In vitro fertilization outcomes after preimplantation genetic testing for chromosomal structural rearrangements comparing fluorescence in-situ hybridization, microarray comparative genomic hybridization, and next-generation sequencing

Chantal B. Bartels, M.D. Reeva Makhijani, M.D., Prachi Godiwala, M.D., Alison Bartolucci, Ph.D., John C. Nulsen, M.D., Daniel R. Grow, M.D., Lawrence Engmann, M.D., and Claudio A. Benadiva, M.D., H.C.L.D.

Center for Advanced Reproductive Services, Department of Obstetrics and Gynecology, University of Connecticut, Farmington, Connecticut

Objective: To compare in vitro fertilization (IVF) outcomes for preimplantation genetic testing for chromosomal structural rearrangements (PGT-SR) using various testing platforms.

Design: Retrospective cohort.

Setting: Large academic IVF center.

Patient(s): Fifty-one balanced translocation carriers undergoing IVF with PGT-SR who completed a total of 91 cycles, including 31 fluorescence in-situ hybridization (FISH), 24 microarray comparative genomic hybridization (aCGH), and 36 next-generation sequencing (NGS) testing cycles.

Intervention(s): PGT-SR.

Main Outcome Measure(s): Primary outcome of live-birth rate and secondary outcomes including implantation rate, clinical loss rate, and percentages of normal or balanced, unbalanced, and aneuploid embryos detected.

Result(s): There was no statistically significant difference in LBR, though there was a tendency toward a higher LBR for NGS testing (14 of 19, 73.7%) compared with FISH (8 of 18, 44.4%) and aCGH (10 of 20, 50.0%). The implantation rate was statistically significantly higher for NGS (16 of 20, 80.0%) compared with FISH (11 of 25, 44.0%) and aCGH (16 of 30, 53.3%). There was no statistically significant difference in clinical pregnancy losses. There was a lower percentage of normal or balanced embryos with FISH (12.5%) compared with aCGH (23.7%) and with NGS (20.7%).

Conclusion(s): This is the first report of PGT-SR outcomes for translocation carriers directly comparing PGT-SR using FISH, aCGH, and NGS. Our findings suggest an improvement in pregnancy outcomes parallel to the advancement in technology and are reassuring for continued use of NGS for this population. (Fertil Steril Rep® 2020;1:249–56. ©2020 by American Society for Reproductive Medicine.)

Key Words: Chromosomal translocation, preimplantation genetic testing for structural rearrangements, Robertsonian translocation, reciprocal translocation, next generation sequencing

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Preimplantation genetic testing for chromosomal structural rearrangements (PGT-SR) is available after in vitro fertilization (IVF) to test embryos in individuals who carry Robertsonian or reciprocal translocations, which are the most common forms of chromosomal structural rearrangements. Translocations, defined as a rearrangement of chromosomal segments between nonhomologous chromosomes, occur in approximately 1 in 500 individuals (1). Robertsonian translocations have abnormal breakage and joining of two acrocentric chromosomes. Reciprocal translocations, which are more common, have an exchange of chromosomes terminal segments (2). Balanced translocation carriers have normal genetic content and are phenotypically normal. Mitosis perpetuates a balanced cell line of somatic cells, but meiosis is disrupted by the rearranged chromosomes, resulting in chromosomal deletions or duplications within gametes (3). Balanced translocation carriers are at risk of creating unbalanced gametes after meiotic segregation (4).

Unbalanced embryos likely result in failed implantation or miscarriage, and often chromosomal translocations are diagnosed as a cause of reduced fertility or recurrent pregnancy loss (5). If successful pregnancy is achieved with an unbalanced embryo, the child may have physical and mental disabilities (5). The outcome of the conception depends on the extent and severity of the genetic imbalance, which in turn depends on the specific chromosome rearrangement (6). After in vitro fertilization (IVF), an embryo can be biopsied by removal of cells that are subsequently analyzed by PGT-SR to determine the chromosome content and structure. Normal or balanced embryos can be selected to increase the chance for live birth, decrease the risk of miscarriage, and avoid translocation-dependent disabilities (7, 8).

Preimplantation genetic testing for chromosomal structural rearrangement evaluates the chromosomes to specifically identify unbalanced embryos. Testing modalities have evolved over time from fluorescence in-situ hybridization (FISH) to microarray comparative genomic hybridization (aCGH) and most recently to next-generation sequencing (NGS). Few publications have assessed the outcomes of PGT-SR for structural chromosomal rearrangements using the NGS platform (2, 9, 10). Next-generation sequencing has been hypothesized to be able to contribute to better pregnancy outcomes and fewer miscarriages (11), attributed to its improved detection of mosaicism and segmental aneuploidy (12, 13). No studies to date have directly compared different chromosome testing modalities in translocation carriers.

We compared the IVF outcomes of patients who underwent PGT-SR for either reciprocal or Robertsonian translocations using one of three methods: FISH, aCGH, and NGS. Though many clinics have adopted NGS testing, this study remains relevant as some countries may be limited by legal restrictions to FISH testing, as described in a recent publication describing PGT-SR outcomes from France (14). The primary objective is to compare live birth outcomes, and the secondary objective is to compare the percentages of unbalanced embryos and the percentages of aneuploid embryos detected. The hypothesis of this study is that PGT-SR using NGS, the most recent testing technology developed, improves live-birth rates associated with greater detection of abnormal embryos compared with FISH and aCGH. The data are important to validate the use of NGS for translocations.

MATERIALS AND METHODS

The institutional review board at University of Connecticut Health approved this study. We included all translocation carriers who underwent PGT-SR between December 2005 and April 2019 at a large university-affiliated IVF center. The inclusion criteria were age >18 years, presence of translocation diagnosed by karyotype, and use of IVF with PGT-SR. The exclusion criteria included transfers of embryos that underwent slow-freeze, transfer of mosaic embryo, and transfer of embryos created with donor gametes.

The primary outcome was live-birth rate. The secondary pregnancy outcomes included implantation rate and clinical pregnancy loss rate. Implantation rate was defined as the number of intrauterine gestational sacs visualized by ultrasound per the total number of embryos transferred. Clinical pregnancy loss rate was defined as the number of spontaneous pregnancy losses per clinical pregnancies defined as a gestational sac visualized by ultrasound. The secondary embryo testing outcomes included the percentages of normal or balanced, unbalanced, and aneuploid embryos detected. Embryos that were unbalanced for the translocation-affected chromosomes and simultaneously aneuploid for other chromosomes were classified as "aneuploid." The aCGH results were separated by day-3 or day-5 biopsy to account for differences due to the day of biopsy.

IVF protocol

The IVF protocols included gonadotropin-releasing hormone (GnRH)-agonist suppression, GnRH antagonist with or without estrogen priming, and microdose flare with leuprolide acetate. The protocols were selected by physician's preference, and these were described elsewhere (15, 16). The treatment medications included recombinant follicle-stimulating hormone (FSH) alone or FSH in combination with human menopausal gonadotropin (Gonal-F, EMD Serono; Follistim, Merck; Menopur, Ferring Pharmaceuticals).

For antagonist protocols, GnRH antagonist (Ganirelix, Merck; Cetrotide, EMD Serono) was started when the lead follicle size reached 13–14 mm or estradiol level was >300 pg/mL. Trigger of final oocyte maturation was performed with either human chorionic gonadotropin (Pregnyl, Merck; Novarel, Ferring Pharmaceuticals) or GnRH agonist (leuprolide acetate, 1 mg; Abbott Laboratories) when three follicles reached at least 17–18 mm in mean diameter. Fertilization was performed by either intracytoplasmic sperm injection or conventional insemination and evaluated 16–18 hours later.

Embryo biopsy

Day-3 cleavage-stage biopsy of a single blastomere was performed for all FISH-tested embryos and most aCGH-tested embryos. Individual embryos were placed into calcium/magnesium–free human tubal fluid–HEPES medium (Sage In Vitro Fertilization) for embryo biopsy. Embryos were positioned so that a nucleated cell was adjacent to the anticipated

biopsy site. A 25–30 μm hole was opened in the zona pellucida with a series of three to five single pulses from an infrared 1.48-μm diode laser using a 1-millisecond pulse duration at 100% power (Hamilton-Thorne Research). Before 2011, an acid Tyrode solution was applied to the zona with an assisted-hatching micropipette (Humagen) to breach a hole in the zona pellucida. Nucleated blastomeres were removed through the opening by applying gentle aspiration with a blastomere biopsy pipette (Humagen). Before processing, the nuclear status of isolated blastomeres from each embryo was verified by light microscopy using Hoffman optics (Nikon).

Individual blastomeres analyzed with FISH were exposed to a potassium chloride solution (KCl) hypotonic solution then fixed to a glass slide using a 3:1 methanol acetic acid fixative solution. Blastomeres analyzed using aCGH or NGS were pipetted into individual polymerase chain reaction tubes, frozen at −20°C, and sent to the PGT laboratory for DNA amplification and analysis. If a normal or balanced embryo was present, the patient underwent fresh embryo transfer at the blastocyst stage on day 5, and supernumerary embryos were cryopreserved by slow-freeze or vitrification technique.

Blastocyst biopsy was performed for a minority of aCGH-tested embryos and for all NGS-tested embryos. Trophectoderm biopsy was performed on day-5 or day-6 on good-quality blastocysts according to the Gardner criteria (3BB or higher). Before trophectoderm biopsy, a 5- to 10-μm hole in the zona pellucida was made on day 3 with a series of 1460 nanometer diode laser using 1-millisecond single pulses from an infrared 1.48-pulse duration at 100% power (Hamilton-Thorne Research). Herniating trophectoderm cells were aspirated into a trophectoderm biopsy pipette (Vitrolife) and detached from the blastocyst by firing several pulses at the constricted area of trophectoderm cells at the end of the pipette.

The biopsied piece of trophectoderm tissue was placed intact into a microcentrifuge tube after several washes through a simple wash buffer. Vitrification became the standard technique at our clinic for embryo freezing in March 2013. Embryos frozen by slow-freeze technique were excluded from the analysis of embryo transfer outcomes. Frozen embryo transfer was performed in either a natural or programmed medicated cycle, as described previously elsewhere (17).

Testing protocols

Our FISH testing was performed by Reprogenetics (Livingston, NJ) using probes specific for the chromosomes involved in the translocation as well as probes specific to chromosomes 13, 16, 18, 21, and 22. The probes were used that bind at the centromere and telomeres when available. All probes were manufactured by Vysis (Abbott Molecular).

Our aCGH testing was performed by Reprogenetics (Livingston, NJ). Whole-genome amplification was performed using array 24Sure (BlueGnome). The array used bacteria artificial chromosomes with >5,000 DNA clones, which covered 30% of the entire genome. Our NGS testing was performed by Cooper Genomics (Livingston, NJ).

For both aCGH and NGS, the genetic material within the embryonic cells was isolated and amplified. A DNA analysis is then performed via aCGH or NGS to detect chromosome aneuploidies (entire extra or missing chromosomes) and some types of segmental aneuploidies (missing or extra segments of chromosomes). Both aCGH and NGS have the ability to detect segments of chromosomes larger than 5 megabases (MB). Neither aCGH nor NGS can distinguish between normal versus balanced embryos. The tests may not detect all forms of polyploidy, balanced structural chromosome abnormalities, or alterations smaller than 5 MB or in a heterochromatic region. Microarray comparative genomic hybridization cannot detect the presence of mosaicism.

Our antimüllerian hormone (AMH) testing practices changed over time. Before 2017 the samples were sent to a third-party laboratory. After 2017 we performed AMH testing in house with the Elecsys AMH assay (Roche Diagnostics) with 2.3% intra-assay and 2.9% interassay coefficients of variability.

Statistical analysis

We used analysis of variance (ANOVA) for continuous variables using Bonferroni correction for multiple comparisons. Chi-square or Fisher's exact test was used for categorical data using Bonferroni correction to account for multiple comparisons. All analyses were conducted with SPSS version 26 (IBM, Inc.). $P < .05$ was considered statistically significant.

RESULTS

In our study, 51 translocation carriers underwent a total of 91 PGT-SR cycles, including 31 FISH cycles, 24 aCGH cycles, and 36 NGS cycles. Of these, nine carriers (five female and four male) had Robertsonian translocations, and 42 carriers (24 female and 18 male) had reciprocal translocations. A total of 644 embryos were tested.

Comparing the cycles in which patients underwent PGT-SR testing, there were no statistically significant differences in baseline characteristics of age, body mass index, day-3 FSH level, or AMH level (Table 1). The day of embryo biopsy was statistically significantly different among the cycles as day-5 or day-6 embryo biopsy was adopted over time. There was a statistically greater percentage of Robertsonian translocation carriers for cycles that underwent aCGH testing (37.5%) compared with FISH (6.4%) and NGS (5.6%).

Embryo transfer characteristics were different among the different testing groups, reflecting changes in clinical practice (Table 2). For tested embryos that underwent transfer, all FISH biopsies were cleavage stage, aCGH biopsies were split (60% biopsied at cleavage stage and 40% biopsied at blastocyst stage), and all NGS biopsies were performed at the blastocyst stage. Fresh embryo transfer was performed for embryos biopsied at the cleavage stage, including those tested by FISH and 60% of those tested by aCGH. Frozen embryo transfer was performed for embryos biopsied at the blastocyst stage using either programmed or natural cycle frozen embryo transfer protocols. A greater number of single-embryo transfers were performed for embryos tested by NGS (94.7%) as compared with FISH (66.7%) and aCGH (50.0%).

TABLE 1

PGT-SR cycle baseline demographics.

Demographics	FISH (n = 31)	aCGH (n = 24)	NGS (n = 36)	*P* value
No. of patients[a]	18	16	21	
Age (y)	34.1 ± 3.7	33.8 ± 3.4	33.7 ± 3.0	.88
BMI	27.5 ± 4.4	27.9 ± 7.0	27.7 ± 5.1	.97
Day 3 FSH (mIU/mL)	5.8 ± 3.2	5.6 ± 1.9	6.3 ± 1.8	.49
AMH	2.1 ± 2.1	2.4 ± 1.0	3.6 ± 3.7	.24
Type of translocation				<.001[b]
Reciprocal	29/31 (93.5%)[c]	15/24b (62.5%)	34/36 (94.4%)[c]	
Robertsonian	2/31 (6.4%)[c]	9/24 (37.5%)[d]	2/36 (5.6%)[c]	
Paternal or maternal carrier				.47
Paternal	16/31 (51.6%)	12/24 (50.0%)	23/36 (63.9%)	
Maternal	15/31 (48.4%)	12/24 (50.0%)	13/36 (36.1%)	
Mean year of cycle (range)	2010 (2005–2013)	2013 (2010–2017)	2017 (2016–2019)	
Day of embryo biopsy				<.001[b]
Day 3	31/31 (100%)[c]	17/24 (70.8%)[d]	0/36 (0)[e]	
Day 5 or 6	0/31 (0)[c]	7/24 (29.2%)[d]	36/36 (100%)[e]	

Note: aCGH = microarray comparative genomic hybridization; AMH = antimüllerian hormone; BMI = body mass index; FISH = fluorescence in-situ hybridization; FSH = follicle-stimulating hormone; NGS = next-generation sequencing; PGT-SR = preimplantation genetic testing for chromosomal structural rearrangements.
[a] Number of total patients who underwent PGT-SR testing and were included, where n is equal to the total number of IVF cycles. Some patients underwent more than one IVF cycle, and some used two different testing modalities. There were 51 total patients who underwent testing.
[b] *P* value < .05 was considered statistically significant.
[c,d,e] Each letter denotes a subset of values that do not statistically significantly differ from each other at the *P* < .05 level.

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Despite similar numbers of oocytes retrieved, there were more embryos biopsied for FISH and aCGH cycles, reflecting the greater number of embryos available on day 3. Despite the fewer embryos available for testing with NGS, there were comparable numbers of cycles with the finding of no euploid embryos: 38.7% for FISH, 20.8% overall for aCGH (17.6% day-3 biopsy and 28.6% day-5 biopsy), and 33.3% for NGS (Table 3). Including all tested embryos, there was overall a similar percentage of normal or balanced embryos with the testing modalities: FISH 12.8%, day-3 biopsy

aCGH 17.4%, and NGS 21.2%, although day-5 biopsy aCGH was associated with more balanced embryos (43.6%) after Bonferroni correction (Table 3).

The embryos with abnormal results were different among the testing modalities (Table 3). For unbalanced embryos, there was a higher percentage of total tested embryos and mean number of embryos per cycle with FISH testing compared with embryos biopsied on day 5 and tested by aCGH and NGS. For day-3 biopsied embryos, FISH resulted a higher percentage of unbalanced embryos than aCGH, while

TABLE 2

Transfer cycle characteristics.

Characteristics	FISH (n = 18)	aCGH (n = 20)	NGS (n = 19)	*P* value
No. of patients[a]	12	12	17	
Type of translocation				<.001[b]
Reciprocal	17/18 (94.4%)[c]	10/20 (50.0%)[d]	17/19 (89.5%)[c]	
Robertsonian	1/18 (5.6%)[c]	10/20 (50.0%)[d]	2/19 (10.5%)[c]	
Day of embryo biopsy				<.001[b]
Day 3	18/18 (100%)[c]	12/20 (60.0%)[d]	0/19 (0)[e]	
Day 5 or 6	0/18 (0)[c]	8/20 (40.0%)[d]	19/19 (100%)[e]	
Transfer type				.03[b]
Fresh	18/18 (100%)[c]	12/20 (60.0%)[d]	0/19 (0)[e]	
Programmed	0/18 (0)[c]	5/20 (25.0%)[c]	13/19 (68.4%)[d]	
Natural cycle	0/18 (0)[c]	3/20 (15.0%)[d]	6/19 (31.6%)[d]	
No. of embryos transferred				.008[b]
Single embryo	12/18 (66.7%)[c]	10/20 (50.0%)[c]	18/19 (94.7%)[d]	
Two embryos	5/18 (27.8%)	10/20 (50.0%)	1/19 (5.3%)	
Three embryos	1/18 (5.6%)	0/20 (0)	0/19 (0)	

Note: aCGH = microarray comparative genomic hybridization; FISH = fluorescence in-situ hybridization; NGS = next-generation sequencing; PGT-SR = preimplantation genetic testing for chromosomal structural rearrangements.
[a] Number of total patients who underwent embryo transfer with normal or balanced embryo, where n is equal to the total number of embryo transfers. Some patients underwent more than one transfer, and some used two different testing modalities. There were 40 total patients who underwent embryo transfer.
[b] *P* value < .05 was considered statistically significant.
[c,d,e] Each letter denotes a subset of values that do not statistically significantly differ from each other at the *P* < .05 level.

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TABLE 3

Preimplantation genetic testing for chromosomal structural rearrangements.

	Day-3 biopsy		Day-5 or day-6 biopsy		
PGT-SR outcomes	FISH (n = 31)	aCGH (n = 17)	aCGH (n = 7)	NGS (n = 36)	P value
Mean no. of oocytes retrieved	18.2 ± 8.6	18.7 ± 8.6	18.7 ± 13.0	16.4 ± 8.8	.77
Mean no. of mature oocytes	13.5 ± 5.7	14.7 ± 5.7	14.7 ± 10.6	12.3 ± 6.0	.56
Mean no. of embryos biopsied	8.6 ± 4.5[a]	9.1 ± 2.8[a]	5.6 ± 2.8[a,b]	5.4 ± 3.5[b]	.001[c]
Mean no. of normal or balanced embryos	1.1 ± 1.4[a]	1.6 ± 1.5[a]	2.1 ± 2.6[b]	1.1 ± 1.1[a]	.006[c]
Percentage of normal or balanced per total embryos tested	12.8% (33/257)[a]	17.4%[a] (27/155)	43.6% (17/39)[b]	21.2%[a] (41/193)	<.001[c]
Mean no. of unbalanced embryos	6.6 ± 3.8[a]	4.6 ± 2.6[a,b]	2.0 ± 1.9[b]	2.9 ± 2.1[b]	<.001[c]
Percentage of unbalanced embryos per total embryos tested	77.0% (198/257)[a]	50.3% (78/155)[b]	35.9% (14/39)[b]	53.4% (104/193)[b]	<.001[c]
Mean no. of aneuploid embryos	0.43 ± 1.2[a]	2.42 ± 1.4[b]	0.57 ± 0.8[a]	0.56 ± 0.7[a]	<.001[c]
Percentage of aneuploid per total embryos tested	5.1% (13/257)[a]	23.2% (36/155)[b]	10.2% (4/39)[a,b]	10.4% (20/193)[a]	<.001[c]
Mean no. of mosaic embryos	NA[d]	NA[d]	0[a]	0.5 ± 1.0[b]	.006[c]
Percentage of mosaic per total embryos tested			0 (0/39)[a]	9.3% (18/193)[b]	.049[c]
Mean no. of "no result" embryos	0.43 ± 0.7	0.82 ± 1.1	0.57 ± 0.8	0.28 ± 0.5	.086
Percentage of "no result" per total embryos tested	5.1% (13/257)	9.0% (14/155)	10.2% (4/39)	5.2% (10/193)	.26
Percentage of cycles with no normal/balanced embryos	38.7% (12/31)	17.6% (3/17)	28.6% (2/7)	33.3% (12/36)	.51

[a,b] Each letter denotes a subset of values which do not statistically significantly differ from each other at the P< .05 level.
[c] P value < .05 was considered statistically significant.
[d] NA = not applicable. Mosaic embryos could not be detected for single blastomere biopsy on day 3.

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there were more aneuploid embryos for aCGH than for FISH. For day-5 biopsied embryos, unbalanced and aneuploid results were similar for aCGH and NGS, while more balanced embryos were associated with aCGH. Mosaic embryos were only detected by NGS. Embryos with "no result" after testing were similar for all testing types.

There were more single-embryo transfers for NGS-tested embryos (Table 1); however, the live-birth rate was highest for NGS (77.8%) compared with FISH (44.4%) and aCGH (50.0%), although the difference was not statistically significant (Table 4). Notably one NGS patient was excluded for transfer of a mosaic embryo, which resulted in a live birth. When pooling together all aCGH results, the implantation rate was statistically significantly highest for NGS (80.0%) compared with FISH (44.0%) and aCGH (53.3%) (P=.045). However, this difference in implantation was no longer statistically significant when distinguishing aCGH results by day of biopsy (Table 4). The clinical pregnancy loss rate was lowest for NGS (no losses out of 14 clinical pregnancies) compared with FISH (20.0%, two losses out of 10 clinical pregnancies) and aCGH (23.1% overall, three losses out of 13 clinical pregnancies), though this finding was again not statistically significant as there were few numbers.

DISCUSSION

This study reflects the advances in genetic testing over time. Fluorescence in-situ hybridization testing of cleavage-stage embryos followed by fresh IVF embryo transfer was replaced by aCGH testing of either cleavage-stage or blastocyst-stage embryos, which most recently has been superseded by NGS testing of blastocyst-stage embryos followed by vitrification and frozen embryo transfer. Single-nucleotide polymorphism (SNP) array, another technique that has been used for PGT-SR in translocation carriers, was not applied in this study population (18–21). The live-birth rate for PGT-SR after transfer of NGS-tested embryos, although not statistically significantly different from FISH or aCGH, was high while adhering to the safe practice of single-embryo transfer. This success correlates with the enhanced detection of abnormal embryos and the high embryo implantation rate.

Fluorescence in-situ hybridization is constrained by the limited number of unique fluorescent probes for each tested chromosome, allowing only five to six chromosomes to be tested (18, 22). Aneuploidy involving untested chromosomes will be undetected. Fluorescence in-situ hybridization is also limited by technical challenges, optical signal splitting, and

TABLE 4

Pregnancy outcomes for embryo transfers.

Outcomes	Day-3 biopsy		Day-5 or day-6 biopsy		P value
	FISH (n = 18)	aCGH (n = 12)	aCGH (n = 8)	NGS (n = 19)	
Implantation rate	44.0% (11/25)	50.0% (9/18)	58.3% (7/12)	80.0% (16/20)	.093
Clinical pregnancy rate	55.6% (10/18)	58.3% (7/12)	75.0% (6/8)	73.7% (14/19)	.59
Clinical pregnancy loss rate	20.0% (2/10)	14.3% (1/7)	33.3% (2/6)	0 (0/14)	.48
Live-birth rate	44.4% (8/18)	50.0% (6/12)	50.0% (4/8)	73.7% (14/19)	.30

Note: Here, n denotes the number of embryo transfers. Implantation rate is the number of visualized gestational sacs per total number of embryos transferred. Clinical pregnancy rate is the number of clinical pregnancies per total number of embryo transfers. Clinical pregnancy loss rate is the number of clinical losses per total number of clinical pregnancies. Live-birth rate is the number of live births per total embryo transfers.

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interpretive artifacts (23, 24). The testing capabilities of aCGH and NGS are improved by whole-genome amplification and simultaneous aneuploidy screening for all 24 chromosomes (25, 26). Higher aneuploidy rates are expected from day-3 embryos due to the decline in the incidence of chromosome abnormalities during development to the blastocyst stage (27, 28). However, fewer aneuploid embryos were detected with FISH testing of cleavage-stage biopsies when compared with aCGH testing performed on cleavage and blastocyst-stage biopsies as well as NGS testing performed solely on blastocyst-stage biopsies. This finding likely reflects the limitations of FISH when compared with the 24-chromosome testing capabilities of aCGH and NGS.

There was a higher percentage of unbalanced embryos detected by FISH testing. Overall there is a high incidence of embryo arrest among translocation carriers, which has been attributed to alterations in chromosomes not directly involved in the translocation (29). Another hypothesis to account for greater embryo arrest is the decreased potential of unbalanced embryos to develop to the blastocyst stage, resulting in a net loss of unbalanced embryos from the day-3 embryo stage to blastocyst stage (28). A retrospective analysis of translocation carriers using FISH, SNP array, aCGH, and NGS testing suggested a natural selection process resulting in more normal or balanced embryos from days 5 or 6, based on the finding that there was an increase in the proportion of genetically normal or balanced embryos from day 3 (38.7%) to day 5 or 6 (74.1%) (30). However, other studies have suggested that the presence of unbalanced chromosomes does not affect the embryo's ability to reach the blastocyst stage (31, 32). The biopsy performed on day 3 removing a totipotent blastomere, as opposed to a day-5 or day-6 trophectoderm biopsy, could have damaged the embryo and impaired its ability to develop further (33).

Alternatively, the lower percentage of unbalanced embryos among embryos tested by aCGH and NGS may be due to their enhanced ability to detect aneuploid embryos, which are also unbalanced and would only be classified as unbalanced by FISH testing. In this study, embryos that were aneuploid for other chromosomes and unbalanced were classified as "aneuploid." Testing limitations may also have contributed to the high number of reported unbalanced embryos by FISH testing, as evidenced by the Northrop et al. (34) study suggesting a high diagnostic inaccuracy rate with FISH; they used

single nucleotide polymorphism microarray to reanalyze 50 embryos previously diagnosed as aneuploid by FISH and found that 58% were euploid. Finally, there were likely more balanced and fewer unbalanced embryos for the patients who underwent aCGH testing due to the greater number of Robertsonian translocation carriers in this group. Robertsonian translocation carriers have been shown to produce more normal or balanced embryos than reciprocal translocation carriers (35).

Next-generation sequencing yields enhanced detection of mosaic embryos, which contain both normal and abnormal cells and are at higher risk for miscarriage (36, 37). There is also improved detection of segmental aneuploidy (12, 13). The advanced sensitivity of NGS may provide translocation carriers with better outcomes, as evidenced in this study by the statistically significant increase in implantation rate and non–statistically significant lower pregnancy loss rate. Importantly, this increased testing sensitivity did not translate into fewer embryos available for transfer: NGS testing detected a similar number of euploid embryos and did not increase the number of cycles in which no normal or balanced embryos were detected. Similar to the data presented here, Cai et al. (9) reported a low miscarriage rate of 2.94% using NGS.

One criticism of PGT-SR is that it may not result in higher live-birth rates when compared with spontaneous pregnancy (38). However, PGT-SR can reduce the miscarriage rate and reduce time to achieve a successful live birth from 4 to 6 years to 4 months or less (4, 7, 39). However, the limitations of NGS testing in the setting of translocation carriers are clinically important. There are a few instances where NGS cannot be used for translocation carriers. This is typically when the unbalanced rearrangement(s) are too small to be seen by the resolution of NGS (~5 MB) (10). In such cases, other methods such as a karyomapping or FISH can be attempted.

Accurate aneuploidy detection is imperative, especially in a population of translocation carriers who are at higher risk for aneuploidy (40). One explanation for this finding is the theory of interchromosomal effect, in which the presence of the translocation negatively affects the segregation of other chromosomes not involved in the translocation (28, 41). Chromosomes involved in rearrangements may interfere with segregation of other chromosomes by disrupting spindle alignment during meiosis. Furthermore, female translocation carriers have been reported to have diminished ovarian

reserve, so there are potentially fewer embryos to test (42–45). One study found that couples with female translocation carriers, when compared with couples with male translocation carriers, had diminished ovarian response and produced a higher rate of unbalanced embryos (14).

The strengths of our study include the ability to interpret outcome data on a large number of embryos, including the evaluation of pregnancy outcomes. All the cycles were performed in one center, with minimal variation in the embryology team through the years. This study is relevant because FISH continues to be used for translocation carriers in some countries (14). Furthermore, this study is clinically important to affirm the practice of NGS testing for translocation carriers, which has been widely adopted without robust data on pregnancy outcomes. Very few publications exist assessing NGS for translocation carriers (2, 9, 10). The miscarriage rate of 0 reported in our study should be interpreted with caution due to the small number of patients; further investigation would be worthwhile for the possible value of this technology for these challenging patients. Overall, this study provides reassuring results to support the use of NGS testing for PGT-SR.

One limitation of the study is the inclusion of many variables that affect outcomes, including biopsy of cleavage-stage versus blastocyst-stage embryos, the inclusion of multiple cycles from the same individual, the inclusion of fresh as well as vitrified embryos, transfer of different numbers of embryos, and the parallel advancements in reproductive technology during the long study time period. Given these variables, it cannot be definitively determined whether differences in outcome are attributable to the testing technique or to other practice changes. For example, the blastocysts chosen for biopsy are high quality, whereas a genetically normal embryo after day-3 biopsy would be more likely to be transferred in the setting of a poor-quality day-5 blastocyst following fresh embryo transfer protocol in the setting of ovarian stimulation. Nevertheless, these data accurately reflect the changes in PGT testing over the past two decades.

Other limitations of the study include the retrospective design, the inclusion of more than one cycle from the same patient, which was not statistically corrected for due to a small sample size, and being underpowered to detect a difference in the primary outcome of live-birth rate also due to the small sample size. Post hoc power analysis indicated that a sample size of 45 in each group would have been necessary to show a statistically significant increase in live-birth rate for NGS over FISH at 80% power and alpha of 0.05. Pooling together data from multiple centers in future studies would enable a more robust assessment.

CONCLUSION

Ours is the first report of outcomes for translocation carriers directly comparing PGT-SR using FISH, aCGH, and NGS. The implantation rate was statistically significantly higher for embryos tested with NGS when compared overall to FISH and aCGH. Live-birth rates were high after single-embryo transfer for individuals who underwent PGT-SR using NGS. Although they are not statistically significant due to the limited sample size from a single center, our findings suggest an improvement in pregnancy outcomes parallel to the advancement in technology and are reassuring for continued use of NGS for this population.

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