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## REVIEW

# Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis


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**Abstract** The development of a modified intracytoplasmic sperm injection (ICSI), called intracytoplasmic morphologically selected sperm injection (IMSI), demonstrated that a profound morphological investigation of the spermatozoon, under the magnification of 6600 $\times$ , enables outcome improvement. The aim of this study was to compare ICSI outcome with IMSI outcome. The meta-analysis results demonstrated no significant difference in fertilization rate between ICSI and IMSI groups. However, a significantly improved implantation (odds ratio (OR) 2.72; 95% confidence interval (CI) 1.50–4.95) and pregnancy rate (OR 3.12; 95% CI 1.55–6.26) was observed in IMSI cycles. Moreover, the results showed a significantly decreased miscarriage rate (OR 0.42; 95% CI 0.23–0.78) in IMSI cycles as compared with ICSI cycles. This is the first meta-analysis of published data to evaluate the potential benefits of IMSI. The pooled data of IMSI cycles demonstrate a statistically significant improvement in implantation and pregnancy rates and a statistically significant reduction in miscarriage rates. However, more randomized controlled trials are needed to confirm these results. 

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**KEYWORDS:** high-magnification ICSI, IMSI, intracytoplasmic morphologically selected sperm injection, intracytoplasmic sperm injection, meta-analysis, MSOME

## Introduction

Since its first introduction in 1992 (Palermo et al., 1992), intracytoplasmic sperm injection (ICSI), whereby one spermatozoon is selected, immobilized, aspirated with a microinjection needle and injected into the oocyte cytoplasm, has been widely used to treat subfertility and has become the treatment of choice in male factor infertility cases. One of the main concerns in ICSI is the selection of a spermatozoon presenting both motility and normal morphology, based on evaluation of its tail, neck and head. ICSI is usually performed under an overall optical magnification of 400 $\times$ , which makes it possible to detect, in the living state, most of the sperm anomalies recognized by the conventional basic sperm analysis, performed on fixed and stained sperm samples. Thus, this system has severe limitations, since the magnification of 400 $\times$  only enables the observation of major sperm morphological defects, whereas minor morphological defects, which seem to be related to the ICSI outcome (Berkovitz et al., 1999) are often not identified.

As a consequence, spermatozoa appearing as morphologically normal at this magnification may, in fact, carry various structural abnormalities (Bartoov et al., 2002) that may negatively influence embryo development and pregnancy establishment (Tesarik, 2005; Tesarik et al., 2002). This fact could possibly explain why, despite its advantages of bypassing male factor infertility and enabling the morphological evaluation of the spermatozoon, ICSI resulting pregnancy rates are only 30–45% and assisted reproduction centres are still facing the challenge of pregnancy rate improvement.

Success rates of ICSI were initially thought to be independent of basic sperm parameters (Kupker et al., 1995; Lundin et al., 1997; Mansour et al., 1995; Nagy et al., 1995; Sukcharoen et al., 1998; Svalander et al., 1996). Many studies addressed the question of whether there is a connection between sperm parameters and IVF outcomes and the percentage of morphologically normal spermatozoa has been recognized as the best predictor of outcome for natural intrauterine insemination (Berkovitz et al., 1999) and conventional IVF (Kruger et al., 1988; Liu and Baker, 1992; Mashiach et al., 1992). However, many studies have reported no relationship between sperm morphology and ICSI (Lundin et al., 1997; Nagy et al., 1995; Svalander et al., 1996). As an attempt to test this hypothesis, a new concept of unstained, real-time, high-magnification observation of spermatozoa, called 'motile sperm organelle morphology examination' (MSOME), has been introduced. It is now possible to examine the nuclear morphology of spermatozoa at a magnification of 6600 $\times$ , using Nomarski differential interference contrast (Bartoov et al., 2001).

Since MSOME is an unstained cytological technique, its incorporation, together with a micromanipulation system, has allowed the introduction of a modified ICSI procedure, intracytoplasmic morphologically selected sperm injection (IMSI). As a consequence, real-time detailed morphological sperm examination at high magnification, ranging from 6600 $\times$  to 13,000 $\times$  (Garolla et al., 2008), enables the selection of the best available motile spermatozoa before oocyte injection.

Application of IMSI in patients undergoing conventional IVF/ICSI treatment demonstrated that a profound morphological investigation of the spermatozoon favours ICSI outcome improvement. Several publications report that the selection of morphologically normal motile spermatozoa at high magnification is positively associated with pregnancy rates in couples with previous and repeated implantation failures (Bartoov et al., 2002, 2003; Berkovitz et al., 2006; Hazout et al., 2006) and in patients with an elevated degree of DNA fragmented spermatozoa (Hazout et al., 2006).

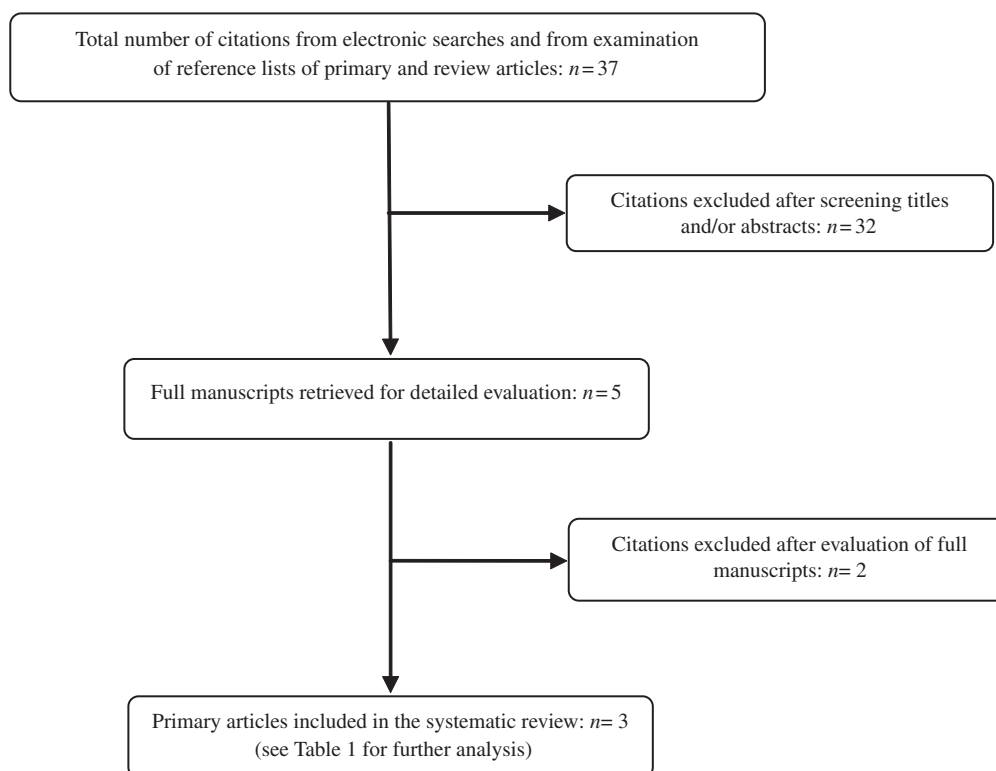
So far, only a few data are available regarding IMSI outcome. Meta-analysis provides an overall consensus from studies, giving a more precise estimate than any one of the individual studies. The aim of this study was to perform the first meta-analysis of published data to compare ICSI and IMSI outcomes.

## Materials and methods

Using the MEDLINE search database, two of the study authors independently searched the literature. The following keywords and combinations were used: 'intracytoplasmic morphologically selected sperm injection', 'IMSI', 'high magnification ICSI' and 'MSOME'. A manual search of reference citations was also performed from reports from the primary search as well as review articles. A total of 37 studies were initially retrieved from the literature. Only five published studies, which analysed the relationship between ICSI and IMSI outcomes, were further considered for inclusion (Bartoov et al., 2001, 2003; Berkovitz et al., 2006; Hazout et al., 2006; Antinori et al., 2008). The articles were scrutinized independently by both reviewers and evaluated for inclusion in the meta-analysis using predetermined criteria. To be included in the analysis, the studies had to be comparative or randomized. Moreover, the intervention and control groups had to be similar. Out of the five studies, three fulfilled the study's predetermined criteria (Antinori et al., 2008; Bartoov et al., 2003; Berkovitz et al., 2006). A meta-analysis of the three selected studies was then conducted. The main outcome measures were fertilization, implantation, pregnancy and miscarriage rates. Two of the three studies analysed the percentage of top-quality embryos (Bartoov et al., 2003; Berkovitz et al., 2006) and this outcome was also included in the meta-analysis. From each study, outcome data were extracted in 2  $\times$  2 tables, statistical heterogeneity was assessed and either a fixed (homogenous) or random (heterogenous) model was used. Heterogeneity of treatment effects was evaluated graphically using forest plot and statistically using chi-squared test. The results are expressed as odds ratio (OR) with 95% confidence intervals (CI). The meta-analysis was conducted using the RevMan 5 Software (Cochrane Collaboration, Oxford, UK).

## Results

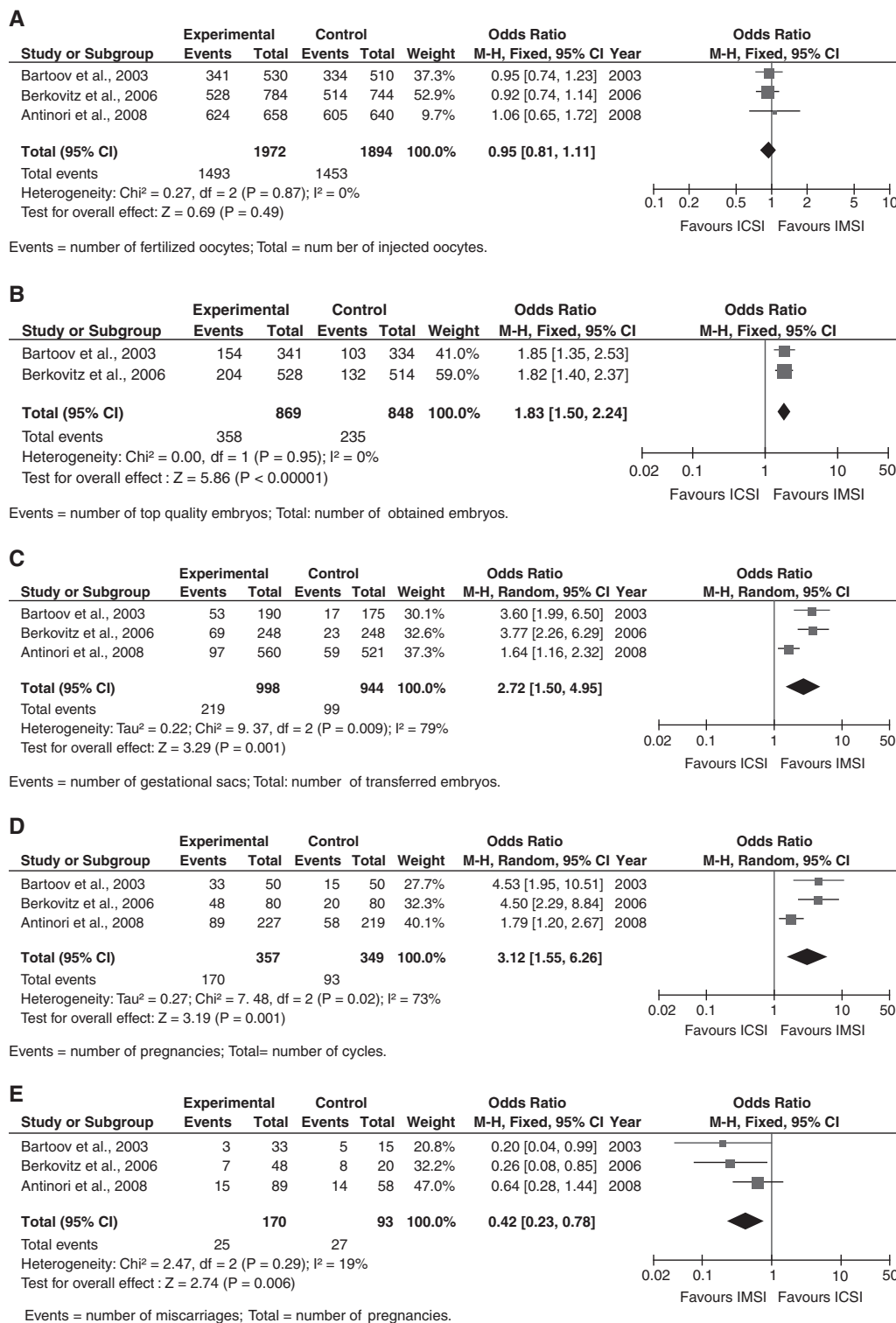
The three selected studies comprised 357 IMSI cycles and 349 ICSI cycles. **Figure 1** illustrates the study selection process. The quality and the main characteristics of the included studies are presented in **Table 1**. The overall result of the meta-analysis is displayed in **Figure 2**.



**Figure 1** Study selection process for systematic review of intracytoplasmic sperm injection (ICSI) versus intracytoplasmic morphologically selected sperm injection (IMSI) outcomes.

**Table 1** Quality and characteristics of studies included in the review of intracytoplasmic sperm injection (ICSI) versus intracytoplasmic morphologically selected sperm injection (IMSI).

Trial	Design	Participants	Numbers		Outcomes
			Experimental (IMSI)	Control (ICSI)	
Bartoov et al. (2003)	Comparative	50 couples undergoing IMSI (male factor infertility, female age <37 years, more than three retrieved metaphase II oocyte in the last ICSI cycle, at least two previous consecutive failed ICSI cycles), matched with 50 couples undergoing ICSI	50	50	Fertilization rate, top-quality embryo rate, implantation rate, pregnancy rate, miscarriage rate
Berkovitz et al. (2006)	Comparative	80 couples (male factor infertility, female age <37 years, at least two previous consecutive failed ICSI cycles), matched with 80 couples undergoing ICSI	80	80	Fertilization rate, top-quality embryo rate, implantation rate, pregnancy rate, miscarriage rate
Antinori et al. (2008)	Randomized	446 couples (at least two previous diagnosis of severe oligoasthenozoospermia, at least 3 years of primary infertility, female age <35 years and undetected female factor) randomly allocated to receive ICSI and IMSI treatments	227	219	Fertilization rate, implantation rate, pregnancy rate, miscarriage rate



**Figure 2** Meta-analysis comparing intracytoplasmic sperm injection (ICSI) versus intracytoplasmic morphologically selected sperm injection (IMSI) outcomes, expressed as odds ratios (OR) with 95% of confidence intervals (CI). (A) Fertilization rate (0.95, 0.81–1.11), (B) top-quality embryo rate (1.83, 1.50–2.24), (C) implantation rate (2.72, 1.50–4.95), (D) pregnancy rate (3.12, 1.55–6.26), and (E) miscarriage rate (0.42, 0.23–0.78). Individual studies are displayed with a square. The horizontal line through the squares indicates the 95% CI. When the CI crosses the vertical line with OR = 1, there is no significant difference between the groups. The diamond in the last row of the graph illustrates the overall result of the meta-analysis. When the diamond does not cross the vertical line, the difference between the groups is considered as statistically significant. Fixed and random models assume homogenous and heterogeneous studies, respectively.

## Fertilization rate

The three studies reported their fertilization rate. A total of 1493 fertilized oocytes were obtained out of 1972 injected oocytes (75.7%) in IMSI group compared with 1453 out of 1894 (76.7%). The overall result of meta-analysis for fertilization rate was not statistically significant (OR 0.95; 95% CI 0.81–1.11; **Figure 2A**).

## Top-quality embryo rate

Two studies reported their top-quality embryo rate. In IMSI group, out of 869 obtained embryos, 358 were considered as top-quality embryos (41.2%). In ICSI group, out of 848 obtained embryos, 235 were considered as top-quality embryos (27.7%). The overall result of meta-analysis for top-quality embryo rate was in favour of IMSI and considered as statistically significant (OR 1.83; 95% CI 1.50–2.24; **Figure 2B**).

## Implantation rate

The three studies reported their implantation rate. A total of 219 gestational sacs resulted from 998 transferred embryos (21.9%) in IMSI cycles compared with 99 out of 944 in ICSI cycles (10.5%). The overall result of meta-analysis was in favour of IMSI and considered as statistically significant (OR 2.72; 95% CI 1.50–4.95; **Figure 2C**).

## Pregnancy rate

The three studies reported their pregnancy rate. In IMSI group, a total of 170 pregnancies were obtained out of 357 cycles (47.6%) and 93 out of 349 ICSI cycles (26.6%). The overall result of meta-analysis was in favour of IMSI and considered as statistically significant (OR 3.12; 95% CI 1.55–6.26; **Figure 2D**).

## Miscarriage rate

The three studies reported their miscarriage rate. In IMSI cycles, 25 miscarriages occurred out of 170 pregnancies (14.7%) compared with 27 out of 93 in ICSI cycles (29.0%). The overall result of meta-analysis was in favour of IMSI and considered as statistically significant (OR 0.42; 95% CI 0.23–0.78; **Figure 2E**).

## Discussion

Since the advent of IMSI, a putative role for sperm morphology in IVF outcome has been the focus of several recent clinical reports. This is the first meta-analysis that draws together the reports on the outcomes of IMSI cycles, addressing the basic question of whether there exists a difference between ICSI and IMSI outcomes.

A strength of systematic reviews is the improved precision of the summary OR estimates compared with the individual studies. The meta-analytical approach is used as a precise investigation for those studies that could fit into the specific criteria.

Information from the studies that failed to fit into the criteria was excluded from this meta-analysis; for example, studies comparing couple's previous ICSI outcome and subsequent IMSI outcome were not included. Out of five relevant studies identified in the current literature, three studies (*Antinori et al., 2008; Bartoov et al., 2003; Berkovitz et al., 2006*) met the eligibility criteria and were identified as suitable for this meta-analysis. These studies comprised 357 IMSI cycles and 349 ICSI cycles and compared their outcomes.

These meta-analysis results demonstrate that IMSI outcomes, such as the percentage of top-quality embryos, implantation rate and pregnancy rate, are significantly improved as compared with ICSI cycles. In addition, the results demonstrate that in IMSI cycles, the miscarriage rate is significantly decreased as compared with ICSI cycles. However, no difference between ICSI and IMSI cycles was observed regarding fertilization rate. It has been suggested that this may be a result of the late paternal effect (*Tesarik, 2005*).

A positive association between high-magnification sperm selection with normal nuclear shape and pregnancy outcome in patients with repeated conventional ICSI failures was recently reported (*Hazout et al., 2006*). Moreover, the same authors also observed a noticeable improvement in implantation and birth rates in patients with normal, moderate and elevated degree of sperm DNA fragmentation. It has been suggested that high-power magnification (6600 $\times$ ) allows the detection and exclusion of sperm cells containing nuclear vacuoles, which could reflect molecular defects responsible for abnormal chromatin remodelling during sperm maturation and result in sperm DNA damage (*Nadalini, et al., 2009*), compromising ICSI outcomes.

The influence of IMSI on early paternal effect was recently evaluated by observing day-2 embryo quality (*Mauri et al., 2010*). The study reported that ICSI and IMSI procedures provided similar top-quality embryo rates; however, the authors did not exclude the possibility that IMSI effects occur as a later paternal effect. Indeed, one previous study (*Vanderzwalmen et al., 2008*) analysed the outcome of embryo development after sibling oocyte injection with different grades of spermatozoa and reported that the number and the size of nuclear vacuoles did not compromise the rate of top-quality day-3 embryos; however, they exerted a negative effect on the competence of an embryo to develop to blastocyst stage. Moreover, the role of vacuoles in sperm cells was addressed in another study that demonstrated a significantly higher degree of sperm DNA fragmentation in spermatozoa presenting vacuoles than in normal spermatozoa (*Franco et al., 2008*).

It is well known that the human spermatozoon has a highly dynamic and essential participation in embryogenesis that clearly extends beyond the fertilization process. The early cleavage divisions of the recently formed zygote depend upon the machinery of the oocyte. Embryo transcription is only initiated at the 4–8-cell stage of the embryonic development. As a result, sperm nuclear deficiencies are usually not detected before the 8-cell stage, when a major expression of sperm-derived genes has begun. Furthermore, a defective spermatozoon may cause arrest of development at multiple levels during embryo development. In addition, this effect can be observed



after implantation, resulting in clinical pregnancy failure or abortion (Barroso et al., 2009).

The current meta-analysis can conclude 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. However, a weakness of this meta-analysis is the variable study's characteristic. Since the advent of IMSI, only one randomized controlled trial was published. Thus, to perform this meta-analysis, comparative studies in which IMSI cycles were matched with ICSI cycles also had to be included.

Although the study characteristics are variable, these data justify the clinical application of IMSI. Moreover, the results also provide a rationale for conducting further research aimed at evaluating IMSI efficacy, through randomized controlled trials, in order to conclusively prove its advantages. Thus, IMSI benefits could be further clarified by updating this meta-analysis.

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