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Gender incidence of intracytoplasmic morphologically selected sperm injection-derived embryos: a prospective randomized study

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Abstract The aim of this prospective randomized study was to determine if the use of intracytoplasmic morphologically selected sperm injection (IMSI) is associated with gender incidence. Couples who underwent IVF-preimplantation genetic screening (PGS) cycles, as a result of advanced maternal age, were randomly allocated into two groups: intracytoplasmic sperm injection (ICSI; $n = 80$) or intracytoplasmic morphologically selected sperm injection (IMSI; $n = 80$). The incidences of genders were compared between ICSI- and IMSI-derived embryos. Considering all the biopsied embryos were characterized as normal for sex chromosome, the results showed that IMSI results in a significantly higher incidence of female embryos as compared with ICSI (65.1% versus 54.0%, respectively, $P = 0.0277$). After analysing only euploid embryos for the eight selected chromosomes, a significantly higher incidence of XX embryos derived from IMSI was also observed compared with ICSI cycles (66.9% versus 52.5%, respectively, $P = 0.0322$). This result was confirmed by logistic regression, which demonstrated a nearly 2-fold increase in euploid XX embryos derived from spermatozoa selected by high magnification (OR 1.83, 95% CI 1.05–3.35, $P = 0.032$). A higher proportion of morphologically normal spermatozoa analysed under high magnification seem to carry the X chromosome. 

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Introduction

In humans, the gender of offspring is determined by the type of spermatozoon, bearing either the X or the Y chromosome,

that fertilizes the oocyte (Schulman and Karabinus, 2005). The balance between genders is known as the sex ratio. Mendelian segregation theoretically implies a sex ratio of 50% (Graffelman et al., 1999). Variation in the sex ratio is

common, and worldwide, there are normally approximately 105 boys to 100 girls at the point of birth (Jacobsen et al., 1999).

Since 1978, when the first IVF baby was born, assisted reproductive technology has been widely used throughout the world. The sex ratio in babies born through assisted conception varies as in any other population (Dean et al., 2010). However, whether assisted reproductive technology has any significant effect on gender is debatable.

With the introduction of a new technique, high-magnification sperm morphology examination, it is now possible to select normal spermatozoa in real time for use in an intracytoplasmic morphologically selected sperm injection (IMSI) cycle (Bartoov et al., 2002). Sperm selection performed by this methodology has shown a significant association with positive outcomes (Bartoov et al., 2003; Cassuto et al., 2009; Figueira Rde et al., 2011; Setti et al., 2011; Souza Setti et al., 2010). However, little is known about how, or even if, IMSI affects the sex ratio in embryos derived from this technique. The aim of this study was to determine whether the use of IMSI is associated with gender incidence.

Materials and methods

Patients

The preliminary study was conducted between May 2009 and December 2010 on couples who underwent their first IVF treatment in conjunction with preimplantation genetic screening (PGS), as a result of advanced maternal age. Couples were randomly allocated to receive one of two sperm selection procedures: intracytoplasmic sperm injection (ICSI, $n = 80$) or IMSI ($n = 80$). To minimize the influence of male factor infertility, all cases of severe spermatogenic alteration were excluded from the study. The couples were eligible to enter the study if the woman had at least six oocytes available upon 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 (029/11).

Ovarian stimulation

Ovarian stimulation was achieved by long pituitary down-regulation using a gonadotrophin-releasing hormone agonist (GnRH agonist, Lupron Kit; Abbott SA Société Française des Laboratoires, Paris, France). This procedure was followed by ovarian stimulation with recombinant FSH (Gonal-F; Serono, Geneva, Switzerland). Oocyte retrieval was performed 35 h after the administration of recombinant human chorionic gonadotrophin (Ovidrel; Serono) through transvaginal ultrasonography.

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, Japan) 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 $\times 6600$. An aliquot of the sperm cell suspension was transferred to a microdroplet of modified human tubal fluid medium containing 8% polyvinyl pyrrolidone (Irvine Scientific, Santa Ana, USA) in a sterile glass dish (FluoroDish; World Precision Instruments, USA). The dish was placed on a microscope stage above an Uplan Apo $\times 100$ oil/1.35 objective lens previously covered by a droplet of immersion oil. The sperm cells exhibiting normally shaped nuclei (smooth, symmetric and oval configuration and no more than one vacuole, which occupied $< 4\%$ of the nuclear area) were selected for injection (Bartoov et al., 1980, 2002, 2003; Berkovitz et al., 2005; Cassuto et al., 2009).

Preimplantation genetic screening and aneuploidy screening

On the morning of day 3 of embryo development, one cell per embryo was biopsied by laser zona drilling using a $1.48 \mu\text{m}$ infrared diode laser (Octax Laser Shot System, MTG, Bruckberg, Germany). Following the biopsies, the embryos were returned to culture. The removed blastomere nuclei were spread using 0.1 mol/l HCl and 0.01% Tween 20 (Sigma, Dorset, UK). Briefly, the blastomeres were placed on a slide in a drop of HCl-Tween spreading solution and observed until the cells had lysed. The slides were then air-dried and dehydrated before fluorescence in-situ hybridization analysis was performed (Munné et al., 1998). All embryos were analysed for chromosomes X, Y, 13, 15, 16, 18, 21 and 22. For the purpose of this study, the blastomeres were classified as normal when two sex and two of each tested autosomal chromosomes were present.

Embryo transfer was performed on day 5 using a soft catheter with transabdominal ultrasound guidance. Only the embryos found to be chromosomally normal were considered for embryo transfer and up to a maximum of three embryos were transferred.

Clinical follow-up

A pregnancy test was performed 12 days after embryo transfer and a positive pregnancy test was considered as defining a biochemical pregnancy. All women with a positive test had a transvaginal ultrasound two weeks after the positive test. A clinical pregnancy was diagnosed when a fetal heartbeat was detected. Miscarriage was defined as a spontaneous abortion before 20 weeks of gestation.

Statistical analysis

The results are expressed as the mean \pm standard deviation for numeric variables and as percentages were used for categorical variables. Mean values were compared using a Student's *t*-test. Associations between sperm selection method and embryo gender were examined using the chi-squared test. The influence of the sperm selection method on embryo gender was assessed using logistic regression analysis. The data were presented as odds ratio (OR) with a 95%

confidence interval (CI) and *P*-value. The results were considered to be significant at the 5% critical level ($P < 0.05$). Data analysis was performed using the GraphPad Prism version 4.0 statistical program (GraphPad Software, La Jolla, CA, USA).

Results

A total of 160 cycles of PGS were included in this study. Patient demographics, stimulation and cycle characteristics for the ICSI and IMSI groups are shown in **Table 1**.

No significant differences were observed between ICSI and IMSI groups in terms of patient demographics, stimulation and cycle characteristics. Clinical pregnancy per transferred cycle (36/76, 47.4% versus 43/79, 54.4%) and implantation rates (number of gestational sacs per transferred embryos (40/96, 41.6% versus 59/128, 46.1%) were also not significantly different. No miscarriages occurred in the IMSI cycles, and only three cases were described in the ICSI cycles. A total of 208 successfully biopsied embryos derived from 80 ICSI and 322 successfully biopsied embryos derived from 80 IMSI cycles were characterized by gender.

Considering that all the biopsied embryos characterized as normal for sex chromosome, the results showed that the IMSI approach resulted in a significantly higher incidence of female embryos when compared with ICSI (65.1% versus 54.0%, respectively, $P = 0.0277$). Analysing only euploid embryos for the eight analysed chromosomes, a significantly higher incidence of XX embryos derived from IMSI was also observed compared with ICSI cycles (66.9% versus 52.5%, respectively, $P = 0.0322$). This result was confirmed by logistic regression, which demonstrated a nearly 2-fold increase in euploid XX embryos derived from spermatozoa selected by high magnification (OR 1.83, 95% CI 1.05–3.35, $P = 0.032$).

Table 1 Demographic data for the ICSI and IMSI preimplantation genetic screening groups.

Characteristic	ICSI (n = 80)	IMSI (n = 80)
Women's age (years)	37.9 ± 5.5	37.5 ± 3.8
Men's age (years)	43.5 ± 7.3	43.8 ± 8.5
FSH administered (IU)	2588 ± 667	2657 ± 651
Follicles	13.1 ± 8.0	16.3 ± 9.1
Oocytes retrieved	8.9 ± 5.1	11.8 ± 9.1
Oocyte recovery	712 (67.9)	944 (72.4)
MII oocyte rate	549 (77.1)	734 (77.8)
Normal fertilization	461 (84.0)	587 (80.0)
Cleaved embryos	441 (95.7)	572 (97.4)
High-quality embryos	214 (48.5)	312 (54.5)
Biopsied embryos ^a	227 (51.5)	350 (61.2)
Embryos with FISH result	208 (91.6)	322 (92.0)
Embryos transferred	1.2 ± 1.0	1.6 ± 1.1

Values are means ± SD or *n* (%).

FISH = fluorescent in-situ hybridization; ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection; MII = metaphase II.

^aSignificantly higher in the IMSI group ($P = 0.002$).

Discussion

The aim of this study was to determine whether the use of IMSI is associated with gender incidence. According to the results, it seems that for morphologically normal spermatozoa analysed under high magnification, a higher proportion carry the X chromosome than the Y chromosome when compared with those selected under conventional magnification.

It has been suggested that the Y chromosome tends evolutionarily to be for reproduction and also for loss. Frequent microdeletions have been shown to occur on the Y chromosome (Pryor et al., 1997). The mutation rate on the Y chromosome is 1.5×10^{-9} per site per year. Cheng et al. (2007) predicted that the whole chromosome will be completely mutated in about 666×10^6 years, and eventually the Y chromosome will no longer exist. The peculiar structure and characteristics of the human Y chromosome, such as microdeletions, polymorphisms, heterogeneity, instability, lack of recombination repair and an accelerated evolutionary rate could be associated with reduced fitness to transmit the Y chromosome. Moreover, spermatozoa are deficient in both antioxidants and DNA-repair systems. (Cheng et al., 2007).

The selection of morphologically normal motile spermatozoa at high magnification is positively associated with pregnancy rates in patients with an elevated degree of DNA fragmented spermatozoa (Hazout et al., 2006) and oligoasthenoteratozoospermia (Balaban et al., 2011). Although morphologically normal spermatozoa from patients with normozoospermia show a significantly higher biological competence, morphologically normal spermatozoa from patients with oligoasthenozoospermia have alterations in their physiological status similar to that found in spermatozoa with head abnormalities (Burrello et al., 2004). The factors that cause these sperm parameter alterations also seem to affect the molecular mechanisms of DNA integrity, chromosome status and chromosome segregation in all germ cells (Setti et al., 2011). Therefore, normal morphology in conventional ICSI cycles does not indicate the selection of competent spermatozoa in patients with oligoasthenozoospermia. However, Setti et al. (2011) demonstrated that in patients with oligoasthenoteratozoospermia (according to the World Health Organization guidelines), the selection based on sperm morphological appearance under high magnification seems to identify competent spermatozoa, resulting in improved outcomes.

Therefore, in infertile men, alterations in the Y chromosome may lead to morphological changes that would prevent spermatozoon selection under high magnification. This would have the effect of creating a bias towards the selection of X chromosome-bearing spermatozoa, which in turn results in a higher proportion of female embryos. Another possible explanation would be that in IMSI the Y chromosome-bearing spermatozoa are omitted due to undetected morphological criteria.

As far as is known, this is the first study of the sex ratio of embryos derived from IMSI cycles. A higher proportion of morphologically normal spermatozoa analysed under high magnification seem to be X chromosome-bearing spermatozoa, suggesting a significantly predictive effect on the incidence of female embryos.

References

- Balaban, B., Yakin, K., Alatas, C., Oktem, O., Isiklar, A., Urman, B., 2011. Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study. *Reprod. Biomed. Online* 22, 472–476.
- Bartoov, B., Eltes, F., Weissenberg, R., Lunenfeld, B., 1980. Morphological characterization of abnormal human spermatozoa using transmission electron microscopy. *Arch. Androl.* 5, 305–322.
- Bartoov, B., Berkovitz, A., Eltes, F., Kogosowski, A., Menezes, Y., Barak, Y., 2002. Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. *J. Androl.* 23, 1–8.
- Bartoov, B., Berkovitz, A., Eltes, F., Kogosovsky, A., Yagoda, A., Lederman, H., Artzi, S., Gross, M., Barak, Y., 2003. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. *Fertil. Steril.* 80, 1413–1419.
- Berkovitz, A., Eltes, F., Yaari, S., Katz, N., Barr, I., Fishman, A., Bartoov, B., 2005. The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. *Hum. Reprod.* 20, 185–190.
- Burrello, N., Arcidiacono, G., Vicari, E., Asero, P., Di Benedetto, D., De Palma, A., Romeo, R., D'Agata, R., Calogero, A.E., 2004. Morphologically normal spermatozoa of patients with secretory oligo-astheno-teratozoospermia have an increased aneuploidy rate. *Hum. Reprod.* 19, 2298–2302.
- Cassuto, N.G., Bouret, D., Plouchart, J.M., Jellad, S., Vanderzwalmen, P., Balet, R., Larue, L., Barak, Y., 2009. A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. *Fertil. Steril.* 92, 1616–1625.
- Cheng, H., Shang, X., He, Y., Zhang, T., Zhang, Y.P., Zhou, R., 2007. Insight into human sex ratio imbalance: the more boys born, the more infertile men. *Reprod. Biomed. Online* 15, 487–494.
- Dean, J., Chapman, M., Sullivan, E., 2010. The effect of human sex ratio at birth by assisted reproductive technology (ART) procedures: an assessment of babies born following single embryo transfers, Australia and New Zealand, 2002–2006. *Br. J. Obstet. Gynaecol.* 117, 1628–1634.
- Figueira Rde, C., Braga, D.P., Setti, A.S., Iaconelli Jr., A., Borges Jr., E., 2011. Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes. *Fertil. Steril.* 95, 990–993.
- Graffelman, J., Fugger, E.F., Keyvanfar, K., Schulman, J.D., 1999. Human live birth and sperm-sex ratios compared. *Hum. Reprod.* 14, 2917–2920.
- Hazout, A., Dumont-Hassan, M., Junca, A.M., Cohen Bacrie, P., Tesarik, J., 2006. High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. *Reprod. Biomed. Online* 12, 19–25.
- Jacobsen, R., Moller, H., Mouritsen, A., 1999. Natural variation in the human sex ratio. *Hum. Reprod.* 14, 3120–3125.
- Munnè, S., Magli, C., Bahce, M., Fung, J., Legator, M., Morrison, L., Cohert, J., Gianaroli, L., 1998. Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. *Prenat. Diagn.* 18, 1459–1466.
- Pryor, J.L., Kent-First, M., Muallem, A., Van Bergen, A.H., Nolten, W.E., Meisner, L., Roberts, K.P., 1997. Microdeletions in the Y chromosome of infertile men. *N. Engl. J. Med.* 336, 534–539.
- Schulman, J.D., Karabinus, D.S., 2005. Scientific aspects of preconception gender selection. *Reprod. Biomed. Online* 10 (Suppl. 1), 111–115.
- Setti, A.S., Figueira, R.D., Braga, D.P., Iaconelli Jr., A., Borges Jr., E., 2011. Intracytoplasmic morphologically selected sperm injection benefits for patients with oligoasthenozoospermia according to the 2010 World Health Organization reference values. *Fertil. Steril.* 95, 2711–2714.
- Souza Setti, A., Ferreira, R.C., Paes de Almeida Ferreira Braga, D., de Cassia Savio Figueira, R., Iaconelli Jr., A., Borges Jr., E., 2010. Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis. *Reprod. Biomed. Online* 21, 450–455.

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