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Minimizing mosaicism: assessing the impact of fertilization method on rate of mosaicism after next-generation sequencing (NGS) preimplantation genetic testing for aneuploidy (PGT-A)

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Abstract

Purpose Advances in preimplantation genetic testing (PGT) have led to practice changes in assisted reproductive technologies (ART), enabling fertility centers to transfer single embryos while maintaining excellent ongoing pregnancy rates, reducing miscarriage rates, and dramatically reducing ART-associated multiple pregnancies. The introduction of next-generation sequencing (NGS) allows PGT laboratories to assess for embryo mosaicism—although the true incidence and reproductive potential of predicted mosaic embryos are controversial. Due to concern for genetic contamination from other spermatozoa, most reference laboratories require use of intracytoplasmic sperm injection (ICSI) for single gene preimplantation genetic diagnosis (PGT-M). However, in PGT for aneuploidy (PGT-A), conventional insemination (IVF) is typically permissible. The purpose of this study was to evaluate rates of euploid, aneuploid, and mosaic in trophectoderm biopsy samples from embryos in IVF versus ICSI PGT-A cycles. Secondary aims were to assess sex ratio, and subtypes of aneuploidy and mosaicism in IVF versus ICSI PGT-A cycles.

Methods We performed a retrospective review of women undergoing PGT-A at a single academic fertility center from July 1, 2015, to September 1, 2017. In all cycles, PGT-A was performed via trophectoderm biopsy on day 5 or 6 and analyzed using NGS at a single reference lab. We collected and compared patient demographics, fertility testing, cycle characteristics, and PGT-A outcomes between IVF and ICSI cycles.

Results Three hundred two PGT-A cycles were included for analysis: 75 IVF and 227 ICSI cycles, resulting in 251 IVF and 724 ICSI biopsied blastocysts. Mean oocyte age of included cycles was 38.6 years (IVF) and 38.5 years (ICSI), $p = 0.85$. Baseline characteristics of IVF and ICSI PGT-A cycles were similar with the exception of semen parameters: IVF cycles had higher sperm concentration and total motility compared to ICSI cycles. PGT-A outcomes did not differ between IVF and ICSI cycles: euploid 27.9% (IVF) versus 30% (ICSI); aneuploid 45.4% (IVF) versus 43.1% (ICSI); no result 4.4% (IVF) versus 6.2% (ICSI). Though not significant, we identified a trend toward higher rate of mosaicism in IVF (25.9%) versus ICSI (20.9%). Among mosaic embryos, a lower percentage of simple mosaic embryos resulted from IVF (53.8%) versus ICSI (70.2%). Among aneuploid embryos, a non-significant higher percentage of complex aneuploidy resulted from IVF (16.3%) versus ICSI (9%). IVF resulted in a non-significant higher proportion of cycles with no transferrable embryos (42.7%) versus ICSI (36.6%). Numerical and sex chromosome involvement in mosaicism and aneuploidy were similar between IVF and ICSI cycles.

Conclusion IVF and ICSI NGS PGT-A have similar rates of euploid, aneuploid, and no result embryos, though IVF may result in higher rates of mosaicism and demonstrates differences in proportions of mosaic and aneuploid subtypes compared to ICSI. ICSI may be preferable to conventional insemination to minimize the rate of mosaic results in NGS PGT-A cycles.

Keywords Mosaicism · Preimplantation genetic testing · Next-generation sequencing · Intracytoplasmic sperm injection · Conventional insemination

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Introduction

Advances in preimplantation genetic testing (PGT) have led to practice changes in assisted reproductive technologies (ART), enabling fertility centers to transfer single embryos while maintaining excellent ongoing pregnancy rates, reducing miscarriage rates, and dramatically reducing ART-associated multiple pregnancies [1, 2]. The benefits of PGT for aneuploidy (PGT-A) have been supported by multiple randomized controlled trials [2, 3] and meta-analyses [4, 5], and hold true for women of all reproductive ages [6, 7]. As a result, the use of PGT-A has become increasingly widespread among US infertility clinics. In 2016, the Society for Assisted Reproductive Technology (SART) indicated that 31% of all ART cycles involved PGT [8], though numerous practices perform PGT in 50% or greater cycles.

In 2012, the practice committees of the American Society for Reproductive Medicine (ASRM) and SART released a committee opinion recommending the use of intracytoplasmic sperm injection (ICSI) for all cycles involving PGT [9]. The rationale behind this recommendation was twofold: to ensure monospermic fertilization and to minimize potential paternal contamination by extraneous sperm attached to the zona pellucida [10, 11]. Beyond these theoretical explanations, review of the literature reveals a dearth of definitive evidence of the superiority of ICSI over IVF in the setting of PGT-A through clinical trials or head-to-head comparisons. At the time of this committee opinion publication, SART indicated that approximately 5% of IVF cycles involved PGT [8]. The dramatic increased use of PGT since this time, coupled with the 2012 recommendation for use of ICSI in these cycles, potentially significantly increases the use of ICSI in reproductive medicine, which is not without risk. In addition to the small increased incidence of imprinting disorders and birth defects [12–14], ICSI substantially increases the cost of ART. Thus, at Columbia University Fertility Center, while ICSI was implemented in all cases of PGT for monogenic diseases (PGT-M), conventional insemination was considered in couples undergoing PGT-A with normal semen parameters or in those patients using donor sperm, when ICSI was not otherwise indicated. Although ICSI was recommended by ASRM, most reference labs accept samples from conventional insemination embryos.

The introduction of next-generation sequencing (NGS) has enabled higher resolution genetic analysis, and consequently, a higher sensitivity in the detection of embryonic mosaicism [15, 16]. While data suggest embryos predicted to be mosaic may have lower implantation rates [17], the true incidence of mosaicism and the reproductive potential of mosaic embryos remain controversial and an active area of study. This study aimed to evaluate the influence of conventional insemination compared to ICSI on outcomes of NGS testing.

Materials and methods

Study design and patient population

We conducted a retrospective cohort study of all PGT-A cycles at Columbia University Fertility Center from July 2015, to September 2017. This timeframe was chosen as our center switched from array comparative genomic hybridization to NGS for PGT testing in July 2015. Cycles undergoing PGT-M were excluded.

Demographic information from in vitro fertilization (IVF) stimulation cycle data was collected, including oocyte age, sperm age, semen parameters, total dose of gonadotropins (IU), peak serum estradiol (pg/mL), fertilization method, number of oocytes retrieved, number of fertilized embryos, and number of biopsied and vitrified blastocysts. PGT-A outcomes were noted, including number of euploid, aneuploid, mosaic, and no result embryos. Data points were obtained through abstraction from existing patient records. The Columbia University Institutional Review Board approved this study, IRB #AAAR5950.

Conventional insemination versus intracytoplasmic sperm injection

Providers' recommendation for ICSI versus conventional insemination was based on partner semen analysis, prior infertility history, and parameters identified on the day of oocyte retrieval. In most circumstances, ICSI was determined prior to cycle start based on prior abnormal semen parameters indicating oligozoospermia, asthenozoospermia, or teratozoospermia, alone or in combination. Additionally, ICSI was recommended for couples with prior failed or poor fertilization following conventional insemination. Finally, ICSI may be advised by the embryologist following assessment of the oocytes or sperm quality on day of oocyte retrieval. Conventional insemination was considered for patients using donor sperm, for couples with normal semen parameters according to 2010 World Health Organization classification [18], or in couples who declined the recommendation for ICSI.

In vitro fertilization stimulation, oocyte retrieval, and laboratory procedures

Gonadotropin releasing hormone agonist and antagonist in vitro stimulation protocols were included. Ovarian stimulation was monitored with regular ultrasound and serum hormonal assays per practice protocol [19]. Oocyte retrieval was performed by transvaginal ultrasound-guided needle aspiration under intravenous sedation. Conventional insemination or ICSI was performed the day of oocyte retrieval according to standard protocols. All normally fertilized embryos were cultured in continuous culture media. Trophectoderm biopsy was performed on embryonic day 5 and day 6 for all high-

quality blastocysts as determined by the criteria established by Gardner and Schoolcraft [20].

Next-generation sequencing preimplantation genetic testing procedures

All biopsy samples were assessed using NGS at a single reference laboratory. The NGS PGT-A methods and analyses implemented by our reference laboratory for determination of euploidy, aneuploidy, and mosaicism have been previously described in detail [17]. Briefly, the VeriseqNGS (Illumina) platform analyzed all samples, which has the ability to detect abnormal chromosome segments as small as 1.5 million base pairs. Biopsy diagnostic decisions were based on copy number variations: a chromosome with two copies was assigned a value of 2 and determined to be euploid, a chromosome with one copy was assigned a value of 1 and determined to be a monosomy, and a chromosome with three copies was assigned a value of 3 and determined to be a trisomy. Values detected between 1 and 2 or 2 and 3 were considered to be mosaic. The reference laboratory states that mosaicism less than 20% or greater than 80% cannot be differentiated from technical noise. Thus, samples with less than 20% mosaicism are classified as euploid, samples with greater than 80% mosaicism are classified as aneuploid, and samples between 20 and 80% mosaicism are classified as mosaic.

Outcome measures

The primary outcomes measures were rates of euploid, aneuploid, and mosaic embryos in conventional insemination versus ICSI cohorts. Secondary outcomes included rates of mosaic subtype (simple, double, or complex), rates of aneuploid subtype (simple, double, complex, or mixed), and sex ratio, defined as number of male embryos divided by number of female embryos, in conventional versus ICSI cohorts. Simple mosaicism was defined as a mosaic embryo with a single mosaic chromosome. Double mosaicism was defined as a mosaic embryo with two mosaic chromosomes. Complex mosaicism was defined as a mosaic embryo with three or more mosaic chromosomes. 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. Mixed aneuploidy was defined as an embryo with both aneuploid and mosaic chromosomes of any quantity.

Statistical analysis

Univariate analyses using Student's *t* test for continuous variables and the chi-square test for categorical variables were implemented to compare conventional insemination and ICSI cohorts. Statistical analyses were performed using GraphPad Prism Software for Mac, version 6 (GraphPad, Inc., San Diego, CA) and Stata/IC 15 (StatCorp LLC, College Station, TX). A p value of less than 0.05 defined statistical significance.

Results

A total of 302 PGT-A cycles were included for analysis: 75 (24.8%) using conventional insemination and 227 (75.6%) using ICSI. These cycles resulted in 975 biopsied blastocysts: 251 (25.7%) following conventional insemination and 724 (74.3%) following ICSI. Baseline demographic profiles were similar between conventional insemination and ICSI cohorts with the exception of sperm concentration and motility (Table 1), which is expected as providers used semen parameters to determine recommended fertilization method. Stimulation outcomes such as oocyte, fertilization, and blastocyst yield were similar between conventional insemination and ICSI cohorts (Table 1).

Conventional insemination resulted in a higher rate of aneuploid embryos compared to ICSI (45.4% versus 43.1%) and mosaic embryos compared to ICSI (25.9% versus 20.9%), though these analyses did not achieve statistical significance (Table 2). Conventional insemination resulted in a significantly lower rate of simple mosaic embryos compared to ICSI (53.8% versus 70.2%, $p = 0.03$) and consequently a higher combined rate of double and complex embryos compared to ICSI (46.2% versus 29.8%, $p = 0.03$) (Table 2). Similarly, conventional insemination resulted in a higher rate of complex aneuploid embryos compared to ICSI, though this did not achieve statistical significance (16.3% versus 9%, $p = 0.06$) (Table 2). There were no differences in sex ratio between conventional insemination and ICSI cohorts (0.54 versus 0.47, $p = 0.09$). There were no differences in numerical or sex chromosome involvement in mosaicism or aneuploidy in conventional insemination versus ICSI cohorts. If euploid and simple mosaic embryos are considered "transferrable embryos," conventional insemination resulted in a higher proportion of cycles with no transferrable embryos compared to ICSI, though this analysis did not achieve statistical significance (42.7% versus 36.6% of cycles, $p = 0.42$).

Discussion

Our findings support the use of ICSI over conventional insemination within the context of NGS PGT-A. We observed a trend toward increased rates of mosaicism with conventional insemination as compared to ICSI, although the reason for this observation is not clear based on the available data.

Table 1 Conventional insemination versus ICSI NGS PGT-A cycle characteristics

Demographics			
	Conventional insemination (75 cycles)	ICSI (227 cycles)	*p* value*
Oocyte age (years)	38.6	38.5	0.85
Peak E2 (pg/mL)	2576	2287	0.08
Total gonadotropins (IU)	3860	4075	0.3
Sperm age (years)	40.2	39.5	0.37
Semen volume (mL)$^{\infty}$	2.3	2.2	0.52
Sperm concentration (million/mL)$^{\infty}$	65.6	46.8	< 0.001
Total motility (%)$^{\infty}$	56.1	49.3	0.002
# Oocytes (mean per person)	15.8	14.5	0.26
2PN per oocyte retrieved (%)	61.8	61.4	0.87
# Embryos biopsied (mean per person)	3.3	3.2	0.76
Blastulation rate (%)	35.7	36.6	0.68

ICSI, intracytoplasmic sperm injection; *E2*, estradiol; *2PN*, two pronuclei (normally fertilized embryo)

*Student's *t* test

$^{\infty}$ Semen parameters as assessed on day of oocyte retrieval. Please note, morphology is not assessed on fresh specimens provided on day of oocyte retrieval

The reproductive potential of mosaic embryos remains controversial and an active area of study as fertility centers are just recently publishing outcomes of transferred mosaic embryos [21–23]. The Preimplantation Genetic Diagnosis International Society (PGDIS) has published recommendations regarding the transfer of mosaic embryos, with a preference for simple mosaic embryos with involvement of a single chromosome over complex mosaic embryos [24]. Thus, ICSI may be preferable to conventional insemination for NGS PGT-A testing not only to minimize the overall rate of mosaicism but also to maximize the number of favorable embryos available for transfer.

The mechanisms explaining our study findings of the differences between conventional insemination and ICSI are unclear at this time. Possible explanations include a truly biological mechanism in that the method of fertilization affects

Table 2 Conventional insemination versus ICSI NGS PGT-A cycle outcomes

Primary outcome			
NGS PGT-A diagnosis	Conventional insemination (251 blastocysts)	ICSI (724 blastocysts)	*p* value*
Euploid	70 (27.9)	217 (30.0)	0.59
Aneuploid	104 (45.4)	312 (43.1)	0.70
Mosaic	65 (25.9)	151 (20.9)	0.12
No result	11 (4.4)	45 (6.2)	0.36
Secondary outcomes			
Mosaic subtypes	Conventional insemination (65 blastocysts)	ICSI (151 blastocysts)	*p* value*
Simple	35 (53.8)	106 (70.2)	0.03
Double	15 (23.1)	25 (16.6)	0.35
Complex	15 (23.1)	20 (13.2)	0.11
Aneuploid subtypes	Conventional insemination (104 blastocysts)	ICSI (312 blastocysts)	*p* value*
Simple	49 (47.1)	125 (40.1)	0.25
Double	13 (12.5)	46 (14.7)	0.69
Complex	17 (16.3)	28 (9.0)	0.06
Mixed aneuploid/mosaic	26 (25.0)	108 (34.6)	0.09

Values expressed as *n* (%)

NGS, next-generation sequencing; *PGT-A*, preimplantation genetic testing for aneuploidy; *ICSI*, intracytoplasmic sperm injection

*Chi-square test

subsequent embryo development and chromosome segregation during subsequent mitoses. Alternatively, our findings may be due to technical artifact related to genetic contamination as suggested by the 2005 ESHRE PGD Consortium [11]. Oocytes planned for ICSI undergo granulosa cell stripping. Therefore, there are fewer maternal somatic cells and adherent sperm cells compared to oocytes planned for conventional insemination. Despite careful technique, it is possible that genetic material from these additional maternal and paternal cells may persist in the culture media and eventual trophectoderm biopsy sample, leading to mosaicism during genetic analysis. Confirmation of our findings through multicenter studies and further investigation into the mechanisms behind mosaicism in the preimplantation embryo are required in order to validate and better understand the reasoning and implications of our study's findings.

Our study is certainly limited by its retrospective nature and limited sample size. However, our study findings may assist fertility providers in counseling couples pursuing PGT-A testing with normal semen parameters who may question the necessity of ICSI. As further information is acquired regarding the short- and long-term outcomes of mosaic embryos, this information will improve the understanding and expectations of both patients and providers.

Conclusion

ICSI may be preferable to conventional insemination for NGS PGT-A cycles in order to minimize the rate of mosaicism and complex mosaic subtypes.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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