Comparison of day 2 embryo quality after conventional ICSI versus intracytoplasmic morphologically selected sperm injection (IMSI) using sibling oocytes

Ana L. Mauri [a], Claudia G. Petersen [a,b], Joao Batista A. Oliveira [a,b], Fabiana C. Massaro [a], Ricardo L.R. Baruffi [a], Jose G. Franco Jr [a,b,c,*]

[a] Centre for Human Reproduction Prof. Franco Jr., Ribeirão Preto, Sao Paulo, Brazil
[b] Department of Gynaecology and Obstetrics, Botucatu Medical School, Sao Paulo State University, UNESP, Brazil
[c] Paulista Centre for Diagnosis, Research and Training, Ribeirão Preto, Sao Paulo, Brazil

ARTICLE INFO

ABSTRACT

Objective: To evaluate whether intracytoplasmic morphologically selected sperm injection (IMSI) could influence early paternal effects by observing embryo quality at day 2.
Study design: The study included 30 couples with at least one of the following criteria: male factor infertility, at least 2 previous failures of implantation or previous miscarriages after IVF/ICSI. Sibling oocytes of each patient were randomly assigned to either the ICSI group or the IMSI group. For IMSI, spermatozoa were selected at 8400× magnification through an inverted microscope equipped with Nomarski differential interference contrast optics, Uplan Apo 100× oil/1.35 objective lens and variable zoom lens. For conventional ICSI, spermatozoa were selected at 400× magnification. An embryo was defined as top quality if there were four identical blastomeres on day 2 with no fragments or multinucleation of blastomeres. Data were analysed using the Wilcoxon and chi-squared tests. The significance level was set at $P < 0.05$. The variables were analysed in relation to the general population and the subpopulations with or without male factor.
Results: A total of 331 MII oocytes (30 oocyte retrievals) were selected and injected by the ICSI (n: 172) or IMSI (n: 159) procedure. For IMSI, only spermatozoa classified as morphologically normal at high magnification were used. No differences ($P > 0.05$) in fertilisation rate (ICSI: 70.9%; IMSI: 70.4%), early embryo cleavage rate (ICSI: 66.9%; IMSI: 60.4%) or cleavage rate (ICSI: 99.2%; IMSI: 99.1%) were observed. On day 2, as compared to ICSI, IMSI provided a similar proportion of top quality embryos (ICSI: 57.8%; IMSI: 52.2%; $P > 0.05$). These results were not influenced by the presence or absence of male factor.
Conclusion: In terms of embryo quality at day 2, IMSI had the same performance as conventional ICSI. However, we cannot exclude the possibility that IMSI effects occur only as a positive later paternal effect.
© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The quality of DNA in spermatozoids evaluated by the presence or absence of fragmentation can influence later embryo development (late paternal effect) [1–3]. Although DNA damage cannot be identified with selection of spermatozoids at high magnification [4], at these resolutions (≥6000×) it is possible to identify sperm with intranuclear vacuoles. These structures may reflect greater probability of molecular defects responsible for DNA damage [5,6] but the vacuoles are not detected at the magnification range commonly employed for conventional ICSI (200–400×). Therefore, it is expected that the exclusion of such spermatozoids could reduce the probability of a spermatozoid with DNA damage being injected into the oocyte [7]. In fact, different reports on blastocyst quality [8,9], implantation, pregnancy and miscarriages rates [7,10–15] and the chance of having a healthy normal child [16], have supported the hypothesis that a late adverse paternal effect on embryo development can be avoided through better selection of spermatozoids at high magnification and intracytoplasmic morphologically selected sperm injection (IMSI).

On the other hand, the spermatozoids also participate in early embryo development (early paternal effect), specifically the centrosome, which tends to provoke a failure of fertilisation or stoppage of embryo development with blockage in stage 2PN, as well as oocyte activation factor capable of inducing delay in the

* Corresponding author at: Av. Prof. João Fiusa, 689–ZIP code 14025-310, Ribeirão Preto, SP, Brazil. Tel.: +55 16 3911 1100; fax: +55 16 3911 1100.
E-mail addresses: franco@crh.com.br, crh@crh.com.br (J.G. Franco Jr).

first cell cycles [1–3,17–19]. Therefore, an early adverse paternal effect may cause increases in the degree of cleaving embryo fragmentation [1,2,20]. Tesarik et al. [20] have shown that the paternal effect can become manifest as early as the 1-cell zygote stage.

In this context we cannot discard the hypothesis that selection of spermatozoids at high magnification ($\geq 6000\times$) and the consequent lower probability of DNA damage, would provoke better early embryo development. The aim of this study was to evaluate whether IMSI could influence embryo quality at day 2 when compared to conventional ICSI.

2. Materials and methods

2.1. Study participants

A total of 30 cycles of 30 couples enrolled in the ICSI program of the Centre for Human Reproduction Prof Franco Jr. were admitted into this study. Inclusion criteria were male factor infertility, at least 2 previous failures of implantation or previous miscarriages after ICSI. The male factor infertility was defined according to WHO/Kruger criteria [21] for sperm concentration, motility and morphology, which have previously been tested. If at least two of these three parameters were abnormal, the couple was considered for ICSI treatment. Written consent was taken from all the patients and the study was performed according to the norms of the Institutional Ethics Committee.

It has been shown that ICSI outcome is positively associated with the morphological state of the sperm nucleus, while the early miscarriage rate was negatively associated with this morphological factor [10,11,13]. On the other hand, it has been suggested that in some cases repeated failure of conventional IVF could be caused by a paternal effect on the early embryo development and this hypothesis has been confirmed in a shared oocyte donation model [1,2,7,20]. In fact, prior failures in ICSI cycles constituted an inclusion criterion in different studies employing IMSI [7,8,10,12,15]. Given these points, it was decided to include in this study, besides male factor infertility, cases presenting previous implantation failures and/or previous miscarriages after ICSI.

2.2. Ovarian stimulation, oocyte recovery and culture protocol

All patients were submitted to the same scheme of controlled ovarian stimulation. After pituitary down-regulation with nafarelin acetate at a dose of 400 μg/day (Synarel®; Pharmacia, São Paulo; SP, Brazil) started in the mid-luteal phase, recombinant human FSH (r-FSH/Gonal F®; Serono, SP, Brazil) was administered at a starting dose of 150–300 IU, depending on the age of the patient and recombinant LH (r-LH/Luveris®, Serono, SP, Brazil) was administered at a dose of 75 IU/day for a period of 7 days. On day 8 of stimulation, follicular development was monitored by 7 MHz transvaginal ultrasound only (Medison Digital Color MT, Medison Co. Ltd, Seoul, Korea) and the FSH dose was adapted according to ovarian response. The r-LH supplementation was increased to 150 IU/day when one or more follicles measuring ≥ 10 mm in diameter were found. When at least three follicles measuring ≥ 17 mm in diameter were observed, recombinant chorionic gonadotropin (HCG/Ovidrel® 250 mg Serono, SP, Brazil) was administered. Transvaginal, ultrasound-guided oocyte retrieval was performed 36 h after HCG.

2.3. Randomization

A double randomization was carried out. First of all, a table was constructed by computer (first randomization) indicating, for each case, from which wells of a Nunc dish (Nunc Multidishes 176740,

Nunc™, Denmark) the oocytes would be chosen to perform conventional ICSI or to perform IMSI (for example: case 1: wells 1 and 2–ICSI/wells 3 and 4–IMSI; case 2: wells 1 and 4–ICSI/wells 2 and 3–IMSI; case 3 wells 2 and 4–ICSI/wells 1 and 3–IMSI; ...). At the moment of the oocyte retrieval, after a sequential distribution of oocytes among the wells (identified numerically from 1 to 4) of the Nunc dish and before maturity analysis, lots were drawn (second randomization) to determine with which case that specific patient would correspond (could be case #1 as well as case #30). In this manner, the sibling oocytes of each patient were randomly allocated into two groups:

- Control group: oocytes to perform conventional ICSI.
- Studied group: oocytes to perform IMSI.

2.4. Semen preparation

Semen samples were collected in sterile containers by masturbation after a sexual abstinence period of 2 to 5 days. The liquefied fresh semen samples were prepared by Isolate (Irvine Scientific, USA) discontinuous concentration gradient. The final pellet was resuspended in 0.2 ml modified HTF medium (Irvine) supplemented with 10% HSA (Irvine Scientific, Santa Ana, CA, USA). Parts of each semen sample were immediately taken for ICSI and IMSI procedures. The remainder of the semen sample was analysed for standard semen quality parameters according to the World Health Organisation [21].

2.5. ICSI procedure

Conventional ICSI was performed utilising a Nikon Eclipse TE 300 inverted microscope equipped with Narishige 231 D-2 (Narishige, Tokyo, Japan) remote control hydraulic micromanipulators and Narishige IM-9B injectors. Spermatozoa were selected at $400\times$ magnification using Hoffman modulation contrast according to a set of previously published guidelines [22].

2.6. IMSI procedure

A 1 μl aliquot of sperm cell suspension was transferred to a 5 μl microdroplet of modified HTF medium containing 7% polyvinylpyrrolidone solution (PVP medium Irvine Scientific, USA). This microdroplet was placed in a sterile glass dish (FluoroDish™-Word Precision Instrument, USA) under sterile paraffin oil (Ovoil-100, Vitrolife, Goteborg, Sweden). The sperm cells, suspended in the microdroplet, were placed on a microscope stage above an Uplan Apo $100\times$ oil/1.35 objective lens previously covered by a droplet of immersion oil. In this manner, suspended motile sperm cells in the observation droplet could be examined at high magnification through an inverted microscope (Eclipse TE 2000 U Nikon, Japan) equipped with high-powered differential interference contrast optics (DIC/Nomarski). The images were captured by a colour video camera containing effective picture elements (pixels) for high quality image production and projected onto a colour video monitor. Morphological evaluation was accomplished on a monitor screen and the total calculated magnification was $8400\times$ (total magnification: objective magnification = $100\times$, magnification selector = $1.0\times$, video coupler magnification = $1.0\times$, calculated video magnification = $84.50\times$).

The spermatozoa used for IMSI were classified into 5 groups. Grade I consisted of spermatozoa free of any morphological abnormality (normal spermatozoa). A spermatozoon was classified as morphologically normal when it exhibited a normal nucleus as well as acrosome, post-acrosomal lamina, neck, tail and mitochondria, besides not presenting a cytoplasmic droplet

or cytoplasm around the head [4]. For the nucleus, the morphological state was defined by the form and content of the chromatin. The criterion for normality of nuclear form was a smooth, symmetric and oval configuration. Normal means for length and width were estimated as 4.75 ± 2.8 and 3.28 ± 0.20 μm [4] respectively, where the form classified as abnormal presented variation of 2 SD in one of the axes (length: ≥ 5.31 and ≤ 4.19 μm, width: >3.7 or <2.9 μm). For rapid evaluation of nuclear form, a fixed, transparent, celluloid form of a sperm nucleus fitting the criteria was superimposed on the examined cell (chablon construction based on ASTM E 1951-2 [23]). In the same manner, the nuclear form was considered abnormal if extrusion or invagination of the nuclear chromatin mass has been detected (regional malformation of nuclear form). Chromatin content was considered abnormal if one or more vacuoles were observed to occupy more than 4% of the nuclear area. A nucleus was considered normal if both nuclear form and chromatin content were normal. When no Grade I spermatozoa were available or the number was insufficient for injection, spermatozoa were classified by head forms as Grade II: large oval: (≥ 5.31 μm), small oval (≤ 4.19 μm), wide (>3.7 μm width) or narrow (<2.9 μm width); Grade III: presence of regional disorders; Grade IV: presence of large vacuoles on 5–50% of head surface and Grade V: presence of large vacuoles on >50% of head surface.

The same technician, blinded to subject identity, performed all sperm selection. The time involved in the selection step was 30–120 min/sample. At least three spermatozoids were selected for each MII oocyte. After the sperm selection, the microinjections were carried out in the same manner as in ICSI. Spermatozoids were still motile when captured for final selection.

2.7. Oocyte and embryo culture, embryo grading and transfer

Sperm injected oocytes, zygotes and embryos from IMSI and ICSI were submitted to the same culture conditions. Oocytes were examined after 17–20 h to assess fertilisation; zygotes with two distinct equal-sized pronuclei were considered normal. 25 to 27 h after injection, on day 1 of culture, early cleavage was evaluated [24]. Embryos, graded on day 2, were deemed top quality if there were four identical blastomeres (44 h after the sperm injection) with no fragments or multinucleation [25,26]. The same technician performed all oocyte/embryo evaluations. On day 2, the two best-scoring embryos were transferred to the patient's uterus, independent of origin (ICSI or IMSI).

2.8. Statistical analysis and sample size

Data are reported as means $\pm$ SD and were analysed using the InStat 3.0 program (GraphPad Software, San Diego, CA, USA) on a Macintosh computer (Apple Computer Inc., Cupertino, CA, USA). The Wilcoxon matched-pairs signed-ranks and chi-squared tests were used where appropriate. The significance level was set at $P < 0.05$. The primary outcome was the percentage of top quality embryos, defined by the number of top quality embryos obtained from the total number of cleaved embryos. The fertilisation, early cleavage and cleavage rates (secondary outcomes) were also analysed. The variables were analysed in relation to the general population and the subpopulations with male factor and without male factor (with at least 2 previous failures of implantation and/or previous miscarriage after ICSI). The sample size (for the general study) was calculated by planning a comparison between two proportions, control and experimental. Usually, our mean proportion of day 2 embryos considered top quality is 55%. Thus a sample size of 100 embryos in each group has 80% power to detect an increase of 20% with an alpha significance level of 0.05 (two-tailed).

3. Results

Table 1 summarises the characteristics of the general study population and of the subpopulations with and without male factor (≥ 2 previous failures or previous miscarriages).

A total of 331 MII oocytes were selected and injected by the ICSI (n: 172) or IMSI (n: 159) procedure. For IMSI, only spermatozoa from group I were used. No differences ($P > 0.05$) in fertilisation rate (ICSI: 70.9%, 122/172; IMSI: 70.4%, 112/159), early embryo cleavage rate (ICSI: 66.9%, 81/121; IMSI: 60.4%, 67/111) or cleavage rate (ICSI: 99.2%, 121/122; IMSI: 99.1%, 111/112) were observed. On day 2, IMSI and ICSI provided similar proportions of top quality embryos (ICSI: 57.8%, 70/121; IMSI: 52.2%, 58/111; $P > 0.05$). Table 2 summarises the results.

No statistically significant differences were found between ICSI and IMSI in the two subpopulations analysed. In the subpopulation of cases with male factor, a total of 155 MII oocytes were selected and injected by the ICSI (n: 81) or IMSI (n: 74) procedure. No differences ($P > 0.05$) were determined in fertilisation rate (ICSI: 66.7%, 54/81; IMSI: 70.3%, 52/74), early embryo cleavage rate (ICSI: 79.6%, 43/54; IMSI: 61.5%, 32/52), cleavage rate (ICSI: 100%, 54/54; IMSI: 100%, 54/54) or proportion of day 2 top quality embryos (ICSI: 57.4%, 31/54; IMSI: 50%, 26/52). In relation to the

Table 1
Characteristics of the general study population and the subgroups.

Characteristic	Value		
	Total	With male factor infertility	Without male factor infertility
Couples (n)	30	14	16
Cycles (n)	30	14	16
Female age (years) (mean $\pm$ SD)	34.0 ± 4.3	32.9 ± 4.8	35.1 ± 3.5
Male age (years) (mean $\pm$ SD)	38.2 ± 7.2	39.6 ± 9.2	36.9 ± 5.0
Duration of infertility (years) (mean $\pm$ SD)	4.3 ± 3.6	5.1 ± 2.9	3.7 ± 4.0
Aetiology			
Male	43.4% (13/30)	92.9% (13/14)	0
Tuboperitoneal	33.3% (10/30)	0	62.5% (10/16)
Idiopathic	16.7% (5/30)	0	31.3% (5/16)
Endometriosis	3.3% (1/30)	0	6.2% (1/16)
Endometriosis + male	3.3% (1/30)	7.1% (1/14)	0
Total sperm count ($\times 10^6$/ml)	82.6 ± 56.4	2.9 ± 4.1	105.4 ± 40.4
Motility (% spermatozoa) (rapid + slow progression)	45.8 ± 10.1	28.1 ± 18.3	54.7 ± 9.8
Normal spermatozoa (%)	10 ± 5.6	3.7 ± 1.9	13.2 ± 3.8
Vitality (%) (mean $\pm$ SD)	60.8 ± 20.1	51.7 ± 26.7	66.6 ± 12.9
Leukocytes in semen ($\times 10^6$) (mean $\pm$ SD)	0.35 ± 0.28	0.4 ± 0.3	0.3 ± 0.3

Table 2
Comparison between the morphologically selected sperm injection (IMSI) and conventional intracytoplasmic sperm injection (ICSI) study groups.

	Total			With male factor infertility			Without male factor infertility		
	IMSI (n: 159 oocytes)	ICSI (n: 172 oocytes)	P	IMSI (n: 74 oocytes)	ICSI (n: 81 oocytes)	P	IMSI (n: 85 oocytes)	ICSI (n: 91 oocytes)	P
Retrieved oocytes (n)	6.1 ± 2.3	6.4 ± 2.4	ns	6.0 ± 2.1	6.5 ± 2.5	ns	6.2 ± 2.4	6.4 ± 2.4	ns
Oocytes MII (n)	5.3 ± 1.8	5.7 ± 2.3	ns	5.3 ± 2.0	5.8 ± 2.2	ns	5.3 ± 1.6	5.7 ± 2.3	ns
Fertilisation rate	70.4%	70.9%	ns	70.3%	66.7%	ns	70.6%	74.7%	ns
Early cleavage rate	60.4%	66.9%	ns	61.5%	79.6%	ns	56.7%	59.3%	ns
Cleavage rate	99.1%	99.2%	ns	100%	100%	ns	98.3%	98.5%	ns
Top quality day 2 embryos	52.2%	57.8%	ns	50%	57.4%	ns	54.2%	58.2%	ns

ns: not significant ($P > 0.05$).

subpopulation without male factor, a total of 176 MII oocytes were selected and injected by the ICSI (n: 91) or IMSI (n: 85) procedure. Again, the statistical analysis demonstrated no differences ($P > 0.05$) in fertilisation rate (ICSI: 74.7%, 68/91; IMSI: 70.6%, 60/85), early embryo cleavage rate (ICSI: 56.7%, 38/67; IMSI: 59.3%, 35/59), cleavage rate (ICSI: 98.5%, 67/68; IMSI: 98.3%, 59/60) or proportion of day 2 top quality embryos (ICSI: 58.2%, 39/67; IMSI: 54.2%, 32/59). Table 2 summarises the results from the both subpopulations.

4. Discussion

Currently, ICSI is performed after morphological selection of spermatozoa at $200\times$ to $400\times$ magnification. In this magnification range, spermatozoa carrying defects of the head, neck or tail can be detected, but not nuclear vacuoles. Therefore, magnification of $6000\times$ to $12,500\times$ to select spermatozoa, particularly by virtue of permitting a perfect identification of vacuoles, appears to be a better strategy [5,6,8,11–13,27,28]. Nevertheless, in relation to early embryo development and consequently, early paternal effect, the high magnification of sperm selection does not appear to exert significant influence.

In the literature, there is a controversy about the influence of high magnification sperm selection for ICSI on the initial embryo development/quality. Hazout et al. [7] in a study that included 125 couples (with male infertility and at least 2 previous failed ICSI attempts) found a similar number of good-morphology embryos (P = ns) on days 2–3 between IMSI cycles (4.0 ± 1.2) and previous conventional ICSI cycles (3.1 ± 1.1). However, in a comparative matched study performed on 50 IMSI and 50 matched ICSI couples (with male infertility and at least 2 failed previous ICSI attempts), Bartoov et al. [10] concluded that the IMSI procedure may significantly increase the number of top quality embryos at days 2–3. Another comparative matched study by the same group [12], including a newly enlarged number of studied patients (80 couples who underwent a single IMSI trial matched with 80 couples who underwent a routine ICSI procedure) confirmed a statistical difference in favour of IMSI. Unfortunately, its lack of randomization hinders any definite conclusion. The only randomized prospective work (446 couples with diagnoses of severe oligoasthenoteratozoospermia) comparing IMSI and conventional ICSI did not analyse embryo quality [15]. However, in our randomized study there was no statistical difference (P = ns) in the rate of top quality embryos at day 2 between IMSI (52.2%) and ICSI (57.8%) groups. In addition, this result was not altered by the presence (ICSI: 57.4% vs. IMSI 50%, P = ns) or absence of male factor (ICSI: 58.2% vs. IMSI 54.2%, P = ns). This observation supports the lack of influence of high magnification sperm selection on early paternal effect.

On the other hand, the stage of embryo development must be considered. The early paternal effect was observed before the major activation of embryonic genome expression, which starts between the 4-cell and 8-cell stage of human pre-implantation development [1,2]. Our study is the only one that limited the

analysis of embryo quality exclusively to day 2 while the others reported this aspect on days 2 and 3. Thus, depending on the number of embryos considered on each day, the embryo quality analysis may represent more of a late paternal effect (activation of embryonic genome expression) than an early one. Given that the late paternal effect appears to be positively correlated with IMSI, the time of embryo quality analysis can justify the divergent results.

In relation to secondary results, again the analysis found no correlation between the high magnification sperm selection and early paternal effect. The fertilisation rates were similar (P = ns) between IMSI (70.4%) and ICSI (70.9%) groups, a result not influenced by the presence or absence of male factor and that confirms previous findings from other studies that also did not detect any significant difference in the fertilisation rate [4,7,8,10,12]. In addition, in a randomized study, Antinori et al. [15] did not find a statistically significant difference in the number of two-PN zygotes between ICSI and IMSI. In a similar manner, we did not observe any statistical difference in early cleavage rate (ICSI: 66.9%, IMSI: 60.4%, P = ns) or cleavage rate (ICSI: 99.2%, IMSI: 99.1%, P = ns) between the groups. Few studies have attempted to analyse cleavage rate. Only Hazout et al. [7] and Bach et al. [8] made this observation and both reported similar cleavage rate between conventional ICSI attempts and the IMSI cycles.

In conclusion, based on prospective and randomized analysis (sibling oocytes) of all 30 treated cases, IMSI had the same performance with respect to embryo quality at day 2 as conventional ICSI. To the best of our knowledge, this paper is the first randomized study that compared IMSI and conventional ICSI with respect to second-day embryo quality.

Conflict of interest

None declared.

Acknowledgment

References

[1] Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 2004;19:611–5.
[2] Tesarik J. Paternal effects on cell division in the human preimplantation embryo. Reprod Biomed Online 2005;10:370–5.
[3] Cohen-Bacrie P. Sperm quality and selection. J Gynecol Obstet Biol Reprod (Paris) 2008;37. Suppl 1:S4–8.
[4] Bartoov B, Berkovitz A, Eltes F, Kogosowski A, Menezo Y, Barak Y. Real-time fine morphology of motile human sperm cells is associated with IVF–ICSI outcome. J Androl 2002;23:1–8.

[5] Franco Jr JG, Baruffi RL, Mauri AL, Petersen CG, Oliveira JB, Vagnini L. Significance of large nuclear vacuoles in human spermatozoa: implications for ICSI. Reprod Biomed Online 2008;17:42–5.

[6] Garolla A, Fortini D, Menegazzo M, De Toni L, Nicoletti V, Moretti A, et al. High-power microscopy for selecting spermatozoa for ICSI by physiological status. Reprod Biomed Online 2008;17:610–6.

[7] Hazout A, Dumont-Hassan M, Junca AM, Cohen Bacrie P, Tesarik J. High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. Reprod Biomed Online 2006;12:19–25.

[8] Bach M, Neyer A, Stecher A, Uher P, Vanderzwalmen P, Zintz M, et al. Morphological integrity of human sperm nuclei and blastocyst formation after intracytoplasmic morphologically selected sperm injection (IMSI). Hum Reprod 2007;22:O273.

[9] Vanderzwalmen P, Hiemer A, Rubner P, Bach M, Neyer A, Stecher A, et al. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reprod Biomed Online 2008;17:617–27.

[10] Bartoov B, Berkovitz A, Eltes F, Kogosovsky A, Yagoda A, Lederman H, et al. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertil Steril 2003;80:1413–9.

[11] Berkovitz A, Eltes F, Yaari S, Katz N, Barr I, Fishman A, et al. The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. Hum Reprod 2005;20:185–90.

[12] Berkovitz A, Eltes F, Lederman H, Peer S, Ellenbogen A, Feldberg B, et al. How to improve IVF–ICSI outcome by sperm selection. Reprod Biomed Online 2006;12:634–8.

[13] Berkovitz A, Eltes F, Ellenbogen A, Peer S, Feldberg D, Bartoov B. Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? Hum Reprod 2006;21:1787–90.

[14] Wittemer C, Pujol A, Boughali H, Viville S. The impact of high-magnification evaluation of sperm on ART outcome. Hum Reprod 2006;21:I59–60.

[15] Antinori M, Licata E, Dani G, Cerusico F, Versaci C, d'Angelo D, et al. Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. Reprod Biomed Online 2008;16:835–41.

[16] Berkovitz A, Eltes F, Paul M, Adrian E, Benjamin B. The chance of having a healthy normal child following intracytoplasmic morphologically-selected sperm injection (IMSI) treatment is higher compared to conventional IVF–ICSI treatment. Fertil Steril 2007;88:S20.

[17] Sathananthan AH, Ratnam SS, Ng SC, Tarin JJ, Gianaroli L, Trounson A. The sperm centriole: its inheritance, replication and perpetuation in early human embryos. Hum Reprod 1996;11:345–56.

[18] Palermo G, Munne S, Cohen J. The human zygote inherits its mitotic potential from the male gamete. Hum Reprod 1994;9:1220–5.

[19] Sutovsky P, Schatten G. Paternal contributions to the mammalian zygote: fertilization after sperm-egg fusion. Int Rev Cytol 2000;195:1–65.

[20] Tesarik J, Mendoza C, Greco E. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. Hum Reprod 2002;17:184–9.

[21] World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge, UK; New York, NY: Published on behalf of the World Health Organization by Cambridge University Press, 1999.

[22] Franco Jr JG, Baruffi RL, Mauri AL, Petersen CG, Campos MG. Semi-programmed ovarian stimulation as the first choice in in vitro fertilization programmes. Hum Reprod 1995;10:568–71.

[23] ASTM. Standard E1951-02 Physical and mechanical testing standards. West Conshohocken, PA 2007, doi: 10.1520/E1951-02R07, www.astm.org.

[24] Petersen CG, Mauri AL, Ferreira R, Baruffi RL, Franco Jr JG. Embryo selection by the first cleavage parameter between 25 and 27 hours after ICSI. J Assist Reprod Genet 2001;18:209–12.

[25] Saldeen P, Sundstrom P. Nuclear status of four-cell preembryos predicts implantation potential in in vitro fertilization treatment cycles. Fertil Steril 2005;84:584–9.

[26] Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, et al. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum Reprod 2007;22:548–57.

[27] Bartoov B, Eltes F, Pansky M, Langzam J, Reichart M, Soffer Y. Improved diagnosis of male fertility potential via a combination of quantitative ultramorphology and routine semen analyses. Hum Reprod 1994;9:2069–75.

[28] Mundy AJ, Ryder TA, Edmonds DK. A quantitative study of sperm head ultrastructure in subfertile males with excess sperm precursors. Fertil Steril 1994;61:751–4.