Consistency in rates of diagnosis of embryonic mosaicism, segmental abnormalities, and "no call" results among experienced embryologists performing preimplantation genetic testing for aneuploidy

Emily K. Osman, M.D.,[a,b] Shelby A. Neal, M.D.,[a,b] Ashley W. Tiegs, M.D.,[a,b] Brent M. Hanson, M.D.,[a,b] Julia G. Kim, M.D., M.P.H.,[a,b] Jason M. Franasiak, M.D., H.C.L.D.,[a] and Richard T. Scott Jr., M.D., H.C.L.D.[a]

[a] IVI/RMA New Jersey, Basking Ridge, New Jersey; and [b] Sidney Kimmel College of Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania

Objective: To determine whether differences exist in rates of subchromosomal abnormalities, mosaicism, and "no call" results among embryologists performing and loading trophectoderm biopsies for preimplantation genetic testing for aneuploidy (PGT-A).
Design: Retrospective cohort.
Setting: Large infertility center.
Patient(s): All patients undergoing in vitro fertilization with PGT-A.
Intervention(s): The NexCCS next generation sequencing platform was used for PGT-A. The χ^2 testing assessed differences in rates of primary outcomes between embryologists. Intraclass correlation coefficients evaluated inter-embryologist reliability in rates of abnormal and no call results. Median absolute performance difference (MAPD) scores, which quantify the impact of technical variation on analytical performance, were averaged for individual embryologists. Analysis of variance assessed differences in mean MAPD scores.
Main Outcome Measure(s): Interoperator variability in rates of mosaic, segmental, and no call results.
Result(s): Four embryologists performed 30,899 biopsies and 6 embryologists loaded specimens into designated tubes. Among individuals performing trophectoderm sampling, rates of mosaicism were 4.3% to 6.1%, segmental errors were 9.0% to 10.7%, and inconclusive results were 1.1% to 2.9%. For those loading, the incidence of mosaicism was 4.2% to 5.9%, subchromosomal abnormalities was 9.7% to 10.4%, and no call results was 1.2% to 2.2%. The intraclass correlation coefficient was 0.978 for embryologists performing biopsies and 0.981 for those loading. Differences in mean MAPD scores were within 0.6% and 0.2% of each other for doing biopsies and loading embryologists, respectively.
Conclusion(s): Rates of mosaicism, segmental, and no call PGT-A results are consistent among experienced embryologists. Due to the large sample size included, differences within 1% of the mean were deemed clinically irrelevant despite statistical significance. (Fertil Steril Rep® 2020;1:119–24. ©2020 by American Society for Reproductive Medicine.)
Key Words: Mosaicism, preimplantation genetic testing for aneuploidy (PGT-A), segmental abnormalities, nonconcurrent and unamplified results, IVF laboratory

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Reprint requests: Emily K. Osman, M.D., IVI/RMA New Jersey, 140 Allen Road, Basking Ridge, New Jersey 07925 (E-mail: eosman@ivirma.com).

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The utilization of the higher sensitivity next-generation sequencing (NGS) platform for preimplantation genetic testing for aneuploidy (PGT-A) has led to results reporting higher resolution and greater depth. Pregnancy and live birth rates have increased as compared to other platforms such as array comparative genomic hybridization (1). Such advancements have also led to increased reporting of abnormalities such as mosaicism and subchromosomal abnormalities, otherwise known as segmental errors.

Differences in reported mosaicism rates have been described between in vitro fertilization (IVF) laboratories, with some studies reporting interlaboratory variance as high as 30% (2). It has been suggested that conditions within the IVF laboratory may contribute to increases in mosaicism and segmental errors (3, 4). Discrepancies between IVF laboratories may be attributed to inconsistencies in aptitude of individual embryologists who are performing biopsies and handling trophectoderm (TE) samples for PGT-A analysis. Technical aspects of the biopsy procedure and specimen handling during loading into designated tubes have the potential to impact analytical performance of the assay being used.

The PGT-A fails to yield a diagnostic result in 0.86%–3.8% of embryo biopsies (5–7). "No call" results include samples that failed to amplify, commonly indicating insufficient DNA in the biopsy specimen for analysis, as well as those labeled nonconcurrent with wide scatter in the data. Variation in biopsy technique has been claimed to contribute to an increased incidence in no-call reads (8). Poor biopsy technique or aggressive use of the laser during the procedure may yield insufficient TE sample size and may increase the risk of uninterpretable results. An excess amount of buffer during the loading procedure effectively changes the reaction volume, thereby decreasing the concentration of DNA from the sample as well as the reagents used in the polymerase chain reaction. This effectively increases the risk for failed or poor quality amplification. Variation in rates of mosaicism, segmental abnormalities, and no call results has only been compared between IVF centers (9–11). Although it has been suggested that laboratory practices and technical aptitude of the embryologist may influence PGT-A results (12), there has been no study thus far to examine whether interembryologist differences exist within the same laboratory while following a uniform protocol.

Alternatively, differences in embryo cohorts or culture systems also have the potential to contribute to increased rates of abnormal PGT-A results. Although rates of blastocyst mosaicism and segmental errors are consistent across age groups, poorer quality embryos are associated with higher rates of no call results (13). Cohorts with a higher proportion of poor quality blastocysts may therefore increase unamplified and nonconcurrent results. It has been hypothesized that altered culture conditions, such as aberrations in osmolality, pH, temperature, or mechanical stress on the embryo may lead to improper chromosomal segregation and disjunction during mitosis (12). Many studies have investigated whether rates of mosaicism and segmental abnormalities increase with the use of monophasic as opposed to sequential media (4, 14, 15) with conflicting results.

There is a need for standardization of individual performance within each laboratory as a means of adhering to strict protocols and ultimately optimizing clinical IVF outcomes. A highly effective way to measure the impact of variation in technique on analytical performance is through monitoring of the median-absolute pairwise difference (MAPD) between log2 ratios of adjacent amplicons of all TE samples biopsied and loaded by each embryologist. With targeted NGS-based PGT-A, log2 ratios are obtained by comparing sample read counts to a reference genome for each amplicon. The MAPD score is an objective assessment of genome-wide copy number noise and provides a measure of how much "scatter" in the data is not due to true biologic differences (16, 17). A lower MAPD score is associated with less deviation in the copy number variation as related to laboratory error.

The objective of this study is to determine whether there are differences in rates of subchromosomal abnormalities, mosaicism rates, and no call results among experienced embryologists performing the TE biopsy and those loading the specimens at a single IVF center. In addition, we wanted to assess whether the same embryologist performing both the loading and the biopsy procedure had similar rates of the aforementioned abnormalities compared with a different embryologist performing the biopsy and loading.

MATERIALS AND METHODS

Study design and patient population

A retrospective cohort study was performed consisting of all embryos that underwent TE biopsy between June 2016 and April 2019 at a single large infertility center. Cases of preimplantation genetic testing being used for monogenic disease detection or structural rearrangements were excluded. Any embryo that underwent a biopsy procedure more than once was not included in the analysis. This study was approved by our Institutional Review Board, and given the retrospective nature, formal consent of study participants was not required.

Embryo biopsy and sample analysis

As per routine laboratory protocol, all high quality blastocysts underwent a biopsy procedure and then vitrified when they were full expanded on day 5, 6, or 7 of development. A blastocyst was determined to be of high quality with a grade of 4BC or better according to a modified Gardner grading system. One embryologist performed the TE biopsy before cryopreservation according to standard operation and either the same or a different embryologist loaded the specimen into designated polymerase chain reaction tubes in an adjacent laminar flow hood immediately after the biopsy procedure. The TE biopsy specimens were sent to a single genetics laboratory for analysis using an NGS platform.

Blastocysts were labeled as mosaic if the DNA copy number deviated from 2.0 by 0.3–0.7 across the genome. Segmental errors were defined as genome-wide chromosome duplications or deletions between 5 and 10 Mb. The threshold for detection was as low as 0.5 Mb in some regions depending on the affected area and the density of coverage in the particular locus. No call reads included biopsy samples in which

there was DNA amplification failure and nonconcurrent results, indicating extensive data scatter and inability to meet quality control standards defined by the reference laboratory. For the purposes of this analysis, if an embryo biopsy was determined to be aneuploid plus mosaic or segmental, it was categorized as a mosaic or subchromosomal abnormality.

Statistical analysis and interpretation of data

The χ^2 testing was used to analyze statistical differences in rates of mosaicism, segmental abnormalities, and no call results between embryologists performing and loading biopsy specimens. Intraclass correlation coefficient testing was performed to assess inter-embryologist agreement in rates of abnormalities between those performing and loading biopsy specimens. Analysis of variance was used to determine whether differences in MAPD scores were present among technicians. Due to the large sample size included in the analysis, variations in rates of abnormal results within 1% of the overall mean or differences in mean MAPD scores of <1% of each other for embryologists loading and performing biopsies were considered to be clinically irrelevant despite statistical significance.

RESULTS

During the study period, 4 embryologists performed 30,899 embryo biopsies and 6 embryologists loaded the samples into designated tubes. Of these biopsies, 1,607 (5.2%) were determined to be mosaic, 3,124 were determined to have segmental abnormalities (10.1%) and 549 (1.7%) of the results were determined to be nonconcurrent or were unamplified.

The mosaicism rates among individual embryologists performing TE sampling ranged from 4.3% to 6.1% ($P<.01$) segmental rates of 9.0% to 10.7% ($P<.01$) and inconclusive result rates of 1.1% to 2.9% ($P<.01$), as demonstrated in Table 1. Among embryologists loading biopsy specimens, the incidence of mosaicism ranged from 4.2% to 5.9% ($P=.03$), segmental errors 9.7% to 10.4% ($P=.28$), and no call results 1.2% to 2.2% ($P<.01$) (Table 2).

One embryologist performed the embryo biopsy and another individual loaded the specimen for 23,210 samples. The same operator performed both the biopsy procedure and loaded 7,689 TE samples. There were no clinical differences in mosaicism rates (5.1% vs 5.4%; $P=.28$), segmental error rates (10.1% vs 10.3%; $P=.51$), and inconclusive results (1.9% vs 1.4%; $P<.01$) between the cases using two embryologists versus those using the same embryologist (Fig. 1). All embryologists (either performing biopsies, loading, or both) had mosaicism and segmental abnormality rates that deviated <1% from the mean. As such, clinical differences were deemed irrelevant despite statistical significance. The intraclass correlation coefficient for embryologists performing embryo biopsies was 0.978 and for loading embryologists was 0.981, indicating excellent reliability between experienced technicians.

Evaluation of mean MAPD scores provided another opportunity to assess technical performance of the embryologists as deviations in their technique might adversely impact analytical performance without making the test result uninterpretable. The MAPD scores for all embryologists performing biopsies and loading samples are demonstrated in Table 3. The deviations in MAPD scores were 0.2% ($P<.01$) for loading embryologists and 0.6% ($P=.04$) for embryologists performing biopsies. These exceedingly small differences of <1% were considered to be clinically irrelevant despite statistical significance.

DISCUSSION

The development and refinement of NGS platforms for PGT-A has led to increased reporting of variants of undetermined significance, including diagnoses of mosaicism and subchromosomal abnormalities. Similarly, obtaining a no call result in which DNA from the biopsy specimen fails to amplify or is uninterpretable is frustrating for the laboratory, the clinician, and most importantly, the patient. In the unlikely event that an embryo biopsy fails to amplify or results in a nonconcurrent result, the clinical team will elect to warm the blastocyst, repeat the performing of the biopsy, and revitrify that embryo in 39.3% of cases (13).

Standardization and adherence to laboratory protocols are key to optimize clinical outcomes during IVF cycles. Successful embryo biopsy results rely on the ability of the embryologist to carefully use mechanical force and the assistance of the laser to sample TE cells, removal of a biopsy specimen of appropriate size and location within the embryo, and suspending the biopsy in an appropriate volume of washing

TABLE 1

Rates of mosaicism, segmental errors, and "no call" preimplantation genetic testing for aneuploidy results based on embryologist performing the biopsy.

	Mosaicism		Segmental error		"No calls"	
Embryologist	**N**	**%**	**N**	**%**	**N**	**%**
A	891/15,803	5.6	1,697/15,803	10.7	302/15,803	1.9
B	428/9,969	4.3	896/9,969	9.0	163/9,997	1.6
C	209/3,754	5.6	395/3,754	10.5	43/3,754	1.1
D	83/1,373	6.1	136/1,373	9.9	40/1,373	2.9
All	1,607/30,899	5.2	3,124/30,899	10.1	548/30,899	1.7

Note: For mosaicism, segmental error, and "no calls," $P<.01$.

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TABLE 2

Rates of mosaicism, segmental errors, and "no call" preimplantation genetic testing for aneuploidy results based on embryologist loading the biopsy sample.

	Mosaicism		Segmental error		"No calls"	
Embryologist	**N**	**%**	**N**	**%**	**N**	**%**
A	313/5,340	5.9	550/5,340	10.3	81/5,340	1.5
B	351/6,566	5.4	673/6,566	10.3	81/6,566	1.2
C	48/917	5.2	112/917	12.2	12/917	1.3
D	201/2,443	4.2	253/2,443	10.4	56/2,443	2.2
E	604/12,184	5.0	1,203/12,184	9.9	260/12,184	2.1
F	189/3,449	5.5	336/3,449	9.7	59/3,449	1.7
All	1,607/30,899	5.2	3,127/30,899	10.1	549/30,899	1.8

Note: Mosaicism, $P = .03$; segmental error, $P = .28$; and "no calls," $P < .01$.

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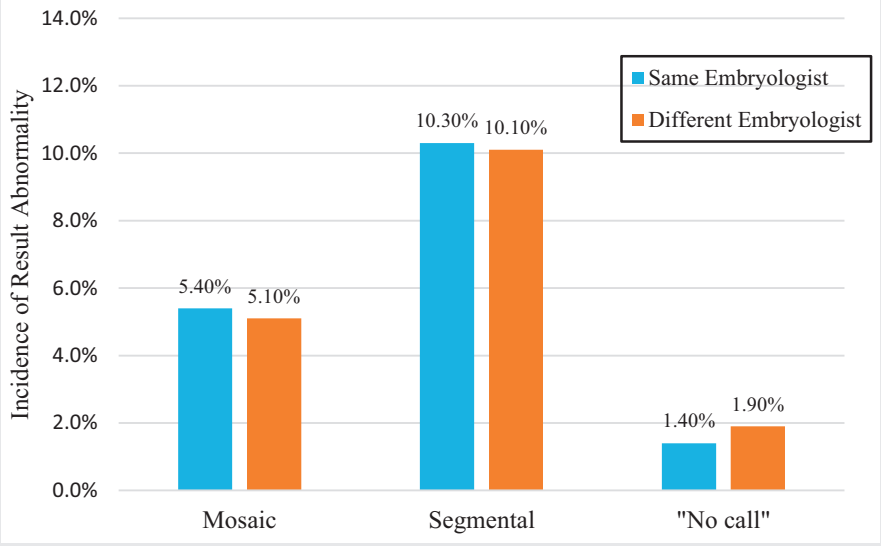
buffer to ensure adequate DNA concentration for amplification and analysis.

Although potential contributors to increased rates of mosaicism, segmental errors, and no call results can be attributed to variations in embryo cohorts and laboratory conditions themselves, we sought to determine whether differences in rates of these abnormalities occur due to discrepancy in the technical aptitude of individual embryologists. Our findings implicate that rates of mosaicism, segmental abnormalities, and no call results do not vary between experienced embryologists performing the biopsy and loading the specimen. Furthermore, there are no clinical differences in outcomes whether the same embryologist versus a different person completes the biopsy and the loading procedure. This is high yield and reassuring information for IVF laboratories that may not have sufficient numbers of experienced embryologists to perform such procedures. Variations in biopsy results between experienced embryologists were uniformly within 1% of the mean, therefore deemed to be clinically irrelevant despite statistical significance as these differences were driven by the large sample size included in the analysis.

Mosaicism and segmental abnormalities occur as a function of mitotic error, and prior studies have suggested that

FIGURE 1



Rates of mosaicism ($P = 28$), segmental abnormalities ($P = 51$), and "no call" biopsy ($P < .01$) preimplantation genetic testing for aneuploidy results between the same embryologist performing trophectoderm biopsy and loading versus different embryologists. Of the 30,899 embryo biopsies included in analysis, 23,210 (75.1%) were performed by one embryologist and loaded by another one, whereas 7,689 (24.9%) of the trophectoderm samples were biopsied and loaded by the same technician. Despite statistically significant variation in the rates of mosaicism, segmental abnormalities, or no calls, including nonconcurrent and unamplified results, these differences were within 1% of the mean and deemed clinically irrelevant.

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TABLE 3

Mean median absolute performance difference scores for individual embryologists performing embryo biopsy and specimen loading.

Embryologist	MAPD of biopsied embryos[a]	MAPD of loaded embryos[b]
A	0.195 ± 0.048	0.195 ± 0.048
B	0.193 ± 0.047	0.195 ± 0.048
C	0.193 ± 0.047	0.194 ± 0.048
D	0.199 ± 0.046	0.195 ± 0.048
E	NA	0.193 ± 0.046
F	NA	0.194 ± 0.048

Note: Data presented as mean ± standard deviation. Median absolute performance difference (MAPD) of biopsied embryos. NA = not available.
[a] $P < .01$.
[b] MAPD of loaded embryos, $P = .04$.

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fluctuating conditions between (18) and within the IVF laboratory (12) may predispose to increased rates. It has been proposed that mechanical and chemical environmental stressors in the IVF laboratory may compromise proper segregation and separation of chromosomes during mitosis. If this theory is valid, the blastocyst is the most mitotically active and presumably prone to mosaicism and segmental error immediately after fertilization until the cleavage stage. It can therefore be assumed that the biopsy procedure itself should not increase mosaicism rates that are otherwise present early on in embryo development, in concordance with our findings.

Previous reports (8) have suggested that the mean number of TE cells was significantly lower in biopsies with unamplified and nonconcurrent results. A poor quality biopsy with an insufficient amount of DNA or an inappropriate volume of media in which the specimen is suspended may lead to an unamplified result, whereas nonconcurrent results may originate from improper technique during biopsy or handling of the specimen. It should be mentioned that our findings are based on highly experienced embryologists, each having performed a minimum of 1,000 biopsies and loaded a minimum of 800 specimens into designated tubes for analysis. These results may not apply to less experienced technicians.

Rigorous internal quality assurance relies on consistent performance of all individuals within the IVF laboratory. An essential laboratory based quality metric is the MAPD score, a metric of data dispersion due to factors not biologic difference. The MAPD score is an objective measure of genome-wide copy number noise and is calculated during biostatistical analysis of TE biopsies undergoing NGS. Higher scores indicate increased genomic scatter and less consistency between amplicon results (16), both of which may be attributed to operator performance. A previous investigation using MAPD scores for validation of a targeted NGS platform had a threshold of 0.5, with any value greater than this indicating an unacceptable amount of genomic noise (17). However, the specific MAPD threshold applied is dependent on the PGT-A platform and number of amplicons used. The MAPD scores for each biopsy analyzed are obtainable from the bioinformatics results generated by the specific platform used. This study is the first to report MAPD scores as a quality assessment of the technical aptitude of individual embryologists. Our laboratory strives for MAPD results to be <0.25 for each technician. In the present study the mean values for all embryologists performing biopsies and loading were below 0.2 and highly consistent with each other. The MAPD assessment is an invaluable tool in evaluating individual competence in the procedures required to successfully perform a biopsy and prepare a TE specimen for PGT-A analysis and should be used consistently between and within IVF laboratories.

Our study is the first of its kind to examine the rates of segmental abnormalities, mosaicism, and no call results between embryologists performing and loading biopsy specimens. Strengths of our study include the large sample size and analysis of samples performed by several experienced embryologists who were trained to follow the same biopsy and loading protocol. In addition, all results were analyzed in one reference laboratory, eliminating any variation that is present between clinical genetics centers that perform PGT-A analysis. Our findings are limited by their retrospective nature.

With the increasing worldwide use of PGT-A, reproducible results are critical for optimizing clinical outcomes during IVF cycles. Our findings are reassuring for patients, laboratory personnel, and clinicians in that experienced embryologists yield consistent and reliable embryo biopsy results.

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