Comparison of aneuploidy rates between conventional in vitro fertilization and intracytoplasmic sperm injection in in vitro fertilization–intracytoplasmic sperm injection split insemination cycles

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Objective: To evaluate the influence of insemination methods on outcomes of preimplantation genetic testing for aneuploidy (PGT-A) by assessing PGT-A results in embryos that derived from conventional in vitro fertilization (IVF) versus intracytoplasmic sperm injection (ICSI) in sibling oocytes.
Design: Retrospective cohort study.
Setting: Single academic IVF center.
Patient(s): A total of 118 couples who underwent 131 split insemination cycles from July 2016–July 2019.
Intervention(s): In all cycles, sibling oocytes were allocated randomly to conventional IVF or ICSI prior to stripping. Preimplantation genetic testing for aneuploidy was performed via trophectoderm biopsy and next-generation sequencing with 24-chromosome screening.
Main Outcome Measure(s): Rates of euploid, aneuploid, and mosaic embryos per biopsy.
Result(s): A total of 2,129 oocytes were randomized to conventional IVF (n = 1,026) and ICSI (n = 1,103). No difference was observed in the aneuploidy rates (50.3% vs. 45.2%) and percentages of mosaic embryos (1.7% vs. 2.4%) per biopsy between conventional IVF and ICSI sibling oocytes. Percentages of different aneuploidy types and aneuploidies that involved sex chromosome abnormalities (6.2% vs. 7.2%) were similar between the two groups. In the end, the overall chance to have an euploid embryo per allocated oocyte in the two groups was similar (13.2% vs. 15.5%).
Conclusion(s): Blastocysts created with conventional IVF and ICSI using sibling oocytes had similar rates of aneuploidy and mosaicism as examined using 24-chromosome screening. It is unlikely that conventional IVF caused significant contamination during PGT-A. We recommend conventional IVF as the preferred insemination method in PGT-A cycles, and ICSI should be indicated only in cases of male-factor infertility. (Fertil Steril Rep® 2020;1:277–81. ©2020 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, insemination, intracytoplasmic sperm injection, in vitro fertilization

Received May 15, 2020; revised July 9, 2020; accepted July 22, 2020.
J.D. has nothing to disclose. O.K. has nothing to disclose. Q.Z. has nothing to disclose. B.B. has nothing to disclose. R.B.L. has nothing to disclose.
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Fertil Steril Rep® Vol. 1, No. 3, December 2020 2666-3341

https://doi.org/10.1016/j.xfre.2020.07.006

Preimplantation genetic testing (PGD) was developed in 1980s as an alternative to prenatal genetic diagnosis to allow parents who are carriers of single-gene disorders or structural chromosome abnormalities to select unaffected embryos for transfer (1, 2). Intracytoplasmic sperm injection (ICSI), which was developed initially to treat couples with male-factor infertility (3), was adopted into PGD to ensure monospermic fertilization and to minimize potential paternal contamination by extraneous sperm attached to the zona pellucida (4). In the last decade, platforms using whole-genome amplification have been applied to PGD, and preimplantation genetic testing for aneuploidy (PGT-A) by analysis of 24-chromosome copy number (5–8) has been used increasingly in embryo selection for patients undergoing in vitro fertilization (IVF) despite controversy surrounding its utility (9). Although there are no randomized controlled trials assessing the impact of fertilization methods on PGT-A diagnostic accuracy, in 2012, the American Society for Reproductive Medicine released a committee opinion recommending the use of ICSI for all cycles involving PGT because of the concerns of inaccurate results due to extraneous sperm contamination (10). Meanwhile, the "overuse" of ICSI in non-male factor infertility has been a concern because ICSI cannot improve the clinical outcome (11–13) in the non-male factor infertility population and it substantially increases the working burden in embryology laboratories and the cost of assisted reproductive treatment. It is also unclear whether the known reproductive risks, such as congenital anomalies and imprinting disorders, are associated with ICSI in non-male factor infertility (10). Currently most reference laboratories recommend ICSI for PGT-A cases but also accept samples from conventionally inseminated oocytes.

A few retrospective cohort studies assessed the accuracy of conventional IVF versus ICSI in PGT-A by looking at the difference in the prevalence of aneuploidy and mosaicism (14, 15). Although no difference in aneuploidy rates were noted in these studies, one indicated the use of standard insemination may increase mosaic cells by 5% compared with use of ICSI (14). One limitation of these studies was the comparison of PGT-A outcomes was performed between separate IVF and ICSI cycles among which patients' clinical/cycle differences and embryology laboratory variations might exist and influence the PGT-A outcomes.

In an IVF laboratory, fertilization failure can occur unexpectedly after conventional insemination even with normal semen parameters. For couples who carry the diagnosis of unexplained infertility or have borderline semen parameters, especially in the first IVF cycle, split insemination by conventional IVF and ICSI are implemented commonly in our clinic to minimize fertilization failure. In this retrospective study, we collected these split IVF cycles and analyzed paired blastocyst PGT-A outcomes of sibling oocytes that were assigned to either conventional insemination or ICSI to evaluate the influence of insemination methods on outcomes of PGT-A. Confounding variations among couples with infertility, different cycles, and different laboratory circumstances were avoided between sibling oocyte groups. Differences in the prevalence of aneuploid and mosaicism between the two insemination methods, potentially as a consequence of parental genetic contamination, were used to reflect the accuracy of each insemination method in PGT-A cases.

MATERIALS AND METHODS

Study Population

We conducted a retrospective cohort study at a single academic fertility center between June 2016 and July 2019. We identified all patients who underwent autologous IVF-ICSI split and PGT-A treatment in this timeframe. Patients who had a known history of chromosomal translocation or single-gene disorders were excluded from the analysis. Demographic information was collected including patient's age at retrieval, paternal age, semen parameters, infertility diagnosis, number of oocytes retrieved, number of fertilized oocytes, number of biopsied blastocysts, and PGT-A outcomes including euploid, aneuploid, mosaic, and no result ("no-call") embryos. This retrospective cohort study was performed under institutional review board approval at Stanford University.

Provider's recommendation for conventional IVF-ICSI split insemination was based on partner's semen analysis, prior infertility history, and semen parameters on the day of oocytes retrieval. In most cases, IVF-ICSI split was determined prior to retrieval based on prior infertility history, for example, unexplained infertility or prior semen parameters indicating borderline oligozoospermia, asthenozoospermia, or teratozoospermia, alone or in combination.

IVF Treatment

The ovarian stimulation protocol was chosen by each patient's primary physician and was based on the patient's ovarian reserve (antimüllerian hormone and antral follicle count). Gonadotropin-releasing hormone (GnRH) antagonist with or without birth control pills, or with estradiol-priming, or GnRH agonist microflare were used. Stimulation medications included follicular stimulating hormone (FSH) (recombinant) and human menopausal gonadotropin. Oocyte maturation was triggered by human chorionic gonadotropin and/or GnRH agonist (Lupron). Medication and doses were adjusted according to patient's response to stimulation as measured using ultrasounds or serum estradiol measurement. Oocyte retrieval procedures were performed as standard protocols at our center.

Laboratory Methods

Oocyte harvest occurred approximately 35 hours after administration of human chorionic gonadotropin. Retrieved oocytes surrounded by cumulus cells were pooled and then assigned alternatively in order into two separate oocyte collection dishes: one for conventional insemination and the other for ICSI. Oocytes allocated to ICSI were denuded immediately after oocyte retrieval. Cumulus cells were removed mechanically by gentle pipetting of oocytes after a short exposure to 80 iu/mL of hyaluronidase (SAGE). The maturation status of the denuded oocytes was evaluated

under inverted microscope. Around 2–6 hours after denudation, mature Metaphase II oocytes (including MI-to-MII oocytes), were injected with sperm and then placed in equilibrated Quinn's Advantage Fertilization (HTF) Medium supplemented with 5 mg/mL human serum albumin overnight. In the conventional IVF group, 5–6 oocytes were placed with 25,000/mL motile spermatozoa in an 80 μL equilibrated Quinn's Advantage Fertilization (HTF) Medium supplemented with 5 mg/mL human serum albumin overnight for fertilization. Sperm was prepared using the density gradient centrifugation method using Puresperm 40/80 (Nadicon). Sperm concentration and motility before and after preparation were analyzed using Hamilton Thorne Sperm Analyzer.

Approximately 18 hours after insemination or ICSI, oocytes were examined for the presence of pronuclei. Zygotes displaying two pronuclei were group cultured at 37°C in separate 80 μL microdrop Sage Single-step media in an atmosphere containing 5% O_2 and 6% CO_2. All embryos were hatched after fertilization check and cultured without interruption up to the blastocyst stage (days 5–7). Embryos that reached blastocysts were graded according to their morphological quality based on the Gardner criteria (16). Blastocysts with a grade of 3CC and above at days 5–7 underwent trophectoderm biopsy. All blastocysts were frozen using vitrification soon after biopsy.

A standardized protocol was used for biopsy as follows: embryos were washed at least three times in droplets containing Quinn's Advantage Medium with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) before biopsy. Approximately 3–8 trophectoderm cells were withdrawn from each embryo and separated using the Hamilton Thorne LYKOS laser. Biopsied trophectoderm cells were rinsed three to four times prior to being placed in 0.2 mL polymerase chain reaction (PCR) tubes containing 2 μL buffer solution that was provided by a diagnostic laboratory. After cell loading, the PCR tubes were immediately frozen at −20°C and kept in the freezer until transportation to the PGT testing center. The Next Generation Sequencing platform used in the current study was Ion GeneStudio S5 with the Ion Chef system (ThermoFisher Scientific), which allowed for an automated chip loading and had then ability to analyze up to 96 samples simultaneously in less than 24 hours. Cell lysis, DNA amplification, and sequencing analysis were performed at a PGT testing center based on the manufacturer's recommendations (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0016712_IonReproSeqPGS_S5_UG.pdf). Diagnosis of ploidy status was based on copy number variations. Simple aneuploidy was defined as an aneuploid embryo with a single abnormal chromosome. Double aneuploidy was defined as an aneuploid embryo with two abnormal chromosomes. Complex aneuploidy was defined as an aneuploid embryo with three or more abnormal chromosomes. Mosaic was defined when the sample had 30%–70% mosaicism. Sample with mosaicism <30% were classified as euploid and samples with >70% were classified as aneuploid. Calling policy for "euploid," "aneuploid," "complex aneuploid," or "mosaic" was not revised by the genetic testing laboratory during the duration of the study.

Outcome Measures

The primary outcomes were rates of euploid, aneuploid, and mosaic embryos per biopsy in conventional insemination versus the ICSI group. Secondary outcomes included rates of aneuploidy subtype (simple, double, or complex) and rates of sex chromosome abnormalities. The fertilization rate was calculated by dividing the number of two pronuclei zygotes obtained by the number of cumulus-oocyte-complexes inseminated (conventional IVF) or the number of mature oocytes injected for ICSI. The blastocyst formation rate was analyzed based on the number of usable blastocysts that were available for biopsy per normally fertilized MII egg (two pronuclear). Aneuploidy rates were calculated per number of biopsied blastocysts.

Statistical Analysis

Independent *t* test or Pearson chi-square test was used for continuous or categorical variables, respectively. Results are presented as mean ± standard deviation unless otherwise stated. $P<.05$ was considered statistically significant.

RESULTS

In a 3-year period (June 2016–July 2019), we identified a total of 131 cycles in 118 couples who underwent split insemination cycles that met the study criteria. Unexplained infertility accounts for 62.3% of the patients, including patients with ovulatory disorder (23.4%) who underwent 3–6 cycles of unsuccessful intrauterine insemination cycles before IVF treatments. The other diagnoses include diminished ovarian reserve (22.3%), borderline male factor (3.1%), and others including uterine and tubal factors (12.3%).

A total of 2,129 oocytes were allocated to conventional IVF (n = 1,026) and ICSI (n = 1,103) cohorts. Mean oocyte age of included cycles was 36.2 years. Mean paternal age was 37.8 years. The average number of eggs retrieved was 16.3 and the number of blastocysts available for biopsy was five per oocyte retrieval cycle. The average semen volume was 2.0 mL and the average sperm concentration was 42.2 million/mL. The average total sperm motility was 65.0%. In the conventional IVF group, normal fertilization (2PN) was observed in 531 of 1,026 cumulus oocytes complexes (51.8%). In ICSI, 845 MII oocytes of the allocated 1,103 cumulus oocytes complexes were injected, and the fertilization rate was 77.2% (652/845). The fertilization rate in the conventional IVF group was significantly lower ($P<.001$) than that in the ICSI group. Although the conventional IVF group had a higher blastocyst formation rate per 2PN embryo than ICSI (62.0% vs. 52.5%: $P<.001$), no difference was observed in the percentages of embryos available for biopsy per 2PN embryo (54.2% vs. 51.5%; $P=.354$) or per allocated oocyte (28.1% vs. 30.5%; $P=.226$) between the conventional IVF and ICSI cohorts (Table 1).

There was no difference of aneuploidy rate per biopsy between the conventional IVF and ICSI groups (50.3% vs. 45.2%; $P=.203$). Similar rates of mosaic embryos (1.7% vs. 2.4%; $P=.723$) and "no-call" (1.0% vs. 1.5%; $P=.621$) between the conventional IVF and ICSI groups were observed.

TABLE 1

Embryological outcomes of conventional in vitro fertilization versus intracytoplasmic sperm injection.

Characteristic	Conventional IVF	ICSI	*P* value
No. of oocytes allocated	1,026	1,103	
No. of oocytes inseminated	1,026	845[a]	
2PN zygote per inseminated oocyte	51.8% (531)	77.2% (652)	<.001
Blastulation rate per 2PN zygote	62.0% (329)	52.5% (341)	<.001
Embryos available for biopsy per 2PN	54.2% (288)	51.5% (336)	.354
Embryos available for biopsy per allocated oocyte	28.1% (288)	30.5% (336)	.226

Note: Data presented as % (n), unless stated otherwise. 2PN = two pronuclear; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.
[a] In the ICSI group, 845 MII oocytes of the allocated 1,103 oocytes were inseminated.

Deng. Aneuploidy rates: conventional IVF vs. ICSI. Fertil Steril Rep 2020.

Percentages of different aneuploidy types including single aneuploidy, double aneuploidy, and complex abnormal aneuploidy were similar between the two groups (Table 2). Aneuploidies that involved sex chromosome abnormalities were similar between the two groups (6.2% vs. 7.2%; $P=.723$) as well. In the end, the overall chance to have an euploid embryo per allocated oocyte in the two groups was similar (13.2% vs. 15.5%; $P=.123$).

DISCUSSION

In this study of sibling oocytes derived from split IVF/ICSI treatment cycles in cases of non-male factor infertility, the use of conventional IVF was not associated with higher prevalence of aneuploidy or mosaic embryos within the context of next-generation sequencing PGT-A. Conventional IVF also yielded comparable results to ICSI in terms of chance of having an euploid embryo per allocated oocyte, which demonstrated that ICSI does not confer benefit during PGT-A or improve embryological outcomes in the absence of male factor subfertility.

Currently, the majority of PGT-A diagnostic platforms are based on whole-genome sequencing, which involves many PCR cycles to amplify the minute initial amount of DNA. It has been recommended to use ICSI for all cycles involving PGT to avoid contamination from insufficient removal of cumulus cells or excess bound spermatozoa during the conventional IVF insemination process, especially with improved analytical platforms and increased testing sensitivity. However, studies assessing IVF versus ICSI in PGT cases have demonstrated similar rates of euploid, aneuploid, and failed diagnosis between the two insemination methods (14, 15). Although one report suggested that standard insemination may increase mosaic cells compared with ICSI (14), it is unclear if this was due to inadequate rinsing of embryos prior to biopsy or sperm DNA packaging. In the IVF laboratory, variance in methods such as biopsy technique and cell handling potentially could impact DNA quality and fidelity of PGT-A results (17). In our laboratory, embryos were washed thoroughly before biopsy and the amount of carry-over medium when moving embryos was limited to be as minimal as possible. The biopsy pieces were rinsed subsequently at least three to four times prior to tubing. Our observations of similar aneuploidy and mosaicism rates between two insemination groups suggest that, with careful embryo and cell washing prior to and after biopsy, conventional IVF does not increase the risk of contamination.

Notably, we did not find an increased rate of sex chromosome aneuploidy in the ICSI group compared with conventional IVF. In prior studies, more sex chromosome anomalies were shown among pregnancies resulting from ICSI compared with spontaneous pregnancies (18–21). The findings of similar sex chromosome aneuploidy rates between conventional IVF and ICSI in patients with non-male factor infertility in our study suggested that the previously reported increased risk for chromosome anomalies in

TABLE 2

Aneuploidy rates in embryos fertilized by conventional in vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes.

Characteristic	Conventional IVF (288 biopsies)	ICSI (336 biopsies)	*P* value
Euploid per biopsy	46.9 (135)	50.9 (171)	.317
Aneuploid per biopsy	50.3 (145)	45.2 (152)	.203
Aneuploid subtypes			
Single aneuploidy	63.4 (92)	57.2 (87)	.274
Double aneuploidy	17.2 (25)	24.3 (37)	.132
Complex abnormal aneuploidy	19.3 (28)	18.4 (28)	.844
Aneuploidy involving sex chromosomes	6.2 (5 trisomies and 4 monosomy X)	7.2 (5 trisomies and 6 monosomy X)	.723
Mosaic per biopsy	1.7 (5)	2.4 (8)	.226
"No-call"	1.0 (3)	1.5 (5)	.621
Euploid per allocated oocytes	13.2 (135)	15.5 (171)	.123

Note: Data presented as % (n), unless stated otherwise. ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.

Deng. Aneuploidy rates: conventional IVF vs. ICSI. Fertil Steril Rep 2020.

ICSI-mediated pregnancies may be due to underlying causes of severe male factors rather than the ICSI technique.

In studies of unexplained infertility, many have reported higher fertilization rate of ICSI than that of conventional IVF in the presence of normal semen parameters, suggesting that ICSI overcomes undiagnosed male factor subfertility, which was not highlighted in a routine semen analysis (22, 23). However, in many studies, higher fertilization rates within the ICSI group did not translate into improved clinical outcomes in the presence of normal sperm parameters (11, 24, 25). Consistent with previous studies, our study showed that ICSI did not improve the blastocyst formation rate or the chance of having an euploid embryo per allocated oocyte, although a higher fertilization rate per inseminated oocyte was observed in the ICSI group. The lower fertilization rate in the conventional IVF group most likely is due to undistinguished insemination of immature oocytes, which led to a larger denominator that was used when calculating the fertilization rate.

Outcomes of PGT-A from sibling oocytes in our study lowered potential heterogeneity among samples between the two different insemination methods as much as possible. Higher cost, more handling of gametes, and more laboratory labor are required for ICSI treatment. Our study findings suggest that conventional IVF should be the preferred insemination method in PGT-A cycles and use of ICSI should be reserved only in cases of male factor infertility. Certainly, our study is limited by its retrospective nature and limited sample size. Different platforms and thresholds are used for diagnosis of normal versus abnormal versus mosaic in different genetic laboratories. Variations of embryo biopsy and sample processing techniques among embryologists or IVF laboratories may affect the cleanness of samples and subsequent diagnosis. As such, data from multicenter and other genetic laboratories are needed for further confirmation of our findings.

REFERENCES

1. Handyside AH, Pattinson JK, Penketh RJ, Delhanty JD, Winston RM, Tuddenham EG. Biopsy of human preimplantation embryos and sexing by DNA amplification. Lancet 1989;1:347–9.
2. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344:768–70.
3. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992; 340:17–8.
4. Thornhill AR, deDie-Smulders CE, Geraedts JP, Harper JC, Harton GL, Lavery SA, et al. ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. Hum Reprod 2005;20:35–48.
5. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet 2012;5:24.
6. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013;100:100–7.e1.
7. Scott RT, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril 2013;100: 697–703.
8. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril 2019;112: 1071–9.e7.
9. Sermon K, Capalbo A, Cohen J, Coonen E, De Rycke M, De Vos A, et al. The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists. Mol Hum Reprod 2016;22:845–57.
10. Technology PCotASfRMaSfAR. Intracytoplasmic sperm injection (ICSI) for non-male factor infertility: a committee opinion. Fertil Steril 2012;98: 1395–9.
11. Bhattacharya S, Hamilton MP, Shaaban M, Khalaf Y, Seddler M, Ghobara T, et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. Lancet 2001;357:2075–9.
12. Check JH, Yuan W, Garberi-Levito MC, Swenson K, McMonagle K. Effect of method of oocyte fertilization on fertilization, pregnancy and implantation rates in women with unexplained infertility. Clin Exp Obstet Gynecol 2011;38:203–5.
13. Tannus S, Son WY, Gilman A, Younes G, Shavit T, Dahan MH. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. Hum Reprod 2017;32:119–24.
14. Palmerola KL, Vitez SF, Amrane S, Fischer CP, Forman EJ. Minimizing mosaicism: assessing the impact of fertilization method on rate of mosaicism after next-generation sequencing (NGS) preimplantation genetic testing for aneuploidy (PGT-A). J Assist Reprod Genet 2019;36:153–7.
15. Feldman B, Aizer A, Brengauz M, Dotan K, Levron J, Schiff E, et al. Pre-implantation genetic diagnosis-should we use ICSI for all? J Assist Reprod Genet 2017;34:1179–83.
16. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000;73:1155–8.
17. Swain JE. Controversies in ART: can the IVF laboratory influence preimplantation embryo aneuploidy? Reprod Biomed Online 2019;34:599–607.
18. Meschede D, Horst J. Sex chromosomal anomalies in pregnancies conceived through intracytoplasmic sperm injection: a case for genetic counselling. Hum Reprod 1997;12:1125–7.
19. Hervé C, Moutel G. Sex chromosome abnormalities after intracytoplasmic sperm injection. Lancet 1995;346:1096–7.
20. Kim JW, Lee WS, Yoon TK, Seok HH, Cho JH, Kim YS, et al. Chromosomal abnormalities in spontaneous abortion after assisted reproductive treatment. BMC Med Genet 2010;11:153.
21. Kushnir VA, Frattarelli JL. Aneuploidy in abortuses following IVF and ICSI. J Assist Reprod Genet 2009;26:93–7.
22. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril 2008;89:823–31.
23. Finch KA, Fonseka KG, Abogrein A, Ioannou D, Handyside AH, Thornhill AR, et al. Nuclear organization in human sperm: preliminary evidence for altered sex chromosome centromere position in infertile males. Hum Reprod 2008; 23:1263–70.
24. Foong SC, Fleetham JA, O'Keane JA, Scott SG, Tough SC, Greene CA. A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. J Assist Reprod Genet 2006;23:137–40.
25. Supramaniam PR, Granne I, Ohuma EO, Lim LN, McVeigh E, Venkatakrishnan R, et al. ICSI does not improve reproductive outcomes in autologous ovarian response cycles with non-male factor subfertility. Hum Reprod 2020;35:583–94.