

Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes

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Objective: To examine the effect of sperm morphology on embryo development at the chromosomal level.

Design: Prospective study.

Setting: Assisted fertilization center.

Patient(s): Couples who underwent IVF-PGS cycle, as a result of advanced maternal age, were randomly allocated into two groups: intracytoplasmic sperm injection (ICSI; n = 60) or intracytoplasmic morphologically selected sperm injection (IMSI; n = 60).

Intervention(s): IVF in conjunction with preimplantation genetic screening (PGS).

Main Outcome Measure(s): Sperm nuclear morphology at high-magnification ICSI and incidence of aneuploidy in derived embryo.

Result(s): There was a significantly increased incidence for sex chromosome aneuploidy in ICSI embryos when compared with IMSI embryos (23.5% vs. 15.0%, respectively). High-magnification sperm selection was associated with a significantly lower risk of sex chromosome abnormalities (odds ratio [OR], 0.57; confidence interval [CI], 0.37–0.90). The incidence of chaotic embryos was also significantly higher with the ICSI procedure (27.5% vs. 18.8%), while the IMSI procedure was associated with a significantly lower risk of chaotic embryos (OR, 0.64; CI, 0.43–0.96). Moreover, the cycle cancellation rate was significantly higher in ICSI cycles (11.8% vs. 2.5%). High-magnification sperm selection was a significant predictor of the likelihood of cycle cancellation (OR, 0.26; CI, 0.11–0.62).

Conclusion(s): Spermatozoa free of nuclear morphological malformations were found to be significantly associated with the lower incidence of aneuploidy in derived embryos, resulting in lower rates of cycle cancellation. (Fertil Steril® 2011;95:990–3. ©2011 by American Society for Reproductive Medicine.)

Key Words: Intracytoplasmic morphologically selected sperm injection, IMSI, preimplantation genetic screening, PGS

Bartoov et al. (1) developed a new method, the motile sperm organelle morphology examination (MSOME), in an attempt to determine the potential of subtle sperm morphological characteristics in predicting intracytoplasmic sperm injection (ICSI) cycle outcomes. The sperm examination, performed in real time using a light microscope equipped with high-power Nomarski optics and enhanced by digital imaging to achieve a magnification of up to $\times 6,300$, showed a significant and positive association with both fertilization rates and pregnancy outcomes. These findings confirm a previous electron microscopy study showing the importance of the normalcy of sperm

head subcellular organelles in achieving pregnancy by ICSI (2). In a subsequent prospective study, Bartoov et al. (3) found that 62% of the couples had conceived after oocyte microinjection of select, motile spermatozoa with morphologically normal nuclei. The average number of previously failed routine ICSI treatments in this study group was 4.1 ± 1.9 cycles. In addition, using this new high-magnification ICSI attempt, named intracytoplasmic morphologically selected sperm injection (IMSI), it was possible to determine whether the increased pregnancy rates were attributable to the preferred nuclear morphology of the selected spermatozoa, rather than to the special sperm preparation technique. The investigators studied cycles in which the oocytes were injected with morphologically affected sperm cells because no spermatozoa with strictly defined morphologically normal nuclei were found. Pregnancy and implantation rates were significantly higher when normal spermatozoa were injected into the ova. Therefore, using a negative control within the IMSI group, the investigators confirmed a positive correlation between the normalcy of the sperm cells and IMSI outcomes. These conclusions were confirmed in

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a larger study group (4), and the implications of using the IMSI technique as the preferred method for identifying individual sperm to be injected are still under study.

In most studies, the investigators noted an improvement in embryo quality that cannot account for the increase in pregnancy and implantation rates. This fact could be related to hidden defects of the embryo at the chromosomal level. Although chromatin abnormalities appear to influence later embryonic development, the role that sperm may play in human embryonic aneuploidy has been largely neglected. In addition, studies attempting to correlate the relationship between sperm morphology and chromosome integrity are inconclusive (5, 6).

The present study was designed to examine the effect of sperm nuclear morphology on embryo development at the chromosomal level. A clarification may better explain the significant impact of the new practice of sperm selection on assisted reproductive technology outcomes. Preliminary results are presented in which the sexual and autosomal ploidy status of the embryos derived from sperm selected by conventional ICSI was analyzed in relation to the high-magnification ICSI methodology.

MATERIALS AND METHODS

Patients

The preliminary study was conducted between May and December 2009 on couples who underwent their first IVF treatment in conjunction with preimplantation genetic screening (PGS) for aneuploidy, as a result of advanced maternal age. Couples were randomly allocated to receive one of two sperm selection procedures (ICSI, n = 60; or IMSI, n = 60) using a computer-generated balanced table in sets of 10. Randomization was done just before the oocyte injection procedure by the laboratory personnel. To minimize the influence of male factor infertility, all cases of sperm concentration less than 1×10^6 M/mL and sperm motility less than 20% were excluded from the study. The couples were eligible to enter the study whenever the woman had at least six oocytes available on oocyte retrieval. Written informed consent was obtained in which the patients agreed to share the outcomes of their cycles for research purposes. The study was approved by the local Institutional Review Board.

Ovarian Stimulation

Controlled ovarian stimulation was achieved by long pituitary down-regulation using a GnRH agonist (GnRH agonist, Lupron Kit, Abbott S.A., Societ  Franaise des Laboratoires, Paris). This was followed by ovarian stimulation with recombinant FSH (Gonal-F, Serono, Geneva). Oocyte retrieval was performed 35 hours after the administration of recombinant hCG (rhCG; Ovidrel, Serono) through transvaginal ultrasound.

Standard and IMSI Sperm Selection

In the ICSI group, sperm morphology selection was assessed using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo) with a Hoffmann modulation contrast system under $\times 400$ magnification. Sperm selection in the IMSI group was examined at high magnification using a similar inverted microscope equipped with high-power differential interference contrast optics (DIC/Nomarski). The total calculated magnification was $\times 6,600$. 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, Santa Ana, CA) in a sterile glass dish (FluoroDish; World Precision Instrument, Sarasota, FL). The dish was placed on a microscope stage above a Uplan Apo $\times 100$ 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 (Bartoov et al. [1, 3, 7, 8]; and Cassuto et al., [9]).

Preimplantation Genetic Diagnosis and Aneuploidy Screening

On the morning of day 3 of embryo development, one cell per embryo was biopsied by laser zona drilling using a 1.48 μ m Infrared Diode Laser (Octax Laser Shot System, MTG, Bruckberg, Germany). After the biopsies, the embryos were returned to culture. The removed blastomere nuclei were spread using 0.1N HCl and 0.01% Tween 20 (Sigma, Dorset, UK). Briefly, the individual nucleus were placed on a slide in a drop of HCl-Tween spreading solution and observed until the cell had lysed. The slides were then air-dried and dehydrated before fluorescent in situ hybridization (FISH) analysis was performed. All embryos were analysed for chromosomes X, Y, 13, 16, 18, 21, and 22 (Abbott Laboratories) following the manufacturer's instructions. For the purpose of this study, the blastomeres were classified as normal when two sexual and two of each tested autosomal chromosomes were present. Blastomeres with two or more chromosomal numerical abnormalities were classified as chaotic. Embryos with abnormal findings in biopsied nuclei were not submitted to reanalysis study.

ET was performed on day 4 using a soft catheter with transabdominal ultrasound guidance. Only the embryos found to be chromosomally normal were considered for ET, and up to a maximum of three embryos were transferred. The cycle was cancelled if normal embryos were absent after FISH.

Clinical Follow-up

A pregnancy test was performed 12 days after ET, and a positive pregnancy test was considered as defining a biochemical pregnancy. All women with a positive test had a transvaginal ultrasound 2 weeks after the positive test. A clinical pregnancy was diagnosed when fetal heartbeat was detected. To calculate the implantation rate, the number of gestational sacs was divided by the number of embryos transferred. Miscarriage was defined as a spontaneous abortion before 20 weeks' gestation.

Statistical Analysis

Patient randomization was carried out using the SAS System for Windows (PROC PLAN, seed=1234) Statistical Program. The results were expressed as mean \pm SD for numeric variables, while proportions (%) were used for categorical variables. Mean values were compared using a Student's *t* parametric test. Associations between the sperm selection method and embryo

TABLE 1

Demographics data of ICSI and IMSI-PGS groups.

Characteristic	ICSI	IMSI	P value
Female's age, y	38.1 \pm 5.4	37.3 \pm 3.2	.5866
Male's age, y	42.6 \pm 8.0	43.6 \pm 8.9	.7330
Total gonadotropin dose, IU	2,601 \pm 676	2,697 \pm 659	.6592
Total aspirated follicles	13.2 \pm 8.2	17.3 \pm 10.7	.1993
Total retrieved oocytes	8.6 \pm 5.2	12.9 \pm 9.7	.0976
No. of retrieved oocytes/ no. of follicles	67.3	75.4	.1705
MII oocyte/total no. of retrieved oocytes	76.3	77.6	.8355
Sperm concentration, M/mL	22.2 \pm 5.3	21.8 \pm 6.7	.7954
Sperm progressive motility	35.6	34.8	.6823
Normal fertilization rate	84.3	76.7	.0638
Embryos	1.1 \pm 1.0	1.7 \pm 1.0	.1705

Note: Values are presented as either mean \pm SD or percent.

Figueira. IMSI approach on PGS cycles. *Fertil Steril* 2011.

chromosomal abnormality rates were examined using the χ^2 cross-tabulation test. The influence of the sperm selection method on aneuploidy rates was assessed using logistic regression analysis. The data are presented as odds ratios (OR) with 95% confidence interval (95% CI) and *P* value. The results were considered to be significant at the 5% critical level (*P*<.05). Data analysis was carried out using GraphPad Prism version 4.0 Statistical Program.

RESULTS

A total of 120 cycles of PGS were included in this study. There were no significant differences between the two groups. Patient demographics, stimulation, and cycle characteristics for the ICSI and IMSI groups are shown in Table 1.

The percentage of embryos showing autosomal aneuploidy was not significantly different between the ICSI and IMSI groups (56.9% vs. 51.3%, respectively; *P*=.0920). However, our results indicate that there was a significantly increased incidence for sex chromosome aneuploidy in embryos arising from ICSI cycles when compared with IMSI cycles (23.5% vs. 15.0%, respectively; *P*=.0139). These data were confirmed by a regression analysis that showed that a high-magnification sperm selection was associated with a significantly lower risk of sex chromosome abnormalities in the derived embryos (OR, 0.57; CI, 0.37–0.90; *P*=.015). The incidence of embryos with two or more chromosomal numerical abnormalities, chaotic embryos, was also significantly higher in embryos derived from ICSI cycles (27.5% vs. 18.8%; *P*=.0193). Our data were confirmed by regression analysis demonstrating that the IMSI procedure was associated with a significantly lower risk of chaotic embryos (OR, 0.64; CI, 0.43–0.96; *P*=.032).

We also noted an unexpected difference in gender incidence rates of euploid embryos. When considering only euploid embryos, there was a significantly higher incidence of XX embryos derived from IMSI cycles compared with from ICSI cycles (30.0% vs. 21.6%, respectively; *P*=.0326). This result was confirmed by logistic regression, which demonstrated a nearly twofold increase in euploid XX embryos derived from sperm selected by high magnification (OR, 1.82; CI, 1.05–3.15; *P*=.033).

Although clinical pregnancy rates (47.1% vs. 53.8%; *P*=.5321) and implantation rates (40.9% vs. 55.6%; *P*=.5911) were not significantly different between the ICSI and IMSI groups, the cycle cancellation rate was significantly higher in ICSI cycles (11.8% vs. 2.5%; *P*=.0016). This finding was confirmed by binary logistic regression, which showed that the high-magnification sperm selection procedure was a significant predictor of the likelihood of cycle cancellation (OR, 0.26; CI, 0.11–0.62; *P*=.001). There was no abortion occurrence in IMSI cycles, and only one case was described in ICSI cycles.

DISCUSSION

With the introduction of this new concept for sperm morphology examination, it is now possible to select normal spermatozoa in real time for use in an IMSI cycle. Of the previous subcellular sperm organelles examined, the morphological nuclear integrity of the sperm cells has been proven to be the most important semen parameter influencing embryo development and IVF treatment outcomes (1, 3, 7). The biochemical mechanism behind this phenomenon is not clear and may reflect some underlying chromosomal or DNA damage of spermatozoa with abnormal nuclei (10).

In this study we showed that the morphological normalcy of the sperm nucleus has a significant effect on aneuploidy occurrence in the developing embryo. The autosomal aneuploidy rate was not affected by sperm selection based on morphological parameters at

high magnification. The present data are further substantiated by previous studies indicating that autosomal aneuploidy rates in ICSI cycles were not different than what was expected based on maternal age in a non-ICSI population (11). Other studies have shown a 0.8%–1% incidence of sex chromosomal anomalies in ICSI offspring, compared with a rate of 0.14%–0.2% in the general population (12, 13). The results of the current study showed that sperm selection at $\times 6,600$ magnification is associated with a significantly lower risk of sex chromosomal aneuploidy when compared with standard sperm morphology selection at $\times 400$ magnification.

As a result, we noted a significantly lower risk of abnormal embryos in ET, which significantly decreased the cycle cancellation rate when using this sperm selection approach. Therefore, as a consequence of the availability of normal embryos to transfer, we observed similar clinical pregnancy and implantation rates in PGS cycles in which the embryos were derived from sperm selected by conventional or high-magnification methods. However, it could be expected that in a non-PGS approach there would be a higher probability of normal embryo selection for transfer, based on morphological criteria, in cases of embryos derived from sperm selected by high magnification. The higher incidence of chromosomally normal embryos, rather than an increase in embryo morphology quality, could explain the increased pregnancy and implantation rates in IMSI cycles that sometimes may not be related to a significant improvement in the quality of embryo morphology.

We also noted an unexpected difference in gender incidence rates of euploid embryos. “Best looking” spermatozoa analyzed by the high-magnification approach seem to carry a higher proportion of the X sexual chromosome. There is no available literature supporting this unexpected occurrence. We will also analyze the incidence of secondary sex (i.e., ratio of male to female births), but these data are not available at this moment.

The correlation of embryo morphology with embryo aneuploidy is still a matter of debate. However, this abnormality is related to the late paternal effect, which disturbs implantation without producing visible alterations in embryo cleavage speed and morphology grade (14). Our results could explain the previous, sharp increase in pregnancy and implantation rates (200% and 300%, respectively) after IMSI (3). The improvement in embryo quality could not, by itself, account for these results. Our results demonstrate that previously related improvement in pregnancy and implantation rates on IMSI (3, 4, 15) may be due to abnormalities at the chromosomal level of the embryo, which are contributed by the sperm cell.

Because the first mitotic division is controlled by the spermatozoon centrosome, irregularities may result in abnormal chromosome distribution among sister cells. In addition, sperm integrity is clearly necessary for normal mitotic division and early embryonic development. For instance, chaotic embryos could be produced by an abnormal number of male centrioles or by suboptimal centriole function (16).

An inverse correlation between sperm aneuploidy and normal sperm cell morphology (4, 15, 18) and normal nuclear shape (4) has previously been reported. In addition, a positive correlation has been found regarding infertile semen samples with apparently normal morphology and DNA fragmentation (19).

Recently, Garolla et al. (17) analyzed, for the first time, mitochondrial function, DNA status, and chromosome number of individual sperm cells isolated using a new apparatus able to magnify the sperm image up to $\times 13,000$. It was demonstrated that the sperm selected on the basis of normal morphology had better mitochondrial function and chromatin structure and lower aneuploidy rates. Our data

suggest that the physiological status of the spermatozoa selected by using high magnification and the resulting effect on embryo development could be associated with IMSI outcomes.

In conclusion, we believe this to be the first study on the chromosomal status of the embryos derived from IMSI cycles. Spermatozoa free of nuclear morphological malformations were found to be

significantly predictive of the lower incidence of aneuploidy in derived embryos, resulting in lower rates in the cancellation of IMSI-PGS cycles. In addition, our results may help to better explain the molecular mechanisms involved in individual sperm morphology at a high magnification as well as improved pregnancy and implantation rates in IMSI cycles.

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