective they focused on prospective trials aiming to distinguish scenarios in which IMSI may improve clinical outcome compared with intracytoplasmic sperm injection (ICSI). *Materials and Methods:* The authors performed this review based on a primary literature search of publications, which as of November 1, 2017, were listed in PubMed under the search phrase <motile-sperm organelle morphology examination>, <MSOME>, <intra-cytoplasmic morphologically selected sperm injection>, <IMSI>, and <sperm vacuoles>. The references of these manuscripts were further reviewed when considered relevant to the subject. *Results:* Inadequate chromatin compaction leads to higher rate of DNA fragmentation and aneuploidies, which may be followed by the appearance of large vacuoles. Distinguishing between large and small vacuoles remains a challenge. IMSI may expand blastocyst formation in cases of previous embryos developmental arrest and may be used in cases of isolated teratospermia and repetitive ICSI cycles failures. *Conclusions:* Solid evidence supports the association between non-adequate sperm DNA compaction and DNA defects, resulting with abnormal vacuoles. Current prospective studies emphasize ICSI as first treatment for male factor during assisted reproductive technology (ART), while the role of IMSI in selective clinical scenarios warrants further large randomized controlled studies.

Key words: MSOME; IMSI; ICSI; Male factor.

Introduction

Male infertility is a major etiology for infertility, accounting for 20-50% among couples referred for in vitro fertilization (IVF) [1]. The revolutionary introduction of intracytoplasmic sperm injection (ICSI) procedure a quarter of a century ago, supplied patients with severe male factor reasonable chance for biological parenthood and opened a new era in the field of assisted reproductive technology (ART). However, 3-5% of patients fail to conceive after IVF-ICSI [2], representing clinical challenge for fertility specialists.

Advanced sperm selection techniques, e.g. sperm apoptosis, sperm birefringence, ability to bind to hyaluronic acid, and sperm morphology under ultra-high magnification, are increasingly being employed in ART, most commonly aiming to improve IVF-ICSI outcome. By a better selection of sperm for intracytoplasmic injection, it is assumed that a structurally intact and mature sperm with high DNA integrity will be chosen with the consequent improved IVF-ICSI outcome. A recent Cochrane review aiming to evaluate the impact of advanced sperm selection techniques on ART outcomes could not find sufficient evidence to allow review authors to determine whether sperm selected by hyaluronanic acid binding, sperm apoptosis, sperm birefringence or surface charge have any additive value over conventional selection [3]. They therefore concluded that further studies of suitable quality are required to evaluate whether any of these advanced sperm selection techniques can be recommended for use in clinical practice.

Motile sperm organelle morphology examination (MSOME) is a real-time high magnification motile sperm examination performed using an inverted microscope equipped with high-power differential interference contrast (DIC) optics (magnification ×150) enhanced by digital imaging (magnification ×44) to achieve a total magnification of over ×6,000 [4]. This approach, primarily introduced by Bartoov *et al.* [5] is currently applied prior to performing intracytoplasmic morphologically selected sperm injection (IMSI), which consists of the intracytoplasmic injection of a spermatozoon that has been selected at high magnification. In their preliminary study of 24 normal-responder young (< 37 years) patients with previous failure of at least five consecutive routine cycles of IVF-

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ICSI, 58% pregnancy rate was achieved following IMSI.

While the first report by Bartoov *et al.* [4] was promising, multiple meta-analyses comparing ICSI with IMSI have yielded conflicting results for pregnancy and birth rates, which in contrast to ICSI, despite all those years of clinical experience, avoid the spread and the routine clinical application of IMSI. Moreover, selecting the patients who might benefit from the IMSI remains a cumbersome obstacle.

In this review, the authors will describe the physiological and pathological rationale for IMSI, discuss the possible clinical scenarios in which IMSI may be considered, and finally will suggest possible directions for future studies.

Materials and Methods

The authors performed this review based on a primary literature search of publications, which as of December 31, 2018, were listed in PubMed under the search phrase <motile-sperm organelle morphology examination>, <MSOME>, <intra-cytoplasmic morphologically selected sperm injection>, <IMSI>, and <sperm vacuoles>. The references of these manuscripts were further reviewed when considered relevant to the subject.

Basic research

Normal sperm morphology - WHO

First sperm morphology investigators examined spermatozoa as a whole (head, mid piece, and tail). Moreover, the definition of "normal" potentially fertilizing spermatozoal morphology was derived from observations on spermatozoa recovered from the female reproductive tract [6] and the surface of the zona pellucida [7].

According to the WHO [2010], "for a spermatozoon to be considered normal, both its head and tail must be normal. The head should be smooth, regularly contoured, and generally oval in shape. There should be a well-defined acrosomal region comprising 40–70% of the head area [8]. The acrosomal region should contain no large vacuoles, and not more than two small vacuoles, which should not occupy more than 20% of the sperm head. The post-acrosomal region should not contain any vacuoles. The midpiece should be slender, regular, and about the same length as the sperm head. The major axis of the midpiece should be aligned with the major axis of the sperm head. Residual cytoplasm is considered an anomaly only when in excess, i.e. when it exceeds one-third of the sperm head size [9]. The principal piece should have a uniform calibre along its length, be thinner than the midpiece, and be approximately about ten times the head length. It may be looped back on itself, provided there is no sharp angle indicative of a flagellar break". Moreover, according to the WHO [2010], an abnormal head morphology should be considered whenever the sperm head is large or small, tapered, pyriform, round, amorphous, vacuolated (more than two vacuoles or >20% of the head area occupied by unstained vacuolar areas), vacuoles in the post-acrosomal region, small or large acrosomal areas (<40% or >70% of the head area), double heads, or any combination of these [10].

Normal sperm morphology – MSOME

MSOME evaluates the morphological state of six subcellular organelles [5]: acrosome, postacrosomal lamina, neck, mitochondria, tail, and nucleus. The first five of these subcellular organelles are considered morphologically normal according to an arbitrary descriptive approach adopted in Bartoov *et al.* [11] studies examining sperm by transmission and scanning electron microscopy. The criteria for a normally shaped nucleus by MSOME were smooth, symmetric, and oval configurations. An extrusion or invagination of the nuclear chromatin mass was defined as a regional nuclear shape malformation. The nuclear chromatin content was considered abnormal if it contained one or more vacuoles that occupied more than 4% of the normal nuclear area. A sperm cell exhibiting a normal nucleus as well as a normal acrosome, postacrosomal lamina, neck, tail, mitochondria, and no cytoplasmic droplet or cytoplasm around the head was classified as morphologically normal. According to Bartoov *et al.* [5], the frequency of morphologically normal spermatozoa as defined by the WHO [12] did not correlate to the frequency of morphologically normal spermatozoa as defined by MSOME.

The development of MSOME classifications

Over the past decade there is an important shift, focusing on the shape of the sperm head and most importantly the presence of vacuoles [13]. The criteria for a normally shaped nucleus by MSOME, were smooth, symmetric, and oval configurations and the nuclear chromatin content was considered abnormal if it contained one or more vacuoles that occupied more than 4% of the normal nuclear area. A sperm cell exhibiting a normal nucleus as well as a normal acrosome, postacrosomal lamina, neck, tail, mitochondria, and no cytoplasmic droplet or cytoplasm around the head was classified as morphologically normal. According to Bartoov et al [5], the frequency of morphologically normal spermatozoa as defined by the WHO [1999] did not correlate to the frequency of morphologically normal spermatozoa as defined by MSOME.

On 2008, Vanderzwalden *et al.* introduced a more sophisticated approach towards sperm vacuoles by MSOME and their clinical significance. That classification system took a further step forward by focusing not only on single criteria for normal vacuole size as suggested by Bartoov *et al.* but also focused on vacuoles number. The authors classified sperm vacuoles to: grade I, no vacuoles, grade II, ≤ two small vacuoles (< 4% of head volume), grade III, ≥ 1 large vacuole, and grade IV, large vacuoles with other abnormalities [14]. The authors reported a significant impact on blastocyst formation among 25 patients after sibling oocyte injection - the occurrence of blastocyst formation was 56.3 and 61.4% with grades I and II spermatozoa, respectively,

Table 1. — *Classifications systems for MSOME - IMSI.*

Reference	Classification	Comments
Bartoov [4]	Normal nucleus - oval shape with a smooth configuration (length, 4.75 ± 0.28 μm; width, 3.28 ± 0.20 μm) and a normal nuclear content (< 4% of the nucleus occupied by vacuoles)	Initial MSOME classification
Vanderzwalmen [14]	Grades: I) no vacuoles; II) ≤ 2 small vacuoles; III) ≥ large vacuole; IV) large vacuoles with other abnormalities	Small vacuoles defined as <4% of head volume
Cassuto [15]	(Normal head = 2) + (lack of vacuole = 3) + (Normal base = 1) = (Total score = 6)	Sperm head according to WHO criteria; vacuoles divided by absent/small (scored 1) *vs*. large (scored 0)
Pedrix [16]	RVA (%)* = [vacuole area (μm(2)) / head area (μm(2))] × 100)]}	RVA of 5.9% and 12.4% for normal and abnormal thresholds, respectively

**RVA - relative vacuole area to sperm head.*

compared with 5.1% with grade III and 0% with grade IV respectively ($p < 0.001$).

In a consequent classification system Cassuto *et al.* added sperm head and base to and introduced the formula: (2 X head) + (3 X vacuoles) + (base). Each of the three components is scored as 1 (normal) or 0 (abnormal), as the highest impact is given to vacuoles component with total score range of 0-6. Sperm head assessment is performed according to WHO criteria (described above) and vacuoles evaluation is divided either absent/small (scored 1) *vs*. large (scored 0) for normal and abnormal, respectively. Accordingly spermatozoa are classified as high- (4-6), medium- (1-3), and low–quality (0). The authors reported not only higher fertilization rate but also improved blastocyst formation by microinjection of higher scored spermatozoa [15].

An interesting study of Pedrix *et al.* have not only distinguished between 'large" and "small' vacuoles but also correlated vacuoles' appearance by digital imaging system and conventional semen parameters in 440 semen samples. They suggested two different cut-offs: 1) relative vacuole area to sperm head (RVA) of 5.9% which represented as normal threshold with a sensitivity of 0.76 and specificity of 0.56, and 2) RVA occupying 12.4% of abnormal threshold with a specificity of 1 and sensitivity of 0.09 [16]. The advantage of that classification system over others is the objective RVA measurement (opposed to conventional subjective selection [17]) and large sample size which supplied adequate statistical analysis. Furthermore, RVA calculation combines both vacuoles' number and size and consequently has the potential to be more reliable than relying on vacuoles' size alone (Table 1).

The lack of consistency and certainty regarding sperm vacuoles limits the value of MSOME in selecting high-quality spermatozoa [18, 19]. Most importantly, vacuoles' nature, origin, localization, and clinical significance is still a matter of debate, resulting with significant efforts to improve our understanding and establish reliable definition criteria.

Vacuoles origin – nucleus or acrosome?

The presence of vacuoles with the sperm head has been reported decades ago [20]. However, its significance is still controversial. Since most vacuoles are located in the anterior aspect of the sperm head, their origin have been speculated to derive either from the nucleus or the acrosome. In their important experiment, Gatimel *et al.* have demonstrated the existence of sperm vacuoles among patients with globozoospermoa – an abnormal sperm shape and function due to the loss of the acrosome – confirming that these substructures derive from the nucleus [21].

Boitrelle *et al.* [18] used MSOME to select 'top' spermatozoa and spermatozoa with a large vacuole but otherwise normal in an attempt to establish a relationship between one type of vacuole morphology and nuclear status. They studied a total of 450 'top' spermatozoa and 450 vacuolated spermatozoa. The rate of non-condensed chromatin was higher for 'vacuolated' spermatozoa than for 'top' spermatozoa. However, 'top' and 'vacuolated' spermatozoa did not differ significantly in terms of DNA fragmentation or aneuploidy. In all vacuolated spermatozoa, the acrosome was intact, the plasma membrane was sunken but intact, and the large vacuole was identified as an abnormal, 'thumbprint'-like nuclear concavity covered by acrosomal and plasmic membrane, suggesting that the large vacuole appears to be a nuclear 'thumbprint' linked to failure of chromatin condensation [18]. On the other hand, Kacem *et al.* [19] have demonstrated that induction of the acrosomal reaction by ionophore significantly increased the percentage of vacuole-free spermatozoa, suggesting that most nuclear vacuoles are of acrosomal origin. Hence, the best spermatozoa selected by MSOME are mostly acrosome-reacted spermatozoa. Thus, IMSI improves IVF outcome by allowing the selection of acrosome-reacted spermatozoa.

Are the vacuoles physiological or pathological features?

MSOME classification development is characterized by increasing focus on sperm vacuoles rather than other sperm organelles and differentiation between small and large (representing normal and abnormal, respectively) vacuoles. However, their characterization remains a prominent challenge mainly due to lack of homogeneous criteria between different studies. On the one hand, several evidences supported their physiological role in sperm production and

function and declined their possible association with infertility.

Tanaka *et al.* investigated the incidence, size, and position of vacuoles in spermatozoa and spermatids from 11 normozoospermic, ten oligozoospermic or asthenozoospermic, four obstructive azoospermic, and three non-obstructive azoospermic men. They reported gradually increasing vacuoles prevalence within the male genital track from 33.7% of spermatids followed by 87.5% in epididymal spermatozoa and 98.3% in the ejaculate [20]. Interestingly, small vacuoles (< 25% of total sperm head) were more frequent in ejaculated spermatozoa opposed to large vacuoles (> 50% of surface area) dominance in testicular spermatids, further emphasizing the differentiation between small and large vacuoles. Montjean *et al.* demonstrated that acrosome reaction (induced either by hyaluronic acid or follicular fluid) is significantly related to vacuoles appearance within the sperm head, suggesting their physiological role in that process among 35 healthy participants [22]. Moreover, Fortunato *et al.* have recently declined the negative correlation between vacuoles and live birth, as well as, disorganization of the sperm DNA among 873 men enrolled for assisted reproduction techniques [23]. Importantly, both Montjean *et al.* [22] and Fortunato *et al.* [23] used Bartoov *et al.*'s criteria [4], as one or multiple vacuoles occupied at least 5% of the total sperm head surface (Bartoov *et al.*'s criteria), whatever their localization.

The association between large sperm vacuoles and DNA abnormalities

Most researchers have chosen to focus on large vacuoles [24], assuming these vacuoles are more likely to have pathological significance. Consequently, while data regarding small vacuoles is currently contradictory between normal and pathological significance [25], accumulating evidence support the notion that large vacuoles are related to several DNA abnormalities. During the sophisticated process of spermatogenesis, the DNA of the differentiating sperm undergoes complex nuclear packaging involving the replacement of histones with protamines, leading to highly condensed and protected DNA to ensure safe passage within the male and female genital tracts [26, 27]. The presence of a large sperm-head vacuole was reported to be associated with failure of chromatin condensation and/or DNA damage and/or aberrant chromosome numbers in spermatozoa [28-30].

Abnormal chromatic compaction

Various studies demonstrated abnormal sperm chromatic compaction in spermatozoa with large vacuoles compared to normal spermatozoa assessed by MSOME. For example, Franco *et al.* described that 67.9% of spermatozoa with large vacuoles (defined as > 50% of sperm head area) had abnormal DNA compaction compared to 33.1% of normal spermatozoa [28]. Similarly, Boitrelle *et al.* reported 36.2% *vs*.7 .6% abnormal compaction, respectively, using large vacuoles cutoff as > 25% of sperm head area. Comparison between sperm with large vacuoles and unselected spermatozoa demonstrated identical findings [18]. Similarly, Pedrix *et al.* assessed by aniline blue staining compared among 20 patients with teratospermia. They reported significantly altered chromatin condensation in spermatozoa with large vacuoles (defined as > 13% of sperm head area) compared to normal spermatozoa [30]. Therefore, in spite of the variable criteria for large vacuoles definition, accumulating evidence support the notion that these features are related to non-efficient chromatin compaction.

Increased risk for DNA fragmentation

Disorganization of sperm chromatin exposes it to damage through various mechanisms such as enzymatically induced DNA breaks, radical oxidants species, and others [31], resulting with DNA fragmentation and male infertility [26]. Recently, Pastuszek *et al.* examined DNA fragmentation among almost 4,000 spermatozoa from ten patients selected by MSOME. They demonstrated that abnormal Vanderzwalden classification was associated with increased DNA fragmentation percentage, especially resulting from double-strand DNA breaks [32]. Hammoud *et al.* classified spermatozoa from eight patients according to motility and morphology alone by either ×200 or ×6,300 magnifications (representing ICSI and IMSI, respectively), and reported significantly lower DNA fragmentation among normal spermatozoa selected by MSOME [33]. Increased DNA fragmentation was reported as well among spermatozoa in with large vacuoles defined either by 5-50% or > 50% of sperm head area [28, 34]. More specifically, Utsuno *et al.* demonstrated statistically significantly higher percentage of DNA fragmentation in spermatozoa with abnormal ellipticity and abnormal angularity than in spermatozoa with normal-shaped heads (6.1% and 5.4% *vs*. 2.8%) on MSOME. Spermatozoa with large nuclear vacuoles also correlated with sperm DNA fragmentation, and had a statistically significantly higher percentage of DNA fragmentation (4.7%) [35]. In their review, Boitrelle *et al.* emphasized that DNA fragmentation association with spermatozoa vacuoles is mainly relevant in semen samples with overall high DNA fragmentation [36].

In light of these multiple and repetitive findings, it seems that large vacuoles are associated with impaired of chromatin organization and increased DNA fragmentation. However, one of the few studies which focused on small vacuoles demonstrated DNA disorganization in cases of multiple compared to single vacuole [25]. Therefore, there is a possibility that both vacuoles' size and number are related to DNA in efficient packaging.

Numerical chromosomal abnormalities

In addition to DNA fragmentation, several researchers evaluated the possible correlation between sperm selection

methodology and sperm aneuploidy. Levron *et al.* reported that motility-based selection by ICSI among eight patients with male factor and repeated implantation failure resulted with significantly lower sperm chromosomes X, Y and 18 aneuploidy compared to morphology-based selection [37]. Garolla *et al.* performed CGH for individual spermatozoa with either large vacuoles, small vacuoles, or no vacuoles. Sperm selected from infertile patients showed a significantly higher percentage of abnormal molecular karyotypes than controls (19.4 *vs.* 7.7%, respectively). Interestingly, sperm with large vacuoles and small vacuoles showed 38.3% and 20.0% abnormal karyotype in infertile men versus 18.3 and 5.0% in controls, respectively ($p < 0.01$). Moreover, complex abnormalities were found only in the large vacuoles category. Abnormal karyotype was never found in normal sperm [38]. Although that study included only three patients in each group, it confirmed the association between vacuoles and DNA abnormalities and further emphasizes the pathological significance of the large vacuoles. Pedrix *et al.* reported similar results with significantly higher aneuploidy and diploidy rates among semen samples with large sperm vacuoles compared to controls [30]. In spite of these findings, these results are not sufficient to confirm an absolute impact on embryo aneuploidy. The present authors have recently reported that abnormal DFI (a possible reflection of abnormal sperm vacuoles) had no correlation with blastocyst aneuploidy examined by PGS-CGH after ICSI [26, 27]. Although PGS limitations have been reported exclusively over the last few years [39, 40], similar study design which will investigate embryo euploidy after IMSI may supply important data on that important subject.

In conclusion, multiple and various studies demonstrates convincing data regarding the association between large sperm vacuoles and DNA abnormalities. Utsuno *et al.* compared 2,400 normal spermatozoa with 2,400 protamine-deficient spermatozoa among 36 infertile men and reported that protamine deficiency is related not only to abnormal sperm head morphology, but also associated with higher DNA fragmentation and large nuclear vacuoles' appearance [41]. It seems that inadequate chromatin compaction leads to higher rate of DNA fragmentation and aneuploidies, which are followed by the appearance of the large vacuoles. On the other hand, small sperm vacuoles are probably physiological phenomenon and may be related to sperm maturation within the male genital track. Distinguishing between large and small vacuoles remains a challenge, and clear cut-off criteria are further to be established and confirmed. In light of that inconsistency, clinical studies have been conducted in efforts to evaluate the clinical significance.

Clinical studies

Can IMSI replace ICSI in selective clinical scenarios?

Various studies have attempted to implement IMSI into clinical usage instead of the gold standard ICSI procedure. While some prospective studies were performed (most of them more than five years ago), most clinical researches had a retrospective design. Overall, these studies are characterized by very wide heterogeneity, specifically with regards to inclusion criteria in which IMSI may be used. A recent Cochrane review comparing the effectiveness and safety of IMSI and ICSI in couples undergoing ART [42] found no effect on live birth or miscarriage nor evidence that IMSI improves clinical pregnancy. They concluded that the results from RCTs do not support the clinical use of IMSI and further trials are necessary to improve the evidence quality before recommending IMSI in clinical practice. However, these results may be attributed to different patient populations and MSOME classification included in the meta-analysis. Although numerous retrospective trials have been investigated the impact of IMSI within the clinical settings, for the purpose of the current review, the present authors decided to focus on prospective studies which obviously supply a higher quality of scientific evidence. Most importantly, substantial egg factor was excluded by either excluding female patients above age of 40 (and even younger age in others) or by including patients with at least 6-8 retrieved eggs. However, as presented in Table 2, these studies lack uniform methodologies such as inclusion criteria, comparison between study and control groups, and MSOME classification criteria.

Late paternal affect and blastocyst formation

Based on the above observations, it is not surprising that abnormal sperm vacuoles, related mainly to sperm DNA abnormalities, have been suggested to have detrimental impact on human reproduction. Specific attention was focused on differentiating between early and late paternal affects, which are attributed to oocyte activation *vs*. paternal DNA activation on day 3 after fertilization, respectively [43].

Vanderzwalmen *et al.* [14] reported significantly higher blastocyst formation among couples with grades I-II MSOME classification (56.3%-61.4%) compared to grade III and IV (5.1% and 0%, respectively, $p < 0.0001$, described above). In their prospective study, Setti *et al.* performed MSOME immediately after ICSI among couples undergoing first IVF-ICSI cycles due to male factor. They reported that large vacuoles (defined as > 13% of sperm head area) were mainly associated with impaired blastocyst formation including lower rates of normal inner cell mass and trophoectoderm development. Additionally, they demonstrated reduced number of blastomeres in earlier days 2 and 3. Importantly, these developmental modifications were not related to conventional sperm morphology assessment [44]. In their randomized prospective study, Knez *et al.* included infertile couples with abnormal semen analysis in whom all embryos in previous ICSI cycle were arrested prior to blastocyst stage. Couples were divided to IMSI group (n=20 using the Cassuto *et al.* classification

Table 2. — *Prospective investigations for possible clinical indications for IMSI.*

Indications	Ref.	Population	MSOME classification	Design	n	Results
Blastocyst formation	[14]	Male factor	Vanderzwalmen [14]	Sibling oocyte injection with different spermatozoa's grading	25	Significantly higher blastocyst formation with grades I and II spermatozoa compared with grades III and IV.
	[44]	Male factor and first ART	Large vacuole occupying >13% of sperm nuclear area	Non-randomized	60	Large vacuoles associated with decreased blastocyst formation / normal tropho-ectoderm and inner cell mass.
	[45]	Male factor and arrested embryos in previous ICSI	Cassuto [15]	Randomized	IMSI 20, ICSI 37	IMSI: higher number of blastocysts per cycle; significantly lower number of cycles with all arrested embryos and cycles with no transfer.
Previous ART failures	[48]	Male factor and 1-3 IVF cycle	Bartoov [4]	Randomized	IMSI 227, ICSI 219	IMSI: higher clinical pregnancy rate selectively in two previous cycles.
	[49]	≥ 2 previous ART failures, not related to semen analysis	Bartoov [4]	Previous cycle as control	75	IMSI: improved day 2 and blastocyst formation; higher clinical pregnancy and birth rates.
	[50]	Male factor, single previous ICSI failure	Poor morphology; vacuoles: multiple / >4% nuclear area; poor midpiece morphology.	Previous cycle as control	8	Significantly improved grade A embryos formation and implantation rate in the subsequent IMSI cycle
	[51]	Male factor, ≥2 previous implantation failures.	Vanderzwalmen [14]	Non-randomized	IMSI 90, ICSI 130	Similar implantation and live birth rates.
Male factor and first ART cycle	[52]	$>0.1\times10^6$ spermatozoa/ml, 1-10 ART cycles (188 in first cycle).	Vanderzwalmen [14]	Sibling oocytes randomized to IMSI *vs*. ICSI	350	Similar fertilization rate, similar blastocyst formation, and clinical outcome (embryo transfer not randomized).
	[53]	$>3\times10^6$ spermatozoa; $<1\times10^6$ motile spermatozoa after density gradient	Pedrix [16]	Randomized	IMSI 116, ICSI 132	Similar clinical outcome, lower fertilization rate, and number of embryos in IMSI group.
	[50]	$1\text{-}20\times10^6$ spermatozoa/ml	Poor morphology; vacuoles: multiple / >4% nuclear area; poor midpiece morphology.	Randomized	IMSI 125, ICSI 125	IMSI: Significantly higher rate of grade I embryos; significantly increased pregnancy and implantation rates.
Isolated teratospermia	[54]	Average of 2.6 and 2.1 previous ART cycles in both subgroups	Vanderzwalmen [14]	Randomized	IMSI 52, ICSI 70	IMSI: significantly higher clinical pregnancy rate, significantly higher number of morulae, and lower number of arrested embryos; significantly higher blastocyst formation using spermatozoa ranked I and/or II compared to III and/or IV.
	[51]	First-second ART cycle	Vanderzwalmen [14]	Non-randomized	IMSI 132, ICSI 126	IMSI: significantly higher number of fertilized oocytes and cleaved embryos; significantly higher top-quality embryos; significantly higher implantation and live birth rates.
Unselected infertile population	[55]		Bartoov [4]	Randomized	IMSI 87, ICSI 81	IMSI: no difference regarding clinical outcome; significantly higher implantation rate among couples with severe male factor.

system) *vs*. repetitive ICSI (n=37). The authors reported significantly lower rate of repetitive arrested embryos in the IMSI compared to ICSI group (0% *vs*. 27%, $p < 0.05$). While clinical pregnancy rate in the IMSI group was higher as well (25% *vs*. 8.1%), it did not reach statistical significance [45]. Therefore, both Vanderzwalmen *et al.*'s [14] and Cassuto *el al.*'s criteria system support the notion that IMSI may improve blastocyst formation by improving the late paternal affect after the activation of the embryonic genome.

Recurrent ICSI failures among couples with male factor

The first report of Bartoov *et al.* [4], which included couples after five ICSI failures, pointed that clinical scenario as a potential to benefit from IMSI. Retrospective studies are generally characterized by different methodologies and therefore their scientific significance is limited. For example, Shalom-Paz *et al.* reported significantly higher pregnancy rate and reduced spontaneous abortions using IMSI compared to previous ICSI after three IVF-ICSI failures [46]. On the other hand, Gatinel *et al.*, while assessing the benefit of IMSI in patients undergoing their third ART attempt, IMSI did not improve the clinical outcomes compared with ICSI, according neither to implantation, clinical pregnancy, nor to live birth rates [47].

Most importantly, few prospective randomized trials have been performed. Antinori *et al.* performed one of the largest randomized studies focused on couples with isolated male factor in their first-third IVF cycle, including 219 couples that were treated by ICSI and compared to 227 couples treated by Bartoov *et al.*'s IMSI criteria using motile spermatozoa with normal head dimensions (length 4.75 ± 0.28 μm, width 3.28 ± 0.20 μm) and shape, and with no or a maximum of one vacuole (0.78 ± 0.18 μm). They reported that IMSI *vs*. ICSI resulted in a higher clinical pregnancy rate (39.2% *vs*. 26.5%, $p = 0.004$) and lower miscarriage rates (17.4% *vs.* 37.5%). Most importantly, a subgroup analysis demonstrated that IMSI clinical pregnancy rate superiority was isolated among patients after two previous cycles (29.9% *vs*. 12.9%, $p = 0.017$), while no significant differences were found between IMSI and ICSI in first and second cycles [48]. In another prospective study in which couples acted as their own controls, 75 infertile couples were offered IMSI after at least two previous IVF or ICSI failures. Semen parameters were not related to inclusion criteria. The researchers used Bartoov *et al.*'s criteria for spermatozoa selection for IMSI. The authors reported improved day 2 and blastocyst formation in IMSI compared to IVF/ICSI cycles (89.8% *vs.* 79.8%, $p = 0.009$ and 41.3% *vs.* 26.7%, $p = 0.04$, respectively), as well as higher clinical pregnancy and birth rates of 29.3% and 18.6%, respectively [49]. A similar study design, which included eight couples after single ICSI failure who had identical ovarian stimulation protocol and IMSI in their subsequent cycle, resulted with significantly improved grade A embryos formation (83.6% *vs*. 60.3%) and implantation rate (20.8% *vs*. 0%) in the subsequent IMSI cycle, resulting with three pregnancies and five live births. It should be noted that the authors selection of MSOME criteria included poor morphology, presence of multiple vacuoles, presence of vacuoles over 4% of area, and poor morphology of midpiece [50]. Opposite to these reports, El Khattabi *et al.* reported a similar implantation rate (16.1% *vs*.16.7%, $p = 0.77$) and live birth rate (21% *vs*. 22%, $p > 0.99$) between IMSI by Vanderzwalmen *et al.*'s criteria (n=90) and ICSI (n=130) among couples with mild male factor who had at least two previous implantation failures after transfers of good-quality embryos [51].

In conclusion, there are is evidence which supports recurrent IVF/ICSI failures with male factor as a possible indication for IMSI. However, data is inconsistent between studies, possibly due to various spermatozoa selection criteria during MSOME and the definition of repeated IVF failure (cycle rank). Further studies with homogenous methodologies should be performed prior to routine applicability.

Couples with male factor during first ART

De vos *et al.* conducted a prominent research, during which sibling oocytes were randomized to IMSI *vs*. ICSI in couples with male infertility ($> 0.1 \times 10^6$ spermatozoa/ml) without female factor. Out of 350 cycles, 188 were first ART cycle while 72, 50, and were 27 second, third, and fourth cycles, respectively. The researchers used Vanderzwalmen *et al.*'s criteria for IMSI. In addition to similar fertilization rate (79.1% and 77.3%, respectively, $p = 0.22$), the authors reported similar preimplantation development up to blastocyst stage and clinical outcome although embryo transfer was not randomized [52]. The researchers should be acknowledged for the strict methodology and large sample size of 350 cycles which supply a high level of scientific reliability to their findings. Although the study included patients with various ART attempts, the present authors believe the results mostly reflect first cycle (53.7% of total cases). Another prospective randomized trial included couples with male factor (at least three million of spermatozoa in the ejaculate and less than one million of motile spermatozoa recovered after density gradient with whatever sperm morphology) during first ART and compared IMSI (n=116) using Pedrix *et al.*'s criteria (described above) *vs.* conventional ICSI (n=139). No statistically significant difference was observed between the two groups with regard to the rates of implantation (IMSI: 24%; ICSI: 22%; NS), ongoing pregnancy (IMSI: 31%: ICSI: 33%, NS) and birth (IMSI: 27% ICSI: 30%; NS). Interestingly, the fertilization rate was significantly lower in the IMSI group (56 ± 25 *vs*. 63 ± 23, $p < 0.05$), as well as the total number of embryos (4.8 ± 3.2 *vs*. 5.8 ± 3.9; $p < 0.05$), and the number of frozen embryos (1.4 ± 2.3 *vs*. 2.2 ± 3.0; $p < 0.05$). The authors speculated that the technical con-

straints of IMSI (time) affect the quality of gametes. However, such a deleterious effect of IMSI has not been reported in other studies [53]. Taken together, these prospective studies decline isolated male factor as an indication for IMSI in first ART cycle. On the other hand, Wilding *et al.* performed a randomized controlled trial comparing between ICSI and IMSI (125 patients in each group) among couples with male factor (sperm count 1-20 million per ml) without describing ART cycles history. Selection MSOME criteria were poor morphology, presence of multiple vacuoles, presence of vacuoles over 4% of area, and poor morphology of midpiece. The rate of fertilization was not significantly different between the cohorts. The authors reported a significantly higher rate of grade I embryos in the IMSI group compared to conventional ICSI (98.6% and 66.0% of transferred embryos, respectively, $p < 0.05$). The pregnancy rate of patients undergoing IMSI was significantly increased with respect to the ICSI controls (65.6% *vs*. 40.0%, $p < 0.05$). The implantation rate of embryos created with IMSI procedures was greater than that of standard ICSI techniques [50]. Unfortunately, the lack of ART cycle number is a major drawback of the study especially as the current literature differentiates IMSI implementation between couples undergoing their first cycle and those with previous ICSI failures.

Isolated teratospermia

An interesting optional indication for IMSI is male factor with isolated teratospermia, since the main theoretical advantage of MSOME is detailed morphological evaluation. In their prospective randomized study, Knez *et al.* reported significantly higher clinical pregnancy rate in the IMSI group (n=52 using Vanderzwalmen *et al.*'s criteria) compared with ICSI group (n=70, 48% *vs*. 24%, respectively, $p < 0.05$). Couples in the IMSI group had an average of 2.6 previous ART cycles compared to 2.1 in the ICSI group. After IMSI, a statistically higher number of morulae developed and a lower number of embryos arrested at low-cell developmental stages compared to ICSI (21% *vs*. 13%, $p < 0.05$; and 44% *vs*. 62%, $p < 0.01$, respectively). In a further analysis the authors demonstrated significantly higher blastocyst formation using spermatozoa ranked I and/or II compared to III and/or IV, supporting previously report of Vanderzwalmen *et al.* [54]. El Khattabi *et al.* compared IMSI (using Vanderzwalmen *et al.*'s criteria) and ICSI among patients with teratospermia in their first-second ART cycle. The number of fertilized oocytes (5.2 *vs*. 4.3, $p = 0.029$) and cleaved embryos (5.3 *vs*. 4.4, $p = 0.03$) were significantly higher in the IMSI subgroup (n=132). Additionally, a significantly higher number of top-quality embryos (2.3 *vs*. 1.7 [$p = 0.009$] at day 2; 1.7 *vs*. 1.1 [$p = 0.003$] at day 3, and good-quality embryos 3.1 *vs*. 2.4 [$p = 0.026$] at day 2, 3.3 *vs*. 2.3, [$p = 0.019$] at day 3) was observed after IMSI compared with the ICSI subgroup (n=126). Most importantly, an implantation rate (30.7 *vs*. 20.1%, p=0.007) and live birth rate (38% *vs*. 20%, $p = 0.002$) were significantly higher after IMSI compared with ICSI [51]. Taken together, both studies supply convincing data regarding the effectiveness of IMSI using Vanderzwalmen *et al.*'s criteria for couples with isolated teratospermia. However, it should be noted that in Knez *et al.* study, the average of previous ART cycles were 2.6 and 2.1 in the IMSI and ICSI groups, respectively. Therefore IMSI superiority may be explained not only by the teratospermia selection but alternatively by previous failures.

Other indications

The rationale for MSOME as improved normal spermatozoa detector compared to ICSI has led few researchers to prospectively investigate IMSI outcome in first ART cycles among *unselected infertile patients*. Balaban *et al.* failed to demonstrate significant improvement in the clinical outcome using IMSI using Bartoov *et al.*'s MSOME criteria. However, specifically severe male factor patients in the IMSI group had significantly higher implantation rates compared with their counterparts in the ICSI group (29.6% *vs*. 15.2%, $p = 0.01$). Unfortunately, number of ART cycle was not reported [55]. The lack of efficiency of MSOME among unselected patients population is further supported by Gatimel *et al.*, who found no sperm head vacuoles differences between fertile and idiopathic infertile patients [56]. In other words, MSOME and IMSI have no diagnostic or therapeutic added values among non-selected or idiopathic infertile patients, on the contrary to couples with male factor in whom these methodologies may be offered under specific indications.

The advantage of IMSI to reduce spontaneous abortions due to declining spermatozoa with DNA abnormalities has been previously suggested [48, 57]. Although theoretically relevant, the correlation between DNA fragmentation (a possible trigger for abnormal vacuoles formation) and spontaneous abortions is a matter of debate [58]. Therefore the notion that IMSI by itself may reduce the risk for spontaneous abortions should be validated by prospective research focused on patients with recurrent pregnancy loss. To the best of the present authors' knowledge, such a study has not been conducted yet.

Summary

Over two and a half decades, ICSI offers clinicians and couples a practical treatment for male infertility [59]. Despite its satisfying reliability and efficiency, ICSI relies on basic sperm assessment (motility and rough morphological evaluation) alone with ×200 to ×400 magnification. The growing evidence for the paternal effect on embryo development and risk for spontaneous abortions [60] emphasize the need to expand our basic understanding regarding the impact of the single fertilizing sperm on reproduction outcome.

Prior to implementing medical technology into routine usage, randomized controlled studies are optimally required and specific indications should be defined and confirmed. Although various retrospective studies reported IMSI advantage over ICSI [5, 57], randomized prospective trials are obviously more reliable and supply important information to scientific data in very selective clinical scenarios. Over the last decade, several studies examined the possible added value of IMSI compared to the "gold standard" conventional ICSI. In general, these trials are characterized by different inclusion criteria, indications, and spermatozoa grading systems. In spite of these heterogeneous methodologies, some clues have suggested a possible advantage of IMSI over ICSI, and should be therefore further validated by larger randomized controlled studies. These are: (a) IMSI (especially using Vanderzwalmen *et al.*'s criteria) for improving blastocyst formation in cases of previous embryos developmental arrest and (b) in cases with isolated teratospermia [51, 54]. On the other hand, current data declines IMSI as a first treatment among couples with male factor undergoing their first or second ART cycles, as well as among unselected infertile patients. Other scenarios such as pregnancy loss should be further clarified and investigated.

While current evidences are quite in favor and support the correlation between large vacuoles and DNA disorganization, specific criteria for "large" vacuole is not certain varying from > 5% to > 50% [4, 28]. Consequently, variable classification systems have been suggested based mainly on vacuoles [14], combined with head shape and base [15] or others. In the lack of such a fundamental universal criteria, it is not surprising that clinical applicable IMSI studies are inconclusive. RVA calculation suggested by Pedrix *et al.*'s [16] combines both vacuoles number and size has a potential to be more reliable than relying on vacuoles' size alone.

Unfortunately, studies focusing on semen analysis have a substantial challenge due to the huge gap between millions of ejaculated sperm cells and the single sperm which eventually fertilizes the egg. In other words, attempting to predict the impact of the fertilizing sperm on reproductive outcome by conventional semen analysis is similar to predicting single patient prognosis by large epidemiological studies. It is not surprising that semen analysis, which is the corner stone for male infertility evaluation, has fundamental drawbacks in male infertility prediction [61]. Under these circumstances, MSOME may be an important methodology to improve diagnostic and therapeutic capabilities. In order to fulfill that promising potential, specific and reliable criteria for sperm selection must be defined and confirmed by basic and clinical studies. Since its introduction, these criteria shifted from sperm organelles to vacuoles and yet, there is a great controversy regarding the definition of "abnormal" vacuole and spermatozoa selection criteria. Accordingly, clinical studies which compared IMSI *vs*. ICSI have used diverse inclusion criteria, consist of various confounders, resulting in conflicting results. Consequently, there is a great debate regarding IMSI reliability, and after more than 15 years since its introduction, that methodology is far from being adopted into routing clinical usage. The present authors' believe it is the time to focus on basic research in order to improve our understanding of sperm vacuoles phenomenon and try to establish reliable distinguishable criteria between physiological and pathological. Future clinical studies, based on well-defined and validated cut-offs, may hopefully authorize the great promise of IMSI for the most appropriate patient populations.

IMSI requires specific equipment and extra time compared to ICSI, during which the semen sample is exposed to room temperature. Therefore it is important to investigate the benefit of the specific lens system compared to ICSI. Previous concerns were raised regarding the possibility that IMSI as a high-powered examination of sperm, actually may cause more damage to sperm than standard ICSI evaluation, thereby causing subtle damage to sperm that may impair reproductive outcomes [62]. The single report of reduced fertilization rate by IMSI compared with ICSI [53] support the aforementioned concern. Laboratories which perform MSOME and IMSI should include specialized embryologists who are capable in performing MSOME efficiently enough to minimize spermatozoa exposure to room temperature.

In conclusion, it appears that ICSI will remain the first treatment choice for male factor during ART. IMSI requires specialized equipment and personnel and might improve clinical outcome only in selected clinical scenarios. Further efforts should focus on homogenous classification MSOME criteria and clinical indications for IMSI.

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