J Assist Reprod Genet (2012) 29:1241–1247
DOI 10.1007/s10815-012-9868-x

ASSISTED REPRODUCTION TECHNOLOGIES

The predictive value of high-magnification sperm morphology examination on ICSI outcomes in the presence of oocyte dysmorphisms

Amanda Souza Setti · Daniela Paes Almeida Ferreira Braga · Rita Cassia Savio Figueira · Assumpto Iaconelli Jr. · Edson Borges Jr.

Received: 10 July 2012 / Accepted: 27 September 2012 / Published online: 6 October 2012

Abstract
Purpose To evaluate the relationship between oocyte dysmorphisms and IMSI outcomes
Methods Data of IMSI cycles performed in 332 patients were included in this study. Patients were included only if presented more than four and less than 30 oocytes upon oocyte retrieval. Patients who underwent IMSI were matched, concerning female age and sperm parameters, with patients who underwent ICSI in the same period (n=332). The two groups (ICSI and IMSI) were compared with regard to treatment outcomes. The influence of IMSI on fertilization and embryo quality on D3 and D5, when oocyte dysmorphisms were present was analyzed.
Results A total of 6444 oocytes were morphologically evaluated and injected. Regardless of the oocyte quality, IMSI performance was a determinant of the increased odds of development to high quality embryo on D3 (OR: 1.98; CI: 1.54–2.56) and D5 (OR: 3.27; CI: 1.61–6.66).
Conclusions The selection of a morphologically normal spermatozoon under high-magnification is associated with increased embryo quality, regardless of the oocyte morphology. The injection of a spermatozoon, selected under high-magnification, into a morphologically normal oocyte leads to the highest probability of developing high quality embryos.

Keywords Embryo quality · Fertilization · Intracytoplasmic morphologically selected sperm injection · Intracytoplasmic sperm injection, oocyte dysmorphisms

Capsule The selection of a morphologically normally spermatozoon under high-magnification is associated with increased embryo quality, regardless of the oocyte morphology.

A. S. Setti · D. P. A. F. Braga · A. Iaconelli Jr. · E. Borges Jr.
Sapientiae Institute – Educational and Research Center in Assisted Reproduction,
Rua Vieira Maciel, 62,
São Paulo, SP, Brazil 04503-040

A. S. Setti · D. P. A. F. Braga · R. C. S. Figueira · A. Iaconelli Jr. · E. Borges Jr. (✉)
Fertility – Assisted Fertilization Center,
Av. Brigadeiro Luis Antonio, 4545,
São Paulo, SP, Brazil 01401-002
e-mail: science@sapientiae.org.br

Introduction

A new concept of observation of sperm at high magnification in real time, known as motile sperm organelle morphology examination (MSOME), was recently introduced [5]. Its incorporation, together with a micromanipulation system, has allowed the introduction of a modified ICSI procedure, called 'intracytoplasmic morphologically selected sperm injection' (IMSI).

Several publications reported that the IMSI procedure is positively associated with pregnancy rates in couples with previous and repeated implantation failures [6, 7, 9, 34] and in patients with an elevated degree of DNA fragmented spermatozoa [27, 48]. Moreover, a recently published meta-analysis showed that the IMSI procedure is associated with improved embryo quality, implantation, pregnancy and miscarriage rates [40].

It is believed that the high magnification allows the detection of sperm containing nuclear vacuoles. Previous studies demonstrated that sperm nuclear vacuoles are associated with sperm DNA fragmentation [11, 23, 24, 35].

It is known that human sperm have highly dynamic and essential participation in embryogenesis that clearly goes beyond the fertilization process. The first divisions of the newly formed embryo depend on the machinery of the oocyte. The activation of the embryonic genome occurs at the stage of 4–

8 cells [13]. As a result, the effects of injection of sperm with nuclear abnormalities are usually not detected before the 8-cell stage, when a high expression of genes derived from the sperm is initiated [4]. Studies suggest that this result reflects a late paternal effect, which is related to blockage of embryonic development observed during / after the implantation of embryos with normal karyotype [10, 43].

The ability of human oocyte to repair sperm DNA damage, has not been fully elucidated, but some studies showed that the oocyte is equipped with mechanisms that can repair some of the paternal DNA abnormalities [25, 31, 47]. However, the ability to repair the oocyte depends on the quality and genetics of the oocyte.

It is generally accepted that good-quality human MII oocytes should have a clear, moderately granular cytoplasm that does not contain inclusions, a small perivitelline space (PVS) containing a single unfragmented IPB and a round, clear, colourless zona pellucida (ZP) [29, 44, 45]. Nevertheless, more than half of all collected oocytes show at least one morphological abnormality [19, 21].

Because the pre-selection of oocytes with the highest developmental potential, based on morphological criteria, is of obvious interest for improving the efficiency of assisted reproduction technology, numerous studies have reported the presence or absence of relationship between oocyte morphological abnormalities and ICSI outcomes [3, 15, 17–19, 22, 26, 28, 30, 36, 39, 44, 46, 49, 50]. However, to date, the relationship between oocyte dysmorphisms and IMSI outcomes has never been investigated. Therefore, that was the aim of this study.

Materials and methods

Experimental design, patients and inclusion criteria

Data of IMSI cycles performed in 332 patients, performed from January 2009 to December 2010, were included in this retrospective cohort study. Patients were included only if presented more than four and less than 30 oocytes upon oocyte retrieval, in order to exclude poor response and hyperstimulation's infuences. To minimize the influence of severe male factor, all cases of sperm concentration less than 1×10^6M/mL and sperm motility less than 20 % were excluded from the study. Patients who underwent IMSI were matched, concerning female age and sperm parameters, with patients who underwent ICSI in the same period (n=332). The matching process was based on four features relevant for IMSI outcomes i.e. female age (±1 year), percentage of morphologically normal spermatozoa forms (±1 %), percentage of sperm motility (±5 %), and total sperm concentration (±5 million/mL). If more than one cycle was eligible the best match was chosen by random selection. The matching procedure was conducted blinded without any information about cycle's outcome.

A written informed consent was obtained, in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the local institutional review board.

Controlled ovarian stimulation

Ovarian stimulation was achieved by the administration of a recombinant follicle-stimulating hormone (r-FSH, Gonal-F®, Serono, Geneve, Switzerland) on a daily basis until the visualization of at least one follicle ≥14 mm, at which time, we began the administration of a gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide; Serono Laboratories, Geneva, Switzerland) 0.25 mg subcutaneous (SC).

The ovulation trigger was given by SC injection of 250 μg of recombinant human chorionic gonadotrophin (hCG, Ovidrel™, Serono, Geneve, Switzerland) when at least three follicles ≥17 mm were observed. Oocyte retrieval was performed 35 h after the administration of hCG, through transvaginal ultrasonography.

Preparation of oocytes

Retrieved oocytes were maintained in culture media (Global® for fertilisation, LifeGlobal, Connecticut, USA) supplemented with 10 % protein supplement (LGPS, LifeGlobal, Connecticut, USA) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, Connecticut, USA) for 2–3 h before removal of cumulus cells. The surrounding cumulus cells were removed after exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/mL, LifeGlobal, Connecticut, USA). The remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

Oocyte morphology was assessed using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon®, Tokyo, Japan) with a Hoffmann modulation contrast system under 400× magnification, just before sperm injection (4 h after retrieval). The following oocyte dysmorphisms were recorded: (i) cytoplasmic granularity, (ii) cytoplasmic color, (iii) vacuoles in the ooplasm, (iv), aggregates of smooth endoplasmic reticulum clusters (SER) in the ooplasm, (v) large perivitelline space (PVS), (vi) PVS granularity, (vii) fragmented polar body (PB), (viii) zona pellucida (ZP) abnormalities and (ix) oocyte shape abnormalities. Oocytes that were observed to have released the first polar body were considered mature and were used for ICSI.

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection (ICSI) was performed in a micro-injection dish prepared with 4 μL droplets of buffered medium (Global® w/HEPES, LifeGlobal, Connecticut, USA)

and covered with paraffin oil on a heated stage at 37.0±0.5 °C of an inverted microscope. Approximately 16 h after ICSI, fertilisation was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were maintained in a 50 μL drop of culture medium (Global®, LifeGlobal, Connecticut, USA) supplemented with 10 % protein supplement covered with paraffin oil in a humidified atmosphere under 6 % CO2 at 37 °C for 3 days.

Intracytoplasmic morphologically selected sperm injection

Sperm selection in the IMSI group was examined at high magnification using an inverted Nikon Diaphot microscope equipped with high-power differential interference contrast optics (DIC/Nomarski). The total calculated magnification was 6,600×. An aliquot of the sperm cell suspension was transferred to a microdroplet of modified human tubal fluid medium containing 8 % polyvinyl pyrrolidone (PVP; Irvine Scientific, SantaAna,CA) in a sterile glass dish (FluoroDish; World Precision Instrument, Sarasota, FL). The dish was placed on a microscope stage above a Uplan Apo × 100 oil/1.35 objective lens previously covered by a droplet of immersion oil. The sperm cells exhibiting normally shaped nuclei ([1] smooth, [2] symmetric, and [3] oval configuration) and [4] normal nuclear chromatin content (if it contained no more than one vacuole, which occupies <4 % of the nuclear area) were selected for injection [5–8, 14, 25, 47].

Embryo quality and transfer

To evaluate cleavage-stage morphology, the following parameters were recorded: the number of blastomeres, the percentage of fragmentation, the variation in blastomere symmetry, the presence of multinucleation and the defects in the zona pellucida and cytoplasm. High-quality cleavage-stage embryos were defined as those with all of the following characteristics: 8–10 cells on day three of development, <15 % fragmentation, symmetric blastomeres, absence of multinucleation, colourless cytoplasm with moderate granulation and no inclusions, absence of perivitelline space granularity and absence of zona pellucida dysmorphism. Embryos lacking any of the above characteristics were considered to be of low quality.

To evaluate blastocyst-stage morphology, the size and compactness of the inner cell mass (ICM) and the cohesiveness and number of trophectoderm (TE) cells were recorded. Embryos were given a numerical score from one to six based on their degree of expansion and hatching status as follows: 1, an early blastocyst with a blastocoel less than half the volume of the embryo; 2, a blastocyst with a blastocoel greater than half of the volume of the embryo; 3, a full blastocyst with a blastocoel completely filling the embryo; 4, an expanded blastocyst; 5, a hatching blastocyst; and 6, a hatched blastocyst. For full, expanded, hatching, and hatched blastocysts, the ICM was classified as either high quality (tightly packed with many cells) or low quality (loosely grouped with several or few cells). Similarly, the TE was also classified as either high quality (many cells forming a cohesive epithelium) or low quality (few cells forming a loose epithelium or very few cells).

Embryo transfer was performed on day 3 by using a soft catheter with transabdominal ultrasound guidance. One to three embryos were transferred per patient. Embryos that were not transferred were cultured until day 5 and vitrified after morphological evaluation.

Clinical follow-up

A pregnancy test was performed 12 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed when the fetal heartbeat was detected. Pregnancy rates were calculated per transfer. Miscarriage was defined as pregnancy loss before 20 weeks.

Data analysis

The two groups (ICSI and IMSI) were compared with regard to: (i) age; (ii) total dose of FSH administered (IU), (iii) number of retrieved oocytes, (iv) oocyte yield, (v) metaphase two (MII) oocyte rate, (vi) normal fertilization rate, (vii) percentage of high quality embryos on the third (D3) and fifth (D5) day of development, (viii) number of transferred embryos, (ix) pregnancy rate, (x) implantation rate and (xi) miscarriage rate.

Fertilization and embryo quality on D3 and D5 were our primary endpoints. Because embryos originating from oocytes presenting both normal and abnormal morphology were transferred, it would not be wise to set implantation and pregnancy as endpoints. The influence of IMSI on fertilization and embryo quality on D3 and D5, when oocyte dysmorphisms were present (isolated and/or in conjunction) was analyzed.

Statistical analysis

Data are expressed as mean ± standard deviation for continuous variables, while percentages were used for categorical variables. Mean values were compared by Student's *t* parametric test or Mann–Whitney non-parametric test. Percentages were compared by the Chi-squared or Fisher exact test, only when expected frequency was five or fewer.

Binary logistic regression, adjusted for maternal age, was used to evaluate the predictive value of IMSI on fertilization and embryo quality when oocytes presenting dysmorphisms

were injected. Results were expressed as odds ratio (OR), with 95 % confidence intervals (CI) and p values, and were considered to be significant at the 5 % critical level ($p<0.05$). Data analysis was conducted using MINITAB Software.

Results

There were no significant differences between ICSI and IMSI groups with regard to patients' characteristics, demographics and distribution of causes of infertility (Table 1). The percentages of high-quality embryos on D3 (51.2 vs 45.1, $p=0.033$) and D5 (37.3 vs 29.1, $p=0.004$) of development were significantly higher in IMSI group as compared to ICSI group.

The influences of IMSI on fertilization and embryo quality are shown in Table 2. Considering all the injected oocytes ($n=6444$), we observed that high-quality embryos on D3 were twice as likely to occur in the IMSI groups as compared to ICSI group (OR: 1.98, $p<0.001$) and the development to blastocyst stage was three times as likely to occur in the IMSI group as compared to the ICSI group (OR: 3.27, $p=0.001$).

Regarding the oocytes presenting normal morphology, the odds of developing to high quality embryos on D3 (OR: 4.53, $p<0.001$) and D5 (OR: 6.14, $p=0.011$) were higher in the IMSI group (Table 2).

When only oocytes presenting abnormal morphology were evaluated, the odds of developing to high-quality embryos on D3 (OR: 1.63, $p=0.001$) and D5 (OR: 2.68, $p=0.013$) were higher in the IMSI group (Table 2).

The predictive value of IMSI on fertilization and embryo quality when oocytes presenting individual dysmorphisms were injected is shown in Table 3. In oocytes presenting exclusively vacuoles, the high-magnification approach was determinant to the increased odds of development to high quality embryo on D3 (OR: 1.80, $p=0.001$). It was not possible to associate individual oocyte dysmorphisms and development into high quality embryo on D5 because there were very few interactions between these variables.

Discussion

In this study we showed that the high-magnification approach has a positive effect on embryo development, regardless of the oocyte quality. High quality embryos on D3 were up to 4,5 times as likely to occur in the IMSI group as compared to the ICSI group. On D5, high quality embryos were up to 6 times as likely to occur in the IMSI group as compared to the ICSI group. Moreover, considering oocytes presenting vacuoles, we demonstrated that a high quality embryo was almost twice as likely to occur in the IMSI group as compared to the ICSI group.

It is generally accepted that semen parameters and chromatin remodeling influence fertilization or early events post fertilization [42]. It has been shown that sperm with protamine deficiency and increased histone remnants, lead to premature chromatin condensation that is the cause of failures in fertilization and embryo development [16, 33].

Immature human sperm show diminished plasma membrane remodeling and zona-binding ability, increased rate of

Table 1 Patient's characteristics, demographics and distribution of causes of infertility in ICSI and IMSI groups

Variable	ICSI group ($n=332$)	IMSI group ($n=332$)	p value
Female age	33.9±4.9	33.9±4.9	0.988
Causes of infertility (%)			
Male factor	40.1	42.2	0.580
Female factor	33.1	33.7	0.869
Male and female factor	16.6	15.7	0.751
Unexplained infertility	10.2	8.4	0.423
Dose of FSH administered (IU)	2205±644	2239±668	0.738
Number of retrieved oocytes	13.0±6.53	13.4±6.32	0.700
Oocyte yield (%)	73.1±18.1	70.3±16.0	0.292
MII oocyte rate (%)	74.3±18.3	70.0±18.1	0.134
Fertilization rate (%)	70.8	68.5	0.461
High quality embryos on D3 (%)	45.1	51.2	0.033
High quality embryos on D5 (%)	29.1	37.3	0.004
Transferred embryos	2.1±0.9	2.2±0.9	0.634
Pregnancy rate (%)	37.0	38.8	0.823
Implantation rate (%)	24.4	28.7	0.470
Miscarriage rate (%)	20.0	12.9	0.453

IU international units; *D3* day 3 of embryo development; *D5* day 5 of embryo development

Table 2 Influence of IMSI on fertilization and high-quality embryos on day 3 and 5 according to oocyte morphological groups

Response variable	Predictors variable Oocyte groups	OR	CI	*P* value
Fertilisation rate				
	Total	1.09	0.87–1.35	0.462
	Normal morphology	0.99	0.61–1.60	0.955
	Abnormal morphology	1.11	0.87–1.42	0.397
High-quality embryo (D3)				
	Total	1.98	1.54–2.56	<0.001
	Normal morphology	4.53	2.44–8.39	<0.001
	Abnormal morphology	1.63	1.22–2.16	0.001
High-quality embryo (D5)				
	Total	3.27	1.61–6.66	0.001
	Normal morphology	6.14	1.24–30.29	0.011
	Abnormal morphology	2.68	1.20–5.99	0.013

OR odds ratio, *CI* confidence intervals

aneuploidies, increased rate of lipid peroxidation and consequential DNA fragmentation; and collectively, these factors cause reduced fertilization rates and adversely affect the early and late paternal contributions to the zygote [41].

ICSI is usually performed under an overall optical magnification of 400×, which only enables the observation of major sperm morphological defects, whereas minor morphological defects are often not identified. As a consequence, spermatozoa appearing as morphologically normal at this magnification may, in fact, carry various structural abnormalities that may negatively influence embryo development [40].

The most predictive factor of sperm quality is the incidence of vacuoles in the sperm head [37]. The MSOME enables the selection of motile spermatozoa with fine nuclear morphology and free of head vacuoles in real time at high magnification, which are then injected into oocytes.

Perdrix et al. [37] demonstrated that sperm vacuoles are exclusively nuclear. The study showed that aneuploidy and chromatin condensation defects were the main alterations observed in spermatozoa presenting large vacuoles. The authors proposed a global impairment of the spermatogenesis process as a common origin of the morphological alterations. Accordingly, Franco Jr. et al. [23] showed an association between large nuclear vacuoles and DNA damage in spermatozoa.

The mammalian oocyte provides gene products that are responsible for repairing DNA damage in both parental genomes after fertilization [1, 12]. These maternal gene products persist to sustain the zygote until its genome is fully activated [13]. However, the ability to repair the oocyte depends on the quality and genetics of the oocyte.

Therefore, we hypothesized that oocytes presenting abnormal morphology possess reduced capability to repair sperm DNA damage. Because high-magnification sperm selection enables the identification of spermatozoa free of DNA damage, we expected that the subsequent embryos would demonstrate an improved development.

In this study, the high-magnification approach was associated with increased embryo development, regardless of the oocyte morphology. Furthermore, we observed that the odds of developing to high quality embryos on D3 and D5 were higher in the group of oocytes presenting normal morphology

Table 3 Influence of IMSI on fertilization and embryo quality on day 3 according to oocyte morphological evaluation

Oocyte dysmorphism	Fertilization			Embryo quality (D3)		
	OR	CI	*p* value	OR	CI	*p* value
Granular cytoplasm (*n*=299)	1.16	0.48–2.77	0.316	1.12	0.41–3.46	0.338
Dark cytoplasm (*n*=291)	0.92	0.31–1.89	0.392	1.92	0.88–4.37	0.862
SER (*n*=303)	0.33	0.11–1.98	0.490	1.21	0.20–7.22	0.839
Vacuoles (*n*=246)	0.90	0.37–2.20	0.825	1.80	1.26–3.07	0.001
PVS granularity (*n*=410)	0.93	0.69–1.25	0.636	1.49	0.97–2.08	0.180
ZP (*n*=516)	1.34	0.67–2.69	0.406	1.94	0.95–3.95	0.068
PVS size (*n*=885)	0.61	0.36–1.05	0.072	1.51	0.84–2.71	0.163
Fragmented PB (*n*=1281)	1.35	0.89–2.04	0.159	1.12	0.67–1.88	0.670
Shape (*n*=222)	1.10	0.42–2.88	0.843	4.07	0.97–17.07	0.140

SER aggregates of smooth endoplasmic reticulum cluster; *PVS* perivitelline space; *ZP* zona pellucida; *PB* polar body; *OR* odds ratio; *CI* confidence intervals; *D3* day 3 of development

than the odds in the group of oocytes presenting dysmorphisms. This finding emphasizes the role of oocyte quality on the embryo development.

When oocytes were divided according to a specific morphological alteration, only those presenting vacuoles were benefited from the high-magnification sperm selection.

Balaban et al., [2] summarized the data in the literature that studied the effect of oocyte morphology on in vitro fertilisation (IVF). The authors suggested that the severe cytoplasmic dysmorphisms, such as SER, centrally severe granulation and excessive vacuolization, should be considered as abnormal and taken into consideration for the selection of a viable oocyte.

It has been proposed that oocyte vacuoles arise either spontaneously or by fusion of vesicles derived from the smooth endoplasmic reticulum and/or Golgi apparatus [20]. How vacuolisation affects fertilisation and pronuclear formation is still unclear. During the completion of meiosis I and the transition to the metaphase II stage, synchronous cytoplasmic maturation, mitochondrial changes, protein synthesis and cytoskeletal changes take place [38]. It has been suggested that the presence of intracytoplasmic vacuoles is associated with severe oocyte degeneration, displacement of the MII spindle from its polar position [45], abnormal cytokinesis pattern [32, 45], compromised embryo development [3] and impaired blastocyst formation [20].

In conclusion, the selection of a morphologically normal spermatozoon under high-magnification positively affects embryo development, regardless of the oocyte quality. Further, our results demonstrate that the injection of a spermatozoon, selected under high-magnification, into a morphologically normal oocyte leads to the highest probability of developing high quality embryos, emphasizing the vital role played by both the oocyte and the spermatozoon on embryo development potential.

References

1. Ashwood-Smith MJ, Edwards RG. DNA repair by oocytes. Mol Hum Reprod. 1996;2:46–51.
2. Balaban B, Urman B. Effect of oocyte morphology on embryo development and implantation. Reprod Biomed Online. 2006;12:608–615.
3. Balaban B, Yakin K, Alatas C, Oktem O, Isiklar A, Urman B. Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study (in press). Reprod Biomed Online. 2011.
4. Barroso G, Valdespin C, Vega E, Kershenovich R, Avila R, Avendano C, et al. Developmental sperm contributions: fertilization and beyond. Fertil Steril. 2009;92:835–48.
5. Bartoov B, Berkovitz A, Eltes F. Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection. N Engl J Med. 2001;345:1067–8.
6. 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.
7. 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.
8. 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.
9. 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.
10. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21:2876–81.
11. Braga DP, Setti AS, Figueira RC, Nichi M, Martinhago CD, Iaconelli A Jr., et al. Sperm organelle morphologic abnormalities: contributing factors and effects on intracytoplasmic sperm injection cycles outcomes. Urology. 2011.
12. Brandriff B, Pedersen RA. Repair of the ultraviolet-irradiated male genome in fertilized mouse eggs. Science. 1981;211:1431–3.
13. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988;332:459–61.
14. Cassuto NG, Bouret D, Plouchart JM, Jellad S, Vanderzwalmen P, Balet R, et al. A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. Fertil Steril. 2009;92:1616–25.
15. Chamayou S, Ragolia C, Alecci C, Storaci G, Maglia E, Russo E, et al. Meiotic spindle presence and oocyte morphology do not predict clinical ICSI outcomes: a study of 967 transferred embryos. Reprod Biomed Online. 2006;13:661–7.
16. Cho C, Jung-Ha H, Willis WD, Goulding EH, Stein P, Xu Z, et al. Protamine 2 deficiency leads to sperm DNA damage and embryo death in mice. Biol Reprod. 2003;69:211–7.
17. Ciotti PM, Notarangelo L, Morselli-Labate AM, Felletti V, Porcu E, Venturoli S. First polar body morphology before ICSI is not related to embryo quality or pregnancy rate. Hum Reprod. 2004;19:2334–9.
18. De Santis L, Cino I, Rabellotti E, Calzi F, Persico P, Borini A, et al. Polar body morphology and spindle imaging as predictors of oocyte quality. Reprod Biomed Online. 2005;11:36–42.
19. De Sutter P, Dozortsev D, Qian C, Dhont M. Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. Hum Reprod. 1996;11:595–7.
20. Ebner T, Moser M, Sommergruber M, Gaiswinkler U, Shebl O, Jesacher K, et al. Occurrence and developmental consequences of vacuoles throughout preimplantation development. Fertil Steril. 2005;83:1635–40.
21. Ebner T, Moser M, Tews G. Is oocyte morphology prognostic of embryo developmental potential after ICSI? Reprod Biomed Online. 2006;12:507–12.
22. Figueira RD, de Almeida Ferreira Braga DP, Semiao-Francisco L, Madaschi C, Iaconelli Jr A, Borges Jr E. Metaphase II human oocyte morphology: contributing factors and effects on fertilization potential and embryo developmental ability in ICSI cycles. Fertil Steril. 2010;94:1115–7.
23. 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.
24. Franco Jr JG, Mauri AL, Petersen CG, Massaro FC, Silva LF, Felipe V, et al. Large nuclear vacuoles are indicative of abnormal chromatin packaging in human spermatozoa. Int J Androl. 2012;35:46–51.

25. Gasca S, Pellestor F, Assou S, Loup V, Anahory T, Dechaud H, et al. Identifying new human oocyte marker genes: a microarray approach. Reprod Biomed Online. 2007;14:175–83.
26. Hassan-Ali H, Hisham-Saleh A, El-Gezeiry D, Baghdady I, Ismaeil I, Mandelbaum J. Perivitelline space granularity: a sign of human menopausal gonadotrophin overdose in intracytoplasmic sperm injection. Hum Reprod. 1998;13:3425–30.
27. 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.
28. Kahraman S, Yakin K, Donmez E, Samli H, Bahce M, Cengiz G, et al. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum Reprod. 2000;15:2390–3.
29. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Veeck LL, et al. New method of evaluating sperm morphology with predictive value for human in vitro fertilization. Urology. 1987;30:248–51.
30. Loutradis D, Drakakis P, Kallianidis K, Milingos S, Dendrinos S, Michalas S. Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. Fertil Steril. 1999;72:240–4.
31. Meseguer M, Santiso R, Garrido N, Garcia-Herrero S, Remohi J, Fernandez JL. Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. Fertil Steril. 2011;95:124–8.
32. Nayudu PL, Lopata A, Jones GM, Gook DA, Bourne HM, Sheather SJ, et al. An analysis of human oocytes and follicles from stimulated cycles: oocyte morphology and associated follicular fluid characteristics. Hum Reprod. 1989;4:558–67.
33. Oliva R. Protamines and male infertility. Hum Reprod Update. 2006;12:417–35.
34. Oliveira JB, Cavagna M, Petersen CG, Mauri AL, Massaro FC, Silva LF, et al. Pregnancy outcomes in women with repeated implantation failures after intracytoplasmic morphologically selected sperm injection (IMSI). Reprod Biol Endocrinol. 2011;9:99.
35. Oliveira JBA, Petersen CG, Mauri AL, Massaro FC, Baruffi R, Franco JG. Clinical outcomes of IMSI in previous ICSI failures. Fertil Steril. 2010;94:S55.
36. Otsuki J, Okada A, Morimoto K, Nagai Y, Kubo H. The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. Hum Reprod. 2004;19:1591–7.
37. Perdrix A, Travers A, Chelli MH, Escalier D, Do Rego JL, Milazzo JP, et al. Assessment of acrosome and nuclear abnormalities in human spermatozoa with large vacuoles. Hum Reprod. 2011;26:47–58.
38. Scott LA. Oocyte and embryo polarity. Semin Reprod Med. 2000;18:171–83.
39. Serhal PF, Ranieri DM, Kinis A, Marchant S, Davies M, Khadum IM. Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum Reprod. 1997;12:1267–70.
40. Souza Setti A, Ferreira RC, Paes de Almeida Ferreira Braga D, de Cassia Savio Figueira R, Iaconelli Jr A, Borges Jr E. Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis. Reprod Biomed Online. 2010;21:450–5.
41. Tavalaee M, Razavi S, Nasr-Esfahani MH. Effects of sperm acrosomal integrity and protamine deficiency on in vitro fertilization and pregnancy rate. Iran J Fertil Steril. 2007;1:27–34.
42. Tavalaee M, Razavi S, Nasr-Esfahani MH. Influence of sperm chromatin anomalies on assisted reproductive technology outcome. Fertil Steril. 2009;91:1119–26.
43. 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.
44. Ubaldi F, Rienzi L. Morphological selection of gametes. Placenta. 2008;29(Suppl B):115–20.
45. Van Blerkom J. Occurrence and developmental consequences of aberrant cellular organization in meiotically mature human oocytes after exogenous ovarian hyperstimulation. J Electron Microsc Tech. 1990;16:324–46.
46. Verlinsky Y, Kuliev A. Preimplantation diagnosis of common aneuploidies in infertile couples of advanced maternal age. Hum Reprod. 1996;11:2076–7.
47. Wells D, Bermudez MG, Steuerwald N, Thornhill AR, Walker DL, Malter H, et al. Expression of genes regulating chromosome segregation, the cell cycle and apoptosis during human preimplantation development. Hum Reprod. 2005;20:1339–48.
48. Wilding M, Coppola G, di Matteo L, Palagiano A, Fusco E, Dale B. Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa. J Assist Reprod Genet. 2011;28:253–62.
49. Xia P. Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. Hum Reprod. 1997;12:1750–5.
50. Zini A, Meriano J, Kader K, Jarvi K, Laskin CA, Cadesky K. Potential adverse effect of sperm DNA damage on embryo quality after ICSI. Hum Reprod. 2005;20:3476–80.