Article

Relationship between visualization of meiotic spindle in human oocytes and ICSI outcomes: a meta-analysis



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Abstract

The objective of this meta-analysis was to investigate the influence of meiotic spindle visualization in human oocytes on intracytoplasmic sperm injection (ICSI) outcomes. Search strategies included on-line surveys of databases (MEDLINE, EMBASE, Science Citation Index, Cochrane Controlled Trials Register and Ovid). The fixed effect was used for odds ratio. Ten trials fulfilled the inclusion criteria comparing in-vitro and clinical ICSI outcomes with or without visualization of meiotic spindle in fresh and in-vivo matured oocytes. According to the meta-analysis, the results showed statistically significant higher fertilization rate ($P < 0.0001$) when the meiotic spindle was viewed than when it was not. Moreover, the percentage of pro-nuclear-stage embryos with good morphology ($P = 0.003$), cleavage rate ($P < 0.0001$), percentage of day-3 top-quality embryos ($P = 0.003$) and percentage of embryos that reached the blastocyst stage ($P < 0.0001$) were statistically significantly better among embryos derived from oocytes in which meiotic spindle was viewed compared with those in which meiotic spindle was not observed. However, these differences were not observed in the clinical pregnancy or implantation rates. This observation has clinical relevance mainly in countries where there is a legal limit on the number of oocytes to be fertilized. However, additional controlled trials are needed to further confirm these results.

Keywords: *human oocyte, ICSI, meiotic spindle, meta-analysis*

Introduction

In assisted reproduction, gamete selection with the aim of achieving better clinical results is an important task for embryologists. This question has particular relevance when ethical and/or legal considerations limit embryo selection after fertilization and, consequently, the formation of supernumerary embryos. Extracellular oocyte morphological characteristics, such as cumulus oophorus, polar body, zona pellucida, and intracellular characteristics (granulations and cytoplasmic inclusions) have been related to fertilization, cleavage, embryo development and clinical outcomes (Coticchio *et al.*, 2004; Borini *et al.*, 2005; Balaban and Urman, 2006; Chamayou *et al.*, 2006; Ebner *et al.*, 2006).

Among the intracellular characteristics, meiotic spindle assessment in mature oocytes has awakened high interest. The first papers about the meiotic spindle supply extensive information on cellular division function. However, the methodology for viewing the spindle, confocal microscopy, was based on fixation and, consequently, its routine application in assisted reproduction procedures and in spindle dynamic studies were frustrated due to the impossibility of applying this method in living oocytes (Eichenlaub-Ritter *et al.*, 2002; Coticchio *et al.*; 2004; Borini *et al.*, 2005). More recently, with the appearance of polarized light microscopy and an integrated imaging processor software, it has been possible to visualize

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the meiotic spindle in living oocytes by a non-invasive method, preserving cell viability (Wang *et al.*, 2001a,b; Cooke *et al.*, 2003; Moon *et al.*, 2003; Rienzi *et al.*, 2003; Cohen *et al.*, 2004; Konc *et al.*, 2004; Chamayou *et al.*, 2006; Montag *et al.*, 2006; Shen *et al.*, 2006; Taylor *et al.*, 2006; Fang *et al.*, 2007; Rama Raju *et al.*, 2007; Varghese *et al.*, 2007; Madaschi *et al.*, 2008).

The meiotic spindle, by controlling chromosomal movements throughout the different stages of meiosis, plays a key role in the successful completion of meiosis. Disturbances of meiotic spindles have been suggested as predisposing oocytes to perturbation of chromosomal segregation and subsequent aneuploidy, maturation arrest, an increased incidence of cell death and subsequent lower fertilization rates (Battaglia *et al.*, 1996; Hardarson *et al.*, 2000; Eichenlaub-Ritter *et al.*, 2002; Cooke *et al.*, 2003; Varghese *et al.*, 2007). In various studies, oocytes in which the meiotic spindles were visualized have demonstrated a significant increase in intracytoplasmic sperm injection (ICSI) outcomes such as fertilization rates (Wang *et al.*, 2001a; Rienzi *et al.*, 2003; Cohen *et al.*, 2004; Shen *et al.*, 2006; Taylor *et al.*, 2006; Rama Raju *et al.*, 2007; Madaschi *et al.*, 2008) and embryo development (Wang *et al.*, 2001a; Cohen *et al.*, 2004; Shen *et al.*, 2006; Rama Raju *et al.*, 2007; Madaschi *et al.*, 2008). However, other studies have not confirmed this correlation (Moon *et al.*, 2003; Fang *et al.*, 2007). The present meta-analysis aimed to investigate the relationship between meiotic spindle presence in human oocytes and ICSI in-vitro and in-vivo outcomes.

Materials and methods

Criteria for considering studies for this meta-analysis

All published and ongoing controlled trials comparing in-vitro and clinical ICSI outcomes between oocytes in which the meiotic spindle was visualized and those in which meiotic spindle was not seen. Only trials that analysed fresh and in-vivo maturated oocytes were included.

Outcome measures

The outcome measures used for this meta-analysis were fertilization rate, embryo development (percentage of pro-nuclear (PN)-stage embryos with good morphology, cleavage rate, percentage of day-3 top-quality embryos and percentage of embryos reaching blastocyst stage), implantation rate and clinical pregnancy rate per transfer.

Identification of studies

Search strategies included online surveys of databases (MEDLINE, EMBASE, Science Citation Index, Cochrane Controlled Trials Register and Ovid) from 1990 to 2008. There was no language restriction. The following medical subject headings and text words were used: meiotic spindle, spindle view, spindle, polarization microscope, polarization light microscopy, PolScope and ICSI guard.

Search results

Among the 15 potentially relevant studies retrieved from online databases, a total of 10 trials fulfilled the inclusion criteria (Wang *et al.*, 2001a; Moon *et al.*, 2003; Rienzi *et al.*, 2003; Cohen *et al.*, 2004; Chamayou *et al.*, 2006; Shen *et al.*, 2006; Taylor *et al.*, 2006; Fang *et al.*, 2007; Rama Raju *et al.*, 2007; Madaschi *et al.*, 2008). A flow diagram for the selection process is shown in **Figure 1**.

Description of the studies

All of the studies used PolScope except for Madaschi *et al.* (2008).

Wang *et al.* (2001a) examined the development of human oocytes with or without meiotic spindles, imaged before ICSI. Oocytes were obtained from stimulated ovaries of consenting patients undergoing oocyte retrieval for ICSI. After imaging and ICSI, oocytes with or without spindles were cultured separately for examination of fertilization and embryo development. A total of 1544 oocytes from 136 cycles were examined and inseminated by ICSI. Spindles were imaged in 82% of oocytes. After ICSI, more oocytes ($P < 0.05$) with spindles (69.4%) fertilized normally, forming 2PN, than oocytes without spindles (62.9%). At day 3, more oocytes ($P < 0.01$) with spindles (66.3%) had developed to cell stages 4–11 than oocytes without spindles (55.4%). Significantly more ($P < 0.001$) oocytes with spindles developed to morula and blastocyst by day 5 (51.1 versus 30.3%) and day 6 (53.2 versus 29.3%) compared with oocytes without spindles.

Moon *et al.* (2003) investigated the relationship between the presence/location of the spindle in metaphase II (MII) oocytes and developmental competence of embryos *in vitro*. The spindles in 626 MII oocytes were examined and divided into six groups based on the presence or absence of spindles and the angle between the spindle and the first polar body. Meiotic spindles were imaged in 523 oocytes (83.5%), while 103 (16.5%) did not have a visible spindle. The rate of normal fertilization was similar in the oocytes with spindles regardless of spindle position (82.2% versus 75.7%).

Rienzi *et al.* (2003) analysed the relationship between the degree of meiotic spindle deviation from the first polar body location and ICSI outcome. Oocytes were divided into four groups according to the angle of meiotic spindle deviation from the polar body position. The angles of deviation were 0–5°, 6–45°, 46–90° and >90° for groups I–IV, respectively. The rate of normal fertilization was higher in the oocytes with spindles regardless of spindle position (74.8% in oocytes with spindle versus 33.3% in oocytes without spindle). The rates of normal (2PN) and abnormal (1PN or >2PN) fertilization did not differ among groups I, II and III. Nevertheless, the rate of normal fertilization was lower among oocytes in which the meiotic spindle deviation angle was >90° (50%); this led to an increased proportion of tripronucleated zygotes that failed to extrude the second polar body. The meiotic spindle was not detected in only 9% of oocytes, and these showed a higher incidence of fertilization abnormalities than did oocytes in which the spindle was detected.

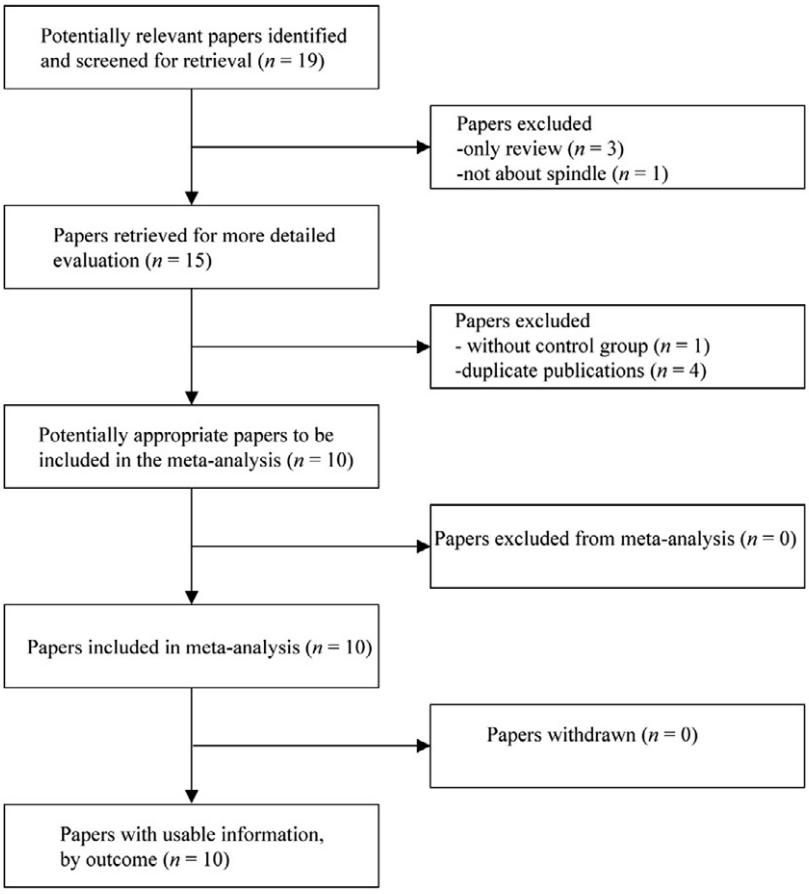


Figure 1. QUOROM statement flow diagram illustrating selection of trials included in the meta-analysis.

Cohen *et al.* (2004) aimed to investigate meiotic spindle assembly in correlation with time elapsed after human chorionic gonadotrophin (HCG) administration, and to determine whether spindle imaging may serve to indicate the likelihood of fertilization and embryo cleavage. A total of 770 MII oocytes from 103 couples who were being treated for male infertility were imaged prior to sperm injection. A spindle was imaged in a significantly higher number of oocytes from ≥38 h after HCG administration compared with those in the <38 h group (78.1–81.5% versus 61.6%, $P < 0.001$). The fertilization rate in oocytes with a visible spindle was statistically higher compared with oocytes in which a spindle could not be detected (70.4% versus 62.2%, $P = 0.035$). There was no relationship between spindle imaging and embryo cleavage on day 3.

Chamayou *et al.* (2006) studied the validity of oocyte morphological criteria such as ooplasm texture, perivitelline space (PVS) size, PVS granulation absence/presence, first polar body shape, and meiotic spindle presence/absence as predictive factors in clinical ICSI outcomes. No relationship was found between meiotic spindle presence or absence and clinical pregnancy per transfer (20.8% versus 17.5%) or implantation rates (11.9% versus 9.7% after ICSI).

Shen *et al.* (2006) aimed this study at improving the non-invasive strategies for selection of high-quality human oocytes prior to ICSI by analysing, independently at two IVF centres, the optical properties of the spindle apparatus quantitatively based on the mean magnitude of light retardance by the oocyte spindle. In one centre, all MII oocytes were fertilized. Accordingly, all fertilized oocytes could be analysed for pronuclear score after ICSI. Fertilization occurred in 91.5% (676/739) of the oocytes possessing a spindle versus 73.4% (116/158) in the group without a spindle, showing a significant difference ($P < 0.001$). The fate of 792 fertilized oocytes with (676) and without spindle (116) was assessed further for development into a high or low PN-stage embryo quality. The proportion of high-quality PN-stage embryos was significantly higher for oocytes possessing a birefringent spindle compared with oocytes without a spindle (34.2% versus 19.9%, $P < 0.001$).

Taylor *et al.* (2006) investigated whether meiotic spindle positioning relative to the polar body and spindle retardance in MII human oocytes constitutes an effective predictor of aneuploidy. The study was conducted in a prospective randomized manner involving 26 patients undergoing ICSI with preimplantation genetic diagnosis (PGD) for aneuploidy screening. When a spindle was present, 109 out of 126 (86.5%) were fertilized, which was significantly higher when compared with fertilization of oocytes that did not show a spindle, 10/17 (58.8%, $P = 0.0099$).

Rama Raju *et al.* (2007) analysed the relationship of meiotic spindle and zona pellucida characteristics to embryonic development potential. A total of 205 oocytes from 25 patients were examined. A meiotic spindle was visualized in 160 oocytes (78.0%), but could not be found in the remaining 45 oocytes (22.0%). Significantly more oocytes with visible spindles were

fertilized and progressed to blastocysts compared with those without visible spindle (82.5% versus 31.1% $P < 0.05$, and 48.5% versus 14.3% $P < 0.05$, respectively).

Fang *et al.* (2007) aimed to investigate the relationship between spindle location and embryonic development of in-vivo and in-vitro maturated human oocytes. The spindles of 134 in-vivo maturated oocytes were examined at the time of ICSI. The spindles were visualized in 83.6% (112/134) and not visualized in 16.4% (22/134) of the oocytes. The normal fertilization rate was similar between spindle (75.9%, 85/112) and non-spindle-group (63.6%, 14/22). The cleavage rate and rate of high-quality embryos were not significantly different between the groups.

Madaschi *et al.* (2008) examined whether the presence of meiotic spindles in living human oocytes can be used as a predictive factor associated with embryo morphology to allow embryo selection before transfer and its association with IVF outcomes. A total of 1097 MII oocytes from 157 women were screened using the polarization imaging software module Octax ICSI Guard® (Octax, Herborn, Germany) at the time of ICSI. The meiotic spindles were detected in 689 (62.8%, spindle-detected group), and spindles were not visualized in 408 (37.2%, spindle-non-detected group). The normal fertilization rate was significantly higher in the spindle-detected group (66.5%) than spindle-non-detected group (58.6%, $P = 0.009$). Although the percentage of embryos with good PN morphology had been similar between the two groups (spindle-detected group, 11.7%; spindle-non-detected group, 9.3%), the rate of early-cleaved embryos was significantly higher in the spindle-detected group than in the spindle-non-detected group (50.9% and 32.6%, respectively, $P = 0.037$). When only embryos from the spindle-detected group were selected for transfer, the pregnancy and implantation rates were 44.4% and 23.0%, and when only embryos from the spindle-non-detected group were transferred, those respective rates were 18.2% and 8.7% ($P = 0.029$ and $P = 0.022$, respectively).

Statistical analysis

Data management and analysis were conducted using the StatsDirect statistical software (Cheshire, UK). The fixed effect model was used for odds ratio (OR). If there was statistical heterogeneity, an additional analysis was performed using the random effects model. Fixed effect effectiveness was evaluated by the Mantel-Haenszel method. A confidence interval (CI) for the Mantel-Haenszel OR in StatsDirect was calculated using the Robins, Breslow and Greenland variance formula. For random effect, a confidence interval for OR was calculated using the DerSimonian-Laird formula. A chi-squared test statistic was used with its associated probability that the pooled OR was equal to 1. The measure of heterogeneity (non-combinability) was evaluated by Cochran's Q, the Breslow-Day and I^2 (Higgins *et al.*, 2003) tests. A non-significant result (i.e. lack of heterogeneity) indicates that no trial has an OR that is significantly worse or better than the overall common OR obtained by pooling the data. Since a fixed effects model has been employed herein, it is important to acknowledge that inferences refer only to the particular studies included in the analysis. Meta-analysis used in this manner is simply a device to pool the information from the various studies to provide a composite finding, but only for those studies. Since many of the preceding analyses contained only two or three studies, it was decided to derive the inferences from a fixed effects model. In the alternative random effect model, the individual studies are regarded as a random sample from the (infinite) population of studies. Global inferences would then be permissible, but the random errors used would need to reflect inter-study variation (heterogeneity).

Results

Fertilization rate

Nine studies were included (Wang *et al.*, 2001a; Moon *et al.*, 2003; Rienzi *et al.*, 2003; Cohen *et al.*, 2004; Shen *et al.*, 2006; Taylor *et al.*, 2006; Fang *et al.*, 2007; Rama Raju *et al.*, 2007; Madaschi *et al.*, 2008). The fertilization rate was statistically significantly higher in the group of oocytes in which the meiotic spindle was viewed (75.6%, 3543/4684) than in the group of oocytes that did not show the meiotic spindle (61.5%, 777/1264) ($P < 0.0001$; OR 1.79, 95% CI 1.57–2.05). There was heterogeneity in this comparison (Breslow-Day 63.6, $df = 8$, $P < 0.0001$; Cochran Q 59.7, $df = 8$, $P < 0.0001$; I^2 86.6%). Using the random effect model, pooling of the results also showed a statistically significantly higher fertilization rate when a meiotic spindle was viewed ($P < 0.0001$, OR 2.52, 95% CI 1.65–3.81) (**Figure 2**).

Embryo development

PN-stage embryo morphology

Two studies were included (Shen *et al.*, 2006; Madaschi *et al.*, 2008). The percentage of embryos with good PN morphology was statistically significantly higher among embryos derived from oocytes in which meiotic spindle was viewed (25.1%, 285/1134) than between embryos derived from oocytes that did not present a meiotic spindle (12.7%, 45/355) ($P = 0.003$; OR 1.71, 95% CI 1.20–2.44). There was no heterogeneity in this comparison (Breslow-Day 1.64, $df = 1$, not significant; Cochran Q 1.64, $df = 1$, not significant) (**Figure 3**).

Cleavage rate

Three studies were included (Wang *et al.*, 2001a; Fang *et al.*, 2007; Madaschi *et al.*, 2008). The cleavage rate was statistically significantly higher among embryos derived from oocytes in which meiotic spindle was viewed (63.1%, 897/1422) compared with embryos derived from oocytes not showing meiotic spindle (43.9%, 188/428) ($P < 0.0001$; OR 1.85, 95% CI 1.47–2.32). There was no heterogeneity in this comparison (Breslow-Day 1.63, $df = 2$, not significant; Cochran Q 1.63, $df = 2$, not significant; I^2 0%) (**Figure 4**).

Day-3 embryo-stage quality

Three studies were included (Wang *et al.*, 2001a; Cohen *et al.*, 2004; Fang *et al.*, 2007). The percentage of embryos classified on day 3 as top quality was statistically higher among those derived from oocytes in which meiotic spindle was viewed (37.3%, 381/1022) than the ones derived from oocytes not presenting meiotic spindle (28.0%, 56/200) ($P = 0.003$; OR 1.70, 95% CI 1.20–2.42). There was no heterogeneity in this comparison (Breslow-Day 0.13, $df = 2$,

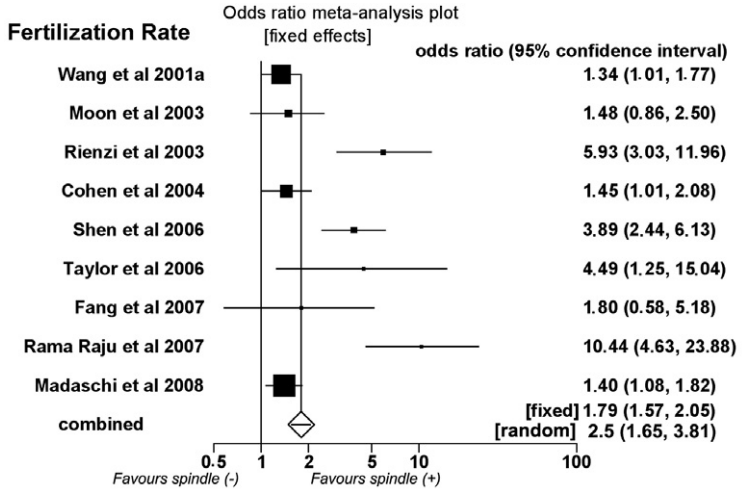


Figure 2. Forest plot for fertilization rate. CI = confidence interval.

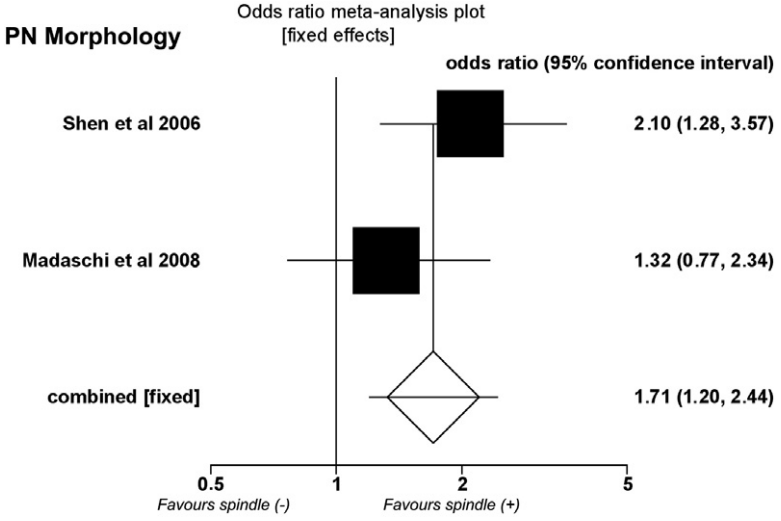


Figure 3. Forest plot for pronuclear (PN) stage morphology.

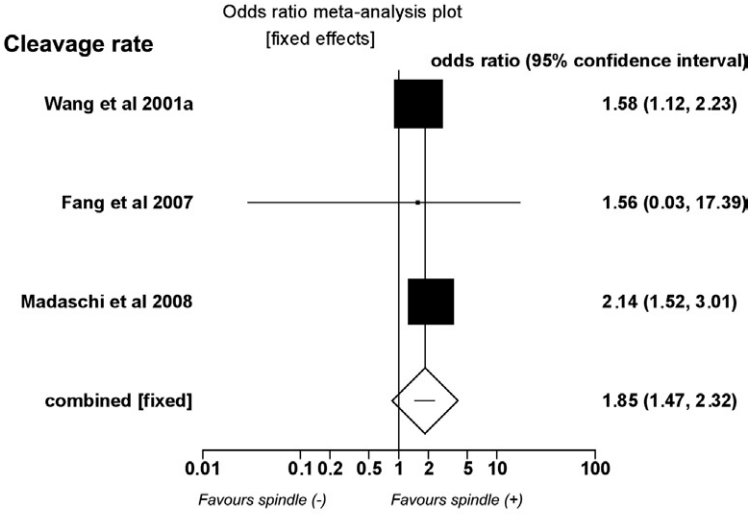


Figure 4. Forest plot for cleavage rate. CI = confidence interval.

not significant; Cochran Q 0.13, *df* = 1, not significant; I^2 0%) (**Figure 5**).

Number of embryos that reached blastocyst stage

Two studies were included (Wang *et al.*, 2001a; Rama Raju *et al.*, 2007). The percentage of embryos that attained the blastocyst stage (on day 5) was statistically significantly higher among those derived from oocytes in which meiotic spindle was viewed (50.7%, 376/742) compared with the ones from oocytes that did not show meiotic spindle (28.6%, 38/133) ($P < 0.0001$; OR 2.61, 95% CI 1.74–3.91), a comparison without heterogeneity (Breslow-Day 1.14, *df* = 1, not significant; Cochran Q 1.10, *df* = 1, not significant) (**Figure 6**).

Implantation rate

Two studies were included (Chamayou *et al.*, 2006; Madaschi *et al.*, 2008). The implantation rate was not significantly different between transferred sets with only embryos derived from oocytes in which meiotic spindle was viewed (15.4%, 64/415) versus sets containing only embryos from oocytes showing no meiotic spindle (9.8%, 24/246) (not significant; OR 1.56, 95% CI 0.94–2.59). There was no heterogeneity in this comparison (Breslow-Day 1.50, *df* = 1, not significant; Cochran Q 1.49, *df* = 1, $P = 0.22$) (**Figure 7**).

Clinical pregnancy rate per transfer

Two studies were included (Chamayou *et al.*, 2006; Madaschi *et al.*, 2008). The clinical pregnancy rate per transfer was not significantly different between sets with only embryos derived from oocytes in which meiotic spindle was viewed (28.5% 55/193) versus embryo sets derived exclusively from oocytes presenting no meiotic spindle (17.6%, 21/119) (not significant; OR 1.65, 95% CI 0.93–2.93), a comparison without heterogeneity (Breslow-Day 2.42, *df* = 1, not significant; Cochran Q 2.36, *df* = 1, not significant) (**Figure 8**).

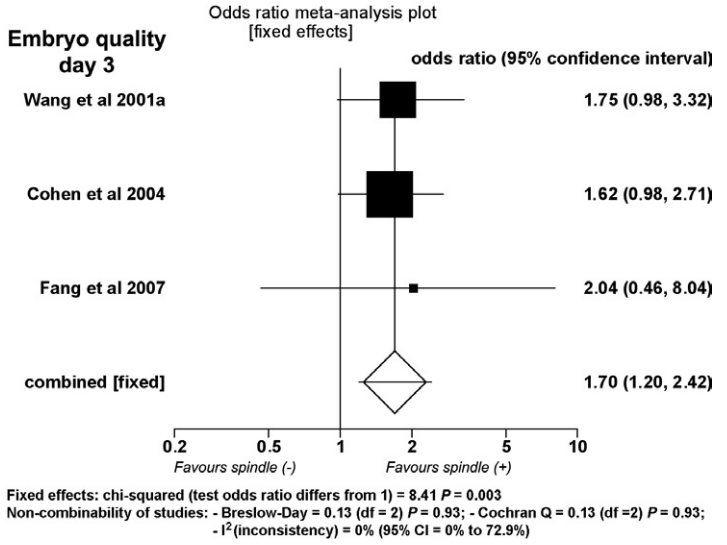


Figure 5. Forest plot for day-3 embryo-stage quality. CI = confidence interval.

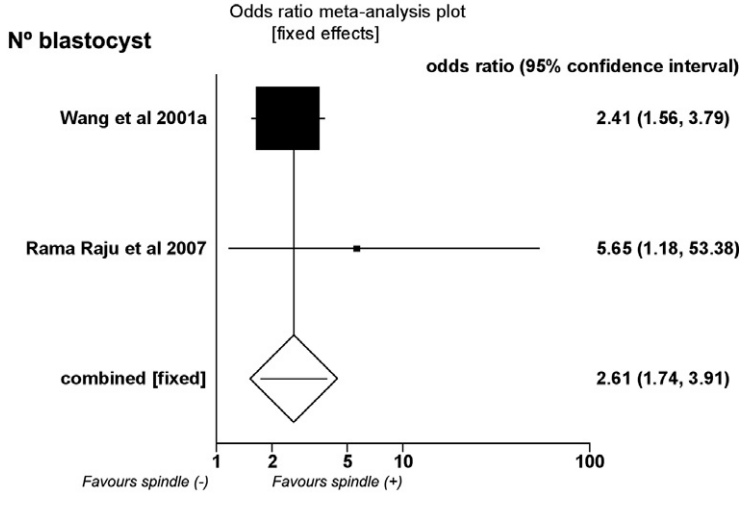


Figure 6. Forest plot for number of embryos at blastocyst stage.

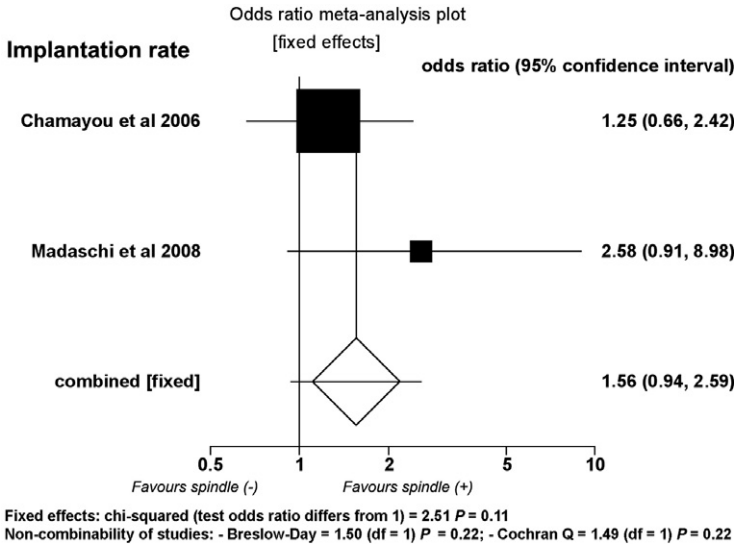


Figure 7. Forest plot for implantation rate.

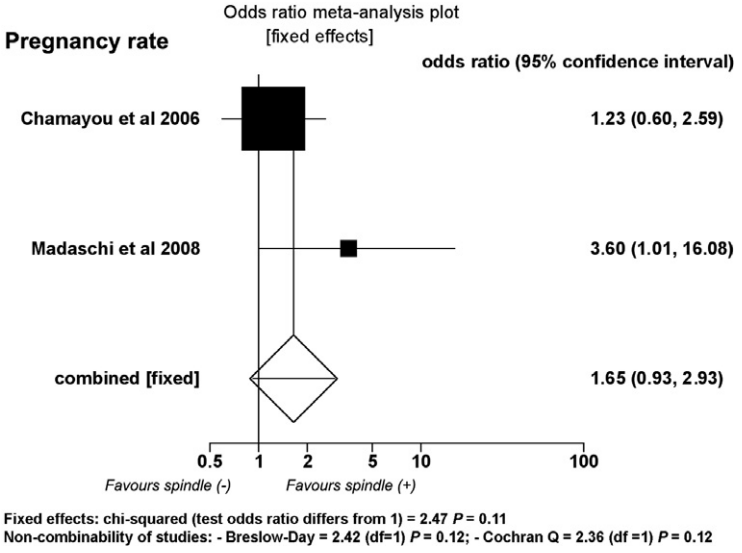


Figure 8. Forest plot for clinical pregnancy rate per transfer.

Discussion

As part of the ongoing search for markers that predict higher success rates in IVF/ICSI cycles by assessment of oocyte maturation and quality, it was suggested that meiotic spindle imaging could contribute valuable information. Different studies reported that many oocytes that had expelled the first polar body immediately after oocyte collection also show the meiotic spindle (Wang *et al.*, 2001a,b; Moon *et al.*, 2003; Rienzi *et al.*, 2003; Cohen *et al.*, 2004; Konc *et al.*, 2004; Taylor *et al.*, 2006; Madaschi *et al.*, 2008; Rama Raju *et al.*, 2007). In most of the cases, the meiotic spindle presence is associated with higher fertilization rates (Wang *et al.*, 2001a,b; Rienzi *et al.*, 2003; Cohen *et al.*, 2004; Shen *et al.*, 2006; Taylor *et al.*, 2006; Madaschi *et al.*, 2008; Rama Raju *et al.*, 2007), and in some cases, with higher embryo development (Wang *et al.*, 2001a; Moon *et al.*, 2003; Rama Raju *et al.*, 2007) and pregnancy rates (Madaschi *et al.*, 2008). In this meta-analysis, significantly higher fertilization was found in 4684 oocytes where a meiotic spindle was viewed compared with 1264 oocytes where no meiotic spindle was observed (75.6% versus 61.5% respectively, $P < 0.0001$; OR 1.79, 95% CI 1.57–2.05). This result indicates that the presence of a meiotic spindle predicts a higher fertilization rate. However, the result of the analysis was impaired due to the heterogeneity observed among some studies (Breslow-Day 63.6, $df = 8$, $P < 0.0001$; Cochran Q 59.7, $df = 8$, $P < 0.0001$; I^2 86.6%). On the other hand, when the data were pooled using a random effects model the difference in fertilization rate was still significant ($P < 0.0001$; OR 2.52, 95% CI 1.65–3.81).

In addition, better results were observed among embryos derived from oocytes in which meiotic spindle was viewed than among those from oocytes not showing a meiotic spindle in the following parameters: cleavage rate ($P < 0.0001$; OR 1.85, 95% CI 1.47–2.32), percentage of PN-stage embryos with good morphology ($P = 0.003$; OR 1.71, 95% CI 1.20–2.44), percentage

of day-3 top-quality embryos ($P = 0.003$; OR 1.70, 95% CI 1.20–2.42) and percentage of embryos reaching the blastocyst stage ($P < 0.0001$; OR 2.61, 95% CI 1.74–3.91). These outcomes showed a strong relationship between meiotic spindle identification and better embryo development. However, these differences were not observed in the implantation rate (not significant; OR 1.56, 95% CI 0.94–2.59) or clinical pregnancy per transfer rate (not significant; OR 1.65, 95% CI 0.93–2.93).

The difference in the percentage of oocytes with spindle meiotic birefringence among the studies (minimum of 62.8% and maximum of 91%) can be related to a variation in the population or to ovarian stimulation protocols. Defects in protein complex, low energy supply or disturbances in the signalling pathway may also be involved in disturbances of spindle structure (Eichenlaub-Ritter *et al*., 2002). However, as thermal control stabilizes the meiotic spindle, this difference can be related to the precision of how this temperature control was performed during oocyte collection, culture or microinjection (Wang *et al*., 2002; Cooke *et al*., 2003; Rienzi *et al*., 2003). Other environmental changes such as pH and culture conditions may also compromise spindle visualization (Hu *et al*., 2001; Roberts *et al*., 2002).

On the other hand, the time at which oocytes were analysed can also contribute to whether or not the spindle is visualized. Cohen *et al*. (2004) showed that the percentage of oocytes with a visible spindle is higher in cases in which the spindle evaluation was performed at least 38 h after the HCG administration, compared with those whose spindle evaluation was performed earlier (81.5% versus 61.6%, respectively). Montag *et al*. (2006) showed that during the transition from metaphase I to metaphase II the spindle completely disappears for approximately 40–60 min. This fact indicates that the absence of the spindle at least in some human MII oocytes is more likely an indicator of the physiological progression through an important developmental stage of meiosis rather than a cellular disturbance. Thus, the poorer ICSI outcomes among oocytes not showing a spindle could simply indicate an incorrect timing of ICSI.

Sometimes the spindles became visible only after oocyte rotation, when the orientation of microtubules had become favourable for the visualization. Therefore, differences in this technique may be responsible for the high degree of spindle absence. By rotating the oocytes with the ICSI micropipette around the axis connecting the centre of the oocyte to the first polar body during the attempts at spindle visualization, Cooke *et al*. (2003) and Rienzi *et al*. (2003) detected the spindle in up to 92.7% and 91% of oocytes examined, respectively. However, Cohen *et al*. (2004), Rama Raju *et al*. (2007) and Madaschi *et al*. (2008) reported lower rates of spindle visualization (76%, 78% and 62.8, respectively) despite rotating the oocytes. On the other hand, some authors have not mentioned how many rotations of the oocyte were viewed to determine presence or absence of a spindle (Wang *et al*., 2001a,b; Wang and Keefe, 2002; Moon *et al*., 2003).

Nuclear oocyte maturation is determined by the absence of the germinal vesicle and the presence of the first polar body. Changes in the maturation promotion factor activity, c-mos kinases and mitogen-activated protein kinases control cellular mitosis by promoting chromosomal condensation, germinal vesicle collapse and spindle formation (Eichenlaub-Ritter and Peschke, 2002; Cohen *et al*., 2004). The lower fertilization rate demonstrated in oocytes without spindle refringence can be attributed to oocyte immaturity. On the other hand, a considerable number of these oocytes were fertilized (61.5% of all studies included in this meta-analysis). Plausible explanations for this number include the occurrence of technical failures during spindle visualization (inexperienced biologist, time of oocyte evaluation) or that spindle absence does not prevent fertilization in all cases. However, according to this meta-analysis, probably the lack of a meiotic spindle may have a subsequent effect during embryonic development.

To avoid damaging the spindle during the ICSI procedure, the embryologists rotated the oocyte by placing a hold pipette based on the first polar body position. However, the movement of the polar body inside the perivitelline space (because of possible physical displacement during oocyte denudation) and/or migration of the spindle inside the oocyte can alter the relationship between these two structures, enabling the microinjection to impair the spindle (Silva *et al*., 1999; Hardarson *et al*., 2000; Wang *et al*., 2001 a,b; Moon *et al*., 2003; Rienzi *et al*., 2003; Cohen *et al*., 2004; Konc *et al*., 2004; Taylor *et al*., 2006; Rama Raju *et al*., 2007). Generally, when there is meiotic spindle visualization, the isolated sperm is injected into the oocyte, taking special care not to damage this structure. Rienzi *et al*. (2003) reported a significantly lower normal fertilization rate and a higher rate of abnormal fertilization with a more frequent development of three pronuclei in oocytes with a higher degree of deviation from the first polar body (>90°). Fang *et al*. (2007) reported that the fertilization rate of in-vivo maturated oocytes with spindles beneath or adjacent to the first polar body (angle of 0–5°) was significantly higher (93.3%) than all other groups of oocytes with greater deviation from the first polar body (6–45°, 46–90° and >90°). However, Moon *et al*. (2003) and Rama Raju *et al*. (2007) did not show the same results. Taylor *et al*. (2006) concluded that the deviation angle of the spindle from the first polar body is not a reliable indicator of embryo quality or subsequent aneuploidy.

Most studies analysing meiotic spindle have sought to draw attention to variables more directly related to laboratory activities. However, some authors suggest that the meta-analysis should be patient-oriented, i.e. primary outcomes should be clinical results (e.g. pregnancy rate); all other outcomes should be listed as secondary outcomes. Fertilization rate, cleavage rate, percentage of PN-stage embryos with good morphology, percentage of top-quality day-3 embryos and percentage blastocyst stage embryos are intermediate end-points, which do not necessarily predict a better outcome and/or a more advantageous cost–effectiveness ratio. Despite the tendency in favour of oocytes with meiotic spindle, this meta-analysis failed to show any statistically significant difference in the most relevant and clinically significant end-points in IVF: the clinical pregnancy rate and implantation rate. This observation can be related to a small cumulative sample size since only two studies supply data about pregnancy or implantation rates. Based on the pregnancy rate per transfer obtained in the group without a visible meiotic spindle (21/119, 17.6%), to detect a difference of 5% with a power of 80%, around 2000 transfers would be necessary for a definitive conclusion, i.e. above the total number included here. On the other hand, an increased number of best-quality embryos may exert possible good effects on cumulative pregnancy rates following the transfer of frozen embryos. Moreover, one must be aware of the fact that a number of other significant predictors of the chance of pregnancy achievement exists in an individual patient, including cause of infertility, chromosomal status of the transferred embryo, as well

as the quality and origin of spermatozoa (Griesinger and Diedrich, 2006). Thus, for a more consistent conclusion, this meta-analysis guides researchers to wait for the results of new controlled trials that have more information about clinical parameters.

In conclusion, the result of this meta-analysis indicates that the presence of a birefringent meiotic spindle in human oocytes can predict better fertilization rate and embryo development. Nevertheless, it failed to show any statistically significant difference in clinically significant end-points in IVF (pregnancy rate and implantation rate). This observation has clinical relevance mainly in countries where there is a legal limit on the number of oocytes that can be fertilized. Additional prospective studies with a large population will be helpful in understanding the importance of spindle presence and its characteristics in laboratory and clinical results.

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Reference

Balaban B, Urman B 2006 Effect of oocyte morphology on embryo development and implantation. *Reproductive BioMedicine Online* **12**, 608–615.

Battaglia DE, Goodwin P, Klein NA, Soules MR 1996 Fertilization and early embryology: influence of maternal age on meiotic spindle assembly oocytes from naturally cycling women *Human Reproduction* **11**, 2217–2222.

Borini A, Lagalla C, Cattoli M *et al*. 2005 Predictive factors for embryo implantation potential. *Reproductive BioMedicine Online* **15**, 653–668.

Chamayou S, Alecci C, Ragolia C *et al*. 2006 Meiotic spindle presence and oocyte morphology do not predict clinical ICSI outcomes: a study of 967 transferred embryos. *Reproductive BioMedicine Online* **13**, 661–666.

Cohen Y, Malcov M, Schwartz T *et al*. 2004 Spindle imaging: a new marker for optimal timing of ICSI. *Human Reproduction* **19**, 649–654.

Cooke S, Tyler JPP, Driscoll GL 2003 Meiotic spindle location and identification and its effect on embryonic cleavage plane and early development. *Human Reproduction* **18**, 2397–2405.

Coticchio G, Sereni E, Serrao L *et al*. 2004 What criteria for the definition of oocyte quality? *Annals of the New York Academy of Sciences* **1034**, 132–144.

Ebner T, Moser M, Tews G 2006 Is oocyte morphology prognostic of embryo potential after ICSI? *Reproductive BioMedicine Online* **12**, 517–512.

Eichenlaub-Ritter U, Peschke M 2002 Expression in in-vivo and in-vitro growing and maturing oocytes: focus on regulation of expression at the translational level. *Human Reproduction Update* **8**, 21–41.

Eichenlaub-Ritter U, Shen Y, Tinneberg H-R 2002 Manipulation of the oocyte: possible damage to the spindle apparatus *Reproductive BioMedicine Online* **5**, 117–124.

Fang C, Mandy T, Li T *et al*. 2007 Visualization of meiotic spindle and subsequent embryonic development in in-vitro and in-vivo matured human oocytes. *Journal of Assisted Reproduction and Genetics* **24**, 547–551.

Griesinger G, Diedrich K 2006 The role of LH in ovarian stimulation: considerations. *Reproductive BioMedicine Online* **12**, 404–406.

Hardarson T, Lundin K, Hamberger L 2000 The position of the metaphase II spindle cannot be predicted by the location of the first polar body in the human oocyte. *Human Reproduction* **15**, 1372–1376.

Higgins JP, Thompson SG, Deeks JJ, Altman DG 2003 Measuring inconsistency in meta-analyses. *British Medical Journal* **327**, 557–560.

Hu Y, Betzendahl I, Cortvrindt R *et al*. 2001 Effects of low O_2 and ageing on spindles and chromosomes in mouse oocytes from pre-antral follicle culture. *Human Reproduction* **16**, 737–748.

Konc J, Kanyo K, Cseh S 2004 Visualization and examination of the meiotic spindle in human oocytes with PolScope. *Journal of Assisted Reproduction and Genetics* **21**, 349–353.

Madaschi C, de Souza Bonetti TC, de Almeida Ferreira Braga DP *et al*. 2008 Spindle imaging: a marker for embryo development and implantation. *Fertility and Sterility* **90**, 194–198.

Montag M, Schimming T, van der Ven H 2006 Spindle imaging in human oocytes: the impact of the meiotic cell cycle. *Reproductive BioMedicine Online* **12**, 442–446.

Moon JH, Hyun CS, Lee SW *et al*. 2003 Visualization of the metaphase II meiotic spindle in living human oocytes using PolScope enables the prediction of embryonic development competence after ICSI. *Human Reproduction* **18**, 817–820.

Rama Raju GA, Prakash GJ, Krishna KM, Madan K 2007 Meiotic spindle and zona pellucida characteristics as predictors of embryonic development: a preliminary study using PolScope imaging *Reproductive BioMedicine Online* **14**, 166–174.

Rienzi L, Ubaldi F, Martinez F *et al*. 2003 Relationship between meiotic spindle location with regard to the polar body position and oocyte development potential after ICSI. *Human Reproduction* **18**, 1289–1293.

Roberts R, Franks S, Hardy K 2002 Culture environment modulates maturation and metabolism of human oocytes. *Human Reproduction* **17**, 2950–2956.

Shen Y, Stalf T, Mehnert C *et al*. 2006 Light retardance by human oocyte spindle is positively related to pronuclear score after ICSI. *Reproductive BioMedicine Online* **12**, 737–751.

Silva CS, Kapura K, Odenberg R, Keefe DL 1999 The first polar body does not predict accurately the location of the metaphase II meiotic spindle in mammalian oocytes. *Fertility and Sterility* **71**, 719–721.

Taylor TH, Gitlin S, Wrigth G *et al*. 2006 The meiotic spindle of human oocytes as viewed under the oosight and its relationship to chromosomal aneuploidy of subsequent embryo. *Human Reproduction* 21(Suppl. 1), i23.

Varghese AC, Goldberg E, Agarwal A 2007 Current and future perspectives on intracytoplasmic sperm injection: a critical commentary. *Reproductive BioMedicine Online* **15**, 719–727.

Wang WH, Keefe DL 2002 Prediction of chromosome misalignment among in-vitro matured human oocytes by spindle imaging with the PolScope. *Fertility and Sterility* **78**, 1077–1081.

Wang WH, Meng L, Hackett RJ *et al*. 2002 Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. *Fertility and Sterility* **77**, 1274–1277.

Wang WH, Meng L, Hackett RJ, Keefe DL 2001a Developmental ability of human oocytes with or without birefringent spindles imaged by PolScope before insemination. *Human Reproduction* **16**, 1464–1468.

Wang W, Meng L, Hackett RJ *et al*. 2001b The spindle observation and its relationship with fertilization after intracytoplasmic sperm injection in living human oocytes. *Fertility and Sterility* **75**, 348–353.

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