ORIGINAL ARTICLE: ASSISTED REPRODUCTION

The concordance rates of an initial trophectoderm biopsy with the rest of the embryo using PGTseq, a targeted next-generation sequencing platform for preimplantation genetic testing-aneuploidy

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Objective: To determine how often the results of a single trophectoderm (TE) biopsy tested by PGTseq, a targeted next-generation sequencing preimplantation genetic testing for aneuploidy technology, reflect the biology of the rest of the embryo.
Design: Blinded prospective cohort study.
Setting: University-affiliated private practice.
Patient(s): A total of 300 blastocysts were donated; 113 of these embryos were euploid; 163 embryos possessed at least one whole chromosome aneuploidy; and 24 embryos were negative for whole chromosome aneuploidy but possessed at least one secondary finding on initial TE biopsy.
Intervention(s): All blastocysts underwent rebiopsy and preimplantation genetic testing for aneuploidy on the PGTseq platform.
Main Outcome Measure(s): Partial concordance rate per embryo, total concordance rate per embryo, and total concordance rate per chromosomal event.
Result(s): An initial TE biopsy result of euploidy or whole chromosome aneuploidy was reconfirmed in >99% of rebiopsied samples, affirming that meiotic errors are manifested in almost the entire embryo. In contrast, results of whole chromosome or segmental mosaicism were confirmed in 15%–18% of subsequent rebiopsies, suggesting that mitotic events are only sporadically seen throughout the embryo. Segmental aneuploidy was confirmed in 56.6% of rebiopsied samples, identifying a mixed meiotic and mitotic etiology for such abnormalities.
Conclusion(s): A euploid or aneuploid result on the PGTseq platform is highly concordant with the rest of the embryo's ploidy status. The rarer confirmation of whole chromosome mosaic and segmental mosaic results suggest that these mosaics are suitable for embryo transfer. Segmental aneuploidy, with higher concordance rates throughout the embryo, may represent a different biologic etiology compared to mosaic embryos. (Fertil Steril® 2021;■:■–■. ©2021 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, mosaicism, segmental abnormality, preimplantation genetic testing, next-generation sequencing

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Preimplantation genetic testing for aneuploidy (PGT-A) has become one of the most used adjuncts of in vitro fertilization treatment, because aneuploidy remains among the most common causes of implantation failure and miscarriage (1). In the early stages of PGT-A, cleavage stage embryos underwent biopsy of one to two cells and could quantify only the number of a limited number of chromosomes with the use of fluorescence in situ hybridization (2). Over time, embryo culture and PGT-A technology have progressed to the point where next-generation sequencing (NGS) can provide comprehensive chromosomal screening of a blastocyst biopsy consisting of approximately 4–5 cells from the trophectoderm (TE) (3).

As NGS technology has increased in resolution and sensitivity, additional errors have been identified; largely in the form of whole chromosome mosaicism and segmental abnormalities (4, 5). With some research reporting as many as 25% of embryos demonstrating evidence of mosaicism, determining the clinical significance of whole chromosome mosaicism (WCM) and segmental errors has emerged as one of the more challenging aspects of PGT-A interpretation. The high resolution of NGS also allows the detection of subchromosomal or segmental abnormalities with greater sensitivity compared to previous methods, introducing an entirely new category of results with unclear significance (6, 7). Some studies have questioned the ability of PGT-A from a single TE biopsy to reflect the chromosomal status of the remainder of the embryo, and claimed that the uncertainty of mosaic results renders PGT-A inaccurate or even detrimental (8). However, other studies have found PGT-A to be highly accurate and predictive of clinical outcomes (9–11).

In addition to the prognostic value gained from nonselection studies, the analytic value of the PGT-A technology itself must be assessed. Embryo rebiopsy can answer this question by gauging how often a result from a single TE biopsy corroborates with the rest of the embryo. Previous rebiopsy studies using whole genome amplification (WGA)-based NGS assays have identified varying concordance rates for findings of euploid, whole chromosome aneuploid, whole chromosome mosaic, and segmental abnormalities (12–14). What remains unanswered is whether these differences in the concordance are a result of assay artifact or whether they reflect true biologic mechanism.

If whole chromosome aneuploidy truly stems from meiotic error, it should necessarily confirm widely throughout the embryo (1). Secondarily, if mitotic error is the driving force behind mosaicism, it should follow that WCM and segmental mosaicism are less likely to be found throughout the entire embryo. The purpose of this study was to determine how concordance rates of a single TE biopsy to the rest of the embryo reflected embryonic biology; first, with regard to findings of either whole chromosome euploidy or aneuploidy, and second, for findings of WCM and segmental abnormalities.

MATERIALS AND METHODS

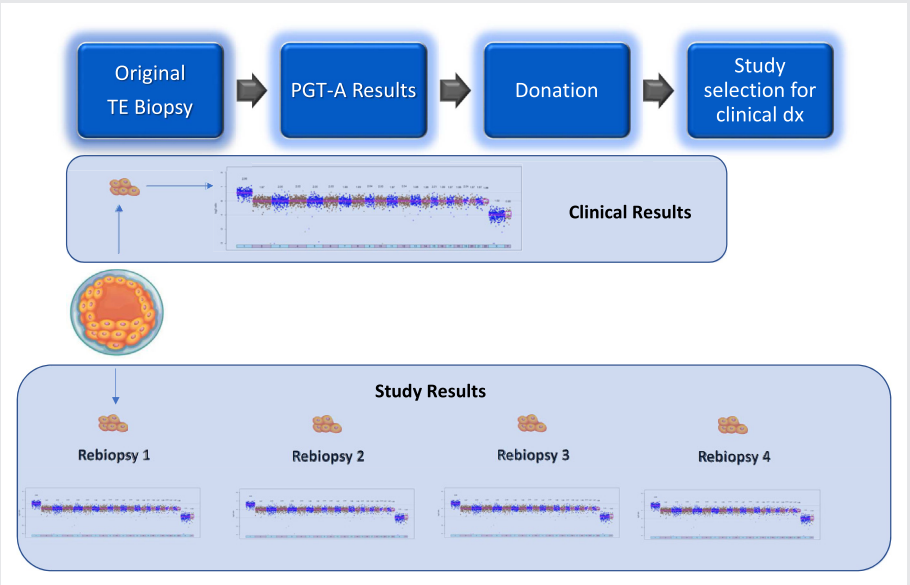
Institutional review board approval was obtained to use aneuploid (WIRB 1053149) and euploid (WIRB 1067121) embryos that had undergone PGT-A by targeted NGS and were then donated for research purposes; embryos that had undergone PGT evaluation for structural rearrangements or monogenic disorders were excluded from the analysis. The donated blastocysts were accumulated from clinical cases after standard protocols for controlled ovarian stimulation, oocyte retrieval, in vitro fertilization/intracytoplasmic sperm injection, and extended culture to the blastocyst stage. The initial TE biopsies were taken when the blastocysts were fully expanded. Thus, biopsies were taken on days 5, 6, or 7 based on the performance of the individual embryo. Biopsies were clinically tested using one of two targeted amplification followed by NGS—either NexCCS or PGTseq-A platforms. Results were reported as euploid or aneuploid, and additional presence of WCM, segmental mosaicism (segM), segmental aneuploidy (segA), or no secondary findings were also reported. A representative number of embryos from each of the aforementioned categories were then specifically chosen for analysis in this study.

NextSeq 500/550 Mid Output Kit v2.5 NGS-based PGT-A was used for TE biopsy chromosome copy number analysis per PGTseq-A protocol instructions. PGTseq-A software (PGTseq Technology, Foundation for Embryonic Competence, Basking Ridge, New Jersey) was used for bioinformatics and auto calls of the chromosome copy number as described previously (10). Although the PGTseq-A platform is capable of quantifying intermediate DNA copy number for mosaic results, we do not report levels of mosaicism, only its presence or absence; this reporting system is because of the fact that such metrics are often influenced by the number of cells in the submitted TE biopsy.

After TE biopsy, the blastocysts were vitrified and stored with the intention to warm any euploid embryos for subsequent frozen embryo transfer. All embryos in this study were donated to research by consenting patients. Donated blastocysts were warmed individually as per a standard protocol for vitrified embryos (Vitrification Thaw kit; Irvine Scientific, Santa Ana, CA). These embryos were then incubated at 37.0°C at 5.0% CO_2 until appropriate blastocyst expansion was observed. Clinical-sized rebiopsies were then performed on these blastocysts, yielding four subsequent pieces (Fig. 1). As the goal of this study was to identify the ability of the initial TE biopsy to predict any part of the entire embryo, TE or inner cell mass (ICM), the ICM was not labeled separately, but was instead simply quantified as another rebiopsy.

All rebiopsied samples were placed in 2 μL of PGTseq loading buffer in Eppendorf polymerase chain reaction tubes and stored at −4°C. The samples were tested according to the same PGTseq protocol used for clinical samples at the Foundation for Embryonic Competence (Basking Ridge, NJ). Preimplantation genetic testing for aneuploidy results of all rebiopsied samples were then reported by a single experienced reviewer who was blinded to the initial TE result. As with the initial TE biopsies, the rebiopsy results reported first as euploid or aneuploid for the primary result, as well as the presence of secondary findings; WCM, segM, segA, or none of the above (Supplemental Figures 1–5). The term WCM was defined as an intermediate DNA whole chromosome

FIGURE 1



Study workflow. PGT-A = Preimplantation genetic testing for aneuploidy; TE = trophectoderm.

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copy number. A segM abnormality was defined as an intermediate DNA copy number in only a segment of a chromosome. A segA abnormality was defined as a monosomic or trisomic DNA copy number variation in only a segment of a chromosome. The PGT-A results of the rebiopsied samples were then unblinded and compared with the initial clinical TE result by a separate reviewer.

Event concordance was determined according to five categories: euploid, aneuploid, WCM, segM, segA. Additionally, because of the presence of >1 abnormality in an embryo even within the same category (e.g., >1 aneuploid chromosome, or >1 secondary finding in the same category), concordance rates of aneuploidy and secondary findings were determined per chromosomal event and per embryo. Analysis was performed to determine the rate of total chromosomal confirmation; at least one subsequent rebiopsied sample confirmed the initial event; and all of the subsequent rebiopsied samples confirmed the initial event. Fisher's exact test was used for comparisons of concordance rates. It was expected that embryos with a meiotic error would have a high degree of confirmation throughout the embryo, and that embryos with mitotic errors would have a lower degree of confirmation in the rest of the embryo.

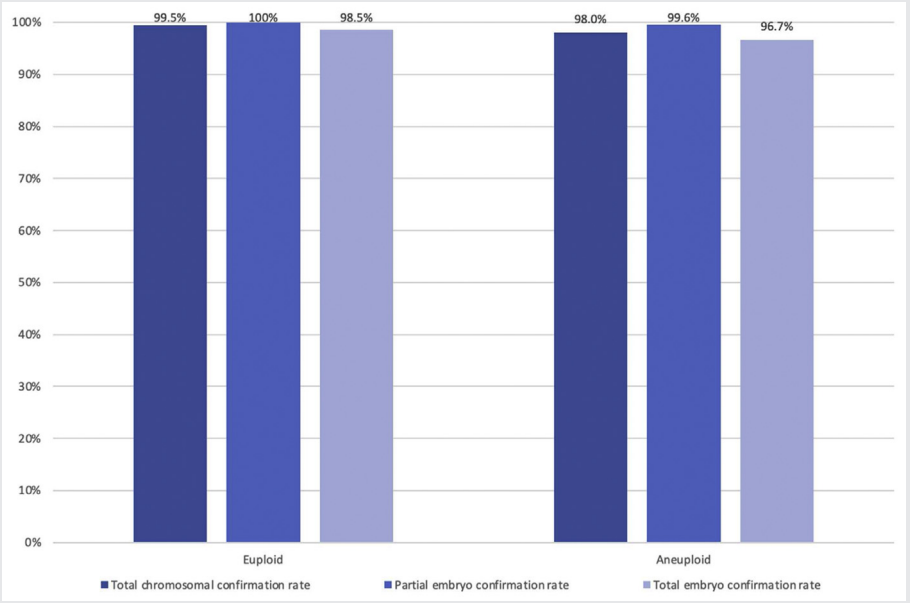
RESULTS

A total of 300 blastocysts were donated for this study; 113 of these embryos were characterized initially as euploid and 163 embryos possessed at least one whole chromosome aneuploidy. An additional 24 embryos did not have any whole chromosome aneuploidy but possessed at least one secondary finding of WCM, segM, or segA on initial TE biopsy; 147 of the aneuploid embryos also had at least one secondary finding identified. The rate of amplification failure was <0.1% of all rebiopsied samples; 1.6% of all rebiopsied samples were deemed "no call." Reasons for a "no call" result included discordant findings between DNA copy number and single nucleotide polymorphism B-allele frequency and elevated median absolute pairwise difference scores, which reflects elevated noise in chromosomal results (15).

Assessment of Whole Chromosome Concordance Rates

The 137 embryos without any whole chromosome aneuploidy yielded 548 rebiopsied samples for analysis. These rebiopsied embryos were considered as concordant when they had the same result as the initial TE biopsy (i.e., same sex and no whole chromosome aneuploidy identified). Of the 548 biopsies, 545 confirmed the initial TE result of no whole chromosome aneuploidy, with a per-biopsy concordance rate of 99.45% (Fig. 2). All of the 137 embryos had at least one subsequent rebiopsied sample confirm the initial euploid result; and 135 embryos confirmed the initial euploid TE result in all rebiopsied samples with a total embryo concordance rate of 98.54%. The discrepant samples were because of mosaicism; one embryo had one (out of four) rebiopsied sample with a whole chromosome aneuploidy and a second embryo had two (out of four) rebiopsied samples with two separate whole chromosome aneuploidies (Table 1). DNA fingerprinting was performed in the above instances to ensure that the discrepant biopsies came from the same embryo and that the differences were not because of labeling errors.

FIGURE 2



Euploid and aneuploid diagnosis confirmation rate.

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The 163 aneuploid embryos yielded 652 rebiopsied samples available for analysis. Because many embryos possessed >1 whole chromosome aneuploidy in the initial TE biopsy, there were a total of 241 independent aneuploid chromosomes initially observed in the 163 embryos. After these aneuploid embryos underwent rebiopsy, a total of 964 aneuploid chromosomes were expected of the rebiopsied samples (Fig. 2). Rebiopsied aneuploid embryos were determined concordant when they had the same exact aneuploid chromosome as the initial TE biopsy (i.e., same sex and same whole chromosome aneuploidy identified); an aneuploid result in a different chromosome would not be considered as concordant. Of the expected 964 aneuploid chromosomes, 944 confirmed, with a concordance rate of 97.9%. On a per embryo basis, 240 of 241 (99.59%) initial aneuploid chromosomes confirmed in at least one subsequent rebiopsied sample, and 234 of the 241 (97.1%) chromosomes confirmed in the entire embryo. There was no evidence of reciprocal whole chromosome aneuploidies among multiple biopsies. The six of seven events in which the initial TE aneuploid result was only partially confirmed in subsequent rebiopsied samples may be evidence of true embryonic mosaicism (Table 1).

The positive predictive value of this PGTseq platform to detect an aneuploid event in the entire remaining embryo was 97.1% (234 confirmed aneuploid chromosomes throughout the whole embryo divided by a total of 241 chromosomes initially called aneuploid) and the negative predictive value was 98.5% (135 confirmed embryos without whole chromosome aneuploidy in the whole embryo divided by a total 137 embryos initially negative for whole chromosome aneuploidy). The high reproducibility seen in embryos with whole chromosome aneuploidy, or lack thereof, demonstrates that the meiotic events of aneuploidy or euploidy can be seen accurately throughout the entire embryo in PGT-A by PGTseq.

Reproducibility of WCM

Of the initial embryo cohort with euploid or aneuploid results, 69 embryos also had a total of 87 WCM chromosomes on initial TE biopsy. A total of 276 rebiopsied samples resulting in 348 total expected WCM chromosomes were evaluated. Rebiopsied WCM chromosomes were classified as concordant when the initial WCM chromosome was noted to confirm as either a WCM gain or loss in the subsequent rebiopsy; to qualify as a confirmatory result, the rebiopsied abnormality had to be in the same chromosome as the initial result. A total of 57 of an expected 352 total WCM events confirmed, with a concordance rate of 16.19% (Fig. 3). When analyzed per embryo, at least one subsequent rebiopsied sample confirmed the initial WCM TE result in 34 of the initial 87 (39.08%) chromosomes. Interestingly, only 2 of the 87 chromosomes confirmed in all subsequent rebiopsied samples with a total embryo concordance rate of 2.29%, highlighting the rarity of a mosaic event seen throughout an entire embryo. A Fisher's exact test calculated among all five categories of PGT-A result for the study found differences in chromosomal confirmation to be statistically significant ($P<.01$).

The lower reproducibility of WCM events throughout the embryo corroborates the dispersed nature of most mitotic error; that it is often seen in another part of the embryo, but seldom in every subsequent biopsy.

TABLE 1

Embryos with discordant rebiopsied results for whole chromosome euploidy and aneuploidy

	Initial result			Rebiopsy result		
Embryono.	Sex	Aneuploidy	2° Finding	Sex	Aneuploidy	2° Finding
E73	XX			XX		WCM +13,16
E73	XX			XX	+13, +16	
E73	XX			XX		WCM -13, -16
E73	XX			XX	+13, +16	
E137	XX		7q31.31q36.3(120546714_156114158)x2~3	XX		WCM -15
E137	XX		7q31.31q36.3(120546714_156114158)x2~3	XX	+15	
E137	XX		7q31.31q36.3(120546714_156114158)x2~3	XX		WCM +15
E137	XX		7q31.31q36.3(120546714_156114158)x2~3	XX		WCM +15
A12	XX	+3,5,6	mos(14:1.69)	XX	+3,+6	WCM +5
A12	XX	+3,5,6	mos(14:1.69)	XX	+3,5,+6	
A12	XX	+3,5,6	mos(14:1.69)	XX	+3,+5,+6	
A12	XX	+3,5,6	mos(14:1.69)	XX	+3,5,+6	
A16	XY	-16,18	mos(17:1.48)	XY	-16	
A16	XY	-16,18	mos(17:1.48)	XY	-16,+18	
A16	XY	-16,18	mos(17:1.48)	XY	-16,-18	WCM -16, -17,18
A16	XY	-16,18	mos(17:1.48)	XY	-16	WCM -17, WCM -18, WCM -X, WCM -Y
A20	XX	+3	mos(4:1.63,15:1.57)	XX		WCM +3
A20	XX	+3	mos(4:1.63,15:1.57)	No Call		
A20	XX	+3	mos(4:1.63,15:1.57)	XX		
A20	XX	+3	mos(4:1.63,15:1.57)	XX		WCM +3, SegM -16
A28	XY	-7,18	mos(19:1.65)	XY	-7, -18	
A28	XY	-7,18	mos(19:1.65)	XY	-7,-18	
A28	XY	-7,18	mos(19:1.65)	XY	-7,-18	
A28	XY	-7,18	mos(19:1.65)	XY	-7,-18	
A54	XY	-16	3p26.3p22.3(760941_33365000)x1	XY	-16	
A54	XY	-16	3p26.3p22.3(760941_33365000)x1	XY		SegA -16, SegM-16
A54	XY	-16	3p26.3p22.3(760941_33365000)x1	XY		WCM -16
A54	XY	-16	3p26.3p22.3(760941_33365000)x1	XY	-16	SegA SegM 3, SegM -12
A91	XY	-13	segA(12:106600663-131664438:3.0)	XY		WCM -13, SegM -12
A91	XY	-13	segA(12:106600663-131664438:3.0)	XY	-13	
A91	XY	-13	segA(12:106600663-131664438:3.0)	XY	-13	SegM +12
A91	XY	-13	segA(12:106600663-131664438:3.0)	XY	-13	
A122	XY	-11	4q13.2q35.2(67585582_189997356)x2~3	XY		WCM -11
A122	XY	-11	4q13.2q35.2(67585582_189997356)x2~3	XY	-11	
A122	XY	-11	4q13.2q35.2(67585582_189997356)x2~3	XY	-11	
A122	XY	-11	4q13.2q35.2(67585582_189997356)x2~3	XY		WCM -11

Note: mos = mosaic; segA = segmental aneuploidy; segM = segmental mosaicism; WCM = whole chromosome mosaicism.

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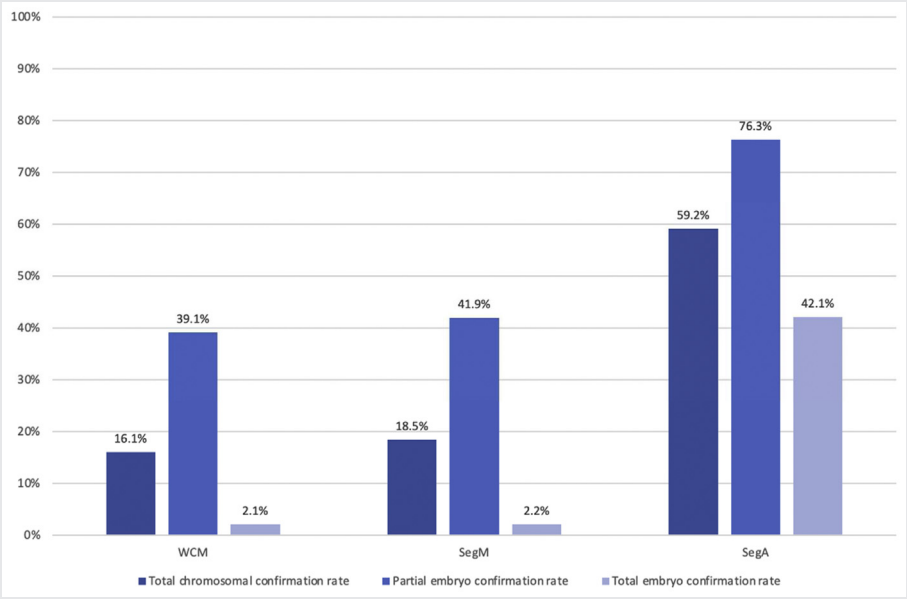
Reproducibility of Segmental Mosaicism

Eighty-five embryos with either an initial euploid or aneuploid result had an additional 93 segM chromosomes on initial TE biopsy. A total of 340 rebiopsied samples resulting in 372 total expected segM chromosomes were assessed. Rebiopsied segM chromosomes were classified as concordant when the initial segM chromosome was noted to confirm as any segmental event (i.e., segM or segA) in a subsequent rebiopsied sample in the same chromosome. A total of 69 of an anticipated 372 segM chromosomes confirmed, generating a concordance rate of 18.55%, slightly higher than the event confirmation rate seen in WCM rebiopsies (Figure 3). Per embryo, 39 of 93 (41.94%) TE segM chromosomes confirmed in at least one subsequent rebiopsied sample, and 2 of the 93 chromosomes confirmed in all subsequent rebiopsied samples with a total embryo concordance rate of 2.15%, similar to the concordance rate seen in WCM biopsies ($P=.10$). The similarity in concordance rates between segM and WCM events may suggest their biologic etiologies to be the same.

Reproducibility of Segmental Aneuploidy

Seventy-five embryos with either an initial euploid or aneuploid result had a total of 76 segA chromosomes reported on initial TE biopsy. A total of 300 rebiopsied samples resulting in 304 total expected segA chromosomes were analyzed. Rebiopsied segA results were classified as concordant when the initial segA chromosome confirmed as any segmental event (i.e., segM or segA) in a subsequent rebiopsied sample in the same chromosome. A total of 180 of an expected 304 total segA chromosomes confirmed, with a concordance rate of 59.21% (Fig. 3). Per embryo, 58 of the initial 76 (76.32%) TE segA chromosomes reconfirmed in at least one subsequent rebiopsied sample, and 34 of the 76 confirmed in all subsequent rebiopsied samples with a total embryo concordance rate of 42.11%, significantly higher than those seen in WCM or segM events ($P<.01$) and significantly lower than seen in euploid or whole chromosome aneuploid events ($P<.01$). The confirmation rates of segA abnormalities may be explained by a combination of mitotic and meiotic etiologies.

FIGURE 3



Whole chromosome mosaic, segmental mosaic, and segmental aneuploid diagnosis confirmation rate. SegA = segmental aneuploidy; SegM = segmental mosaicism; WCM = whole chromosome mosaicism.

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DISCUSSION

This study identifies the striking differences among the concordance rates of euploid or aneuploid results, vs. those of WCM, segM, and segA results, consistent with the biology of the abnormalities that are being identified. The very high concordance rates seen in euploid and aneuploid events endorse the meiotic nature of whole chromosome aneuploidy, and the ability of PGT-A to correctly identify any errors consistently. Critically, every aneuploid confirmatory event qualified as a true monosomy or trisomy as originally identified, whereas another rebiopsy study deemed high-level mosaics in rebiopsied samples as confirmatory of aneuploidy (12). Conversely, the much lower confirmation rates of WCM and segmental results reaffirm the differing mechanisms behind meiotic errors and mitotic errors. The clear differences in concordance rates among mosaic events demonstrate the ability if PGTseq to eliminate technical noise and truly reflect the various biologic processes as they exist in the embryo.

Origins of Embryonic Error

It has been well-established that the whole chromosome aneuploidy observed in human embryos is attributed to meiotic error. The high reproducibility of euploid and aneuploid results after rebiopsy emphasizes their meiotic origin: the presence or absence of whole chromosomal error is so highly conserved because it is predicated on events that start before embryogenesis. It is expected that true euploidy or whole chromosome aneuploidy should be seen throughout the entire embryo, and the PGTseq rebiopsy results confirm these expectations.

In stark contrast to these meiotic errors is the phenomenon of embryonic mosaicism: it has been noted that WCM and segmental abnormalities may arise from either meiotic or mitotic errors, but are primarily mitotic in etiology and originate from early cell divisions after fertilization (5, 6, 16, 17). Surveillance mechanisms that monitor cell-cycle control have been proposed as being more prone to error in the early days of embryogenesis because of the rapid mitotic activity regulated by maternal RNA and proteins; consequently, an increased number of double-stranded DNA breaks unidentified by corrective mechanisms are the genesis of segmental duplications or deletions (6). This study's analysis, which separates segmental mosaic results from segmental aneuploid results, suggests mitotic error to be the common etiology of mosaicism, both whole chromosomal and segmental. The much lower concordance rates of WCM and segM events in the rest of the embryo support a mitotic etiology. The sporadic confirmation of mosaic events supports what is known about patterns of mosaicism: that it generally is scattered throughout an embryo as opposed to being in one cluster, but rarely uniformly found in every cell (17).

How then, to explain segmental aneuploidy? The fact that segA results had whole-embryo concordance rates in between whole chromosome aneuploidy and mosaicism suggests that segmental aneuploidy arises from either meiotic or mitotic events. It may be that the segA events with lower concordance rates are primarily mitotic in nature, caused by terminal imbalances that result from double-stranded DNA breaks followed by nondisjunction of an acentric chromosomal

segment (18). In contrast, the segA events with higher concordance rates may be evidence of meiotic origin; nonallelic homologous recombination in particular represents one such mechanism demonstrated as a cause of segA events in meiosis (19). Concern has also been raised that the segA results could be due to technical error: namely, the use of WGA may generate an artifact that could be interpreted as a segA error, although this study used a targeted NGS platform (20). Further work must be done to elucidate the mechanisms behind subchromosomal aneuploidy while ensuring that the results are attributed to biologic events as opposed to assay artifacts (4).

Clinical Consequences

One of the greatest concerns regarding PGT-A is its potential to misdiagnose truly euploid and mosaic embryos as aneuploid, and vice versa. This analysis finds that the incidence of such mosaicism with regard to a euploid or aneuploid call is very rare. Additionally, the significance of whole chromosome mosaicism reconfirming is likely to be negligible, because truly "uniform mosaic" only occurred in 3% of all WCM chromosomes.

The increasing number of reports describing healthy live births after the transfer of putative mosaic embryos casts aspersions on the practice of rendering embryos ineligible for transfer based solely on a WCM result (21–23). Moreover, nonselection studies evaluating the reproductive competence of embryos with blinded PGT-A results at the time of embryo transfer found that live birth rates were equivalent to those seen in embryos classified as euploid without any WCM (11, 24).

The clinical consequences of segmental results remain controversial: some studies have identified a higher loss rate and lower sustained implantation rate in embryos with segmental results, whereas others have found them to have outcomes similar to WCM (23, 24). Based on the findings of this analysis, segM errors originate from the same mitotic etiology as WCM errors, and thus, may be similar in terms of pregnancy prognosis and outcomes. By extension, some segA abnormalities that are mitotic in origin may similarly pose limited risk to the rest of the embryo and be eligible for transfer; however, because some segA embryos of meiotic origin may affect the whole embryo, it may be prudent to deprioritize such embryos for transfer after WCM or segM embryos.

Prior studies have proposed clinical rebiopsy of an embryo with a segmental result because of these results having a lower concordance rate. The findings of this study (which has a greater number of initial segM and segA events) contradict this idea (13, 14). Segmental mosaicism events, like WCM events, are uncommonly concordant with the rest of the embryo, and segA events are often seen in at least another portion of the embryo: both scenarios render the use of a rebiopsy limited. Additionally, some studies have demonstrated that a clinical rebiopsy can negatively impact pregnancy outcomes because of the additional warming/vitrification process to which the embryo is subjected, although other publications did not find the same negative impact (25–28). The clinical significance of segA results remains to be further elucidated, but may warrant discussion with a genetic counselor; it should be noted, however, that the lower confirmation rate of segA results in this study suggests a lower positive predictive value for subsequent clinical outcomes.

Strengths and Limitations

This study's strengths lie in its large numbers of chromosomal events in each of the aforementioned categories, as well as the largest number of embryos in any rebiopsy study (29). This study also has greater clarity in what constituted a confirmatory result for subsequent rebiopsied samples. Some studies considered a confirmatory result to be merely the confirmation of the same chromosome, even if the abnormality was different (e.g., an initial WCM result that subsequently was called aneuploid on the same chromosome was considered to be a confirmation); whereas others required an identical match of karyotypes (12–14).

The blinded nature of the rebiopsied sample review was also unique: the reviewing individual had no role in selecting the embryos, nor was he aware of the initial TE results. Additionally, this is the only rebiopsy study in which all PGT-A results were run on an NGS platform with adequate clinical validation. One possible limitation of this study is the lack of separation of the ICM from the rest of the embryo. This was done intentionally, because the goal of this study was to address how an initial TE biopsy would reflect any part of the remaining embryo. From a technical perspective, it was also felt that a biopsy of solely the ICM without a single TE cell present was not possible to guarantee. Furthermore, given that PGT-A is never used clinically with a biopsy from the ICM, it was determined that the ICM should not be treated differently in the calculation of statistical concordance.

CONCLUSION

Like any diagnostic test, PGT-A remains susceptible to potential biologic and technical limitations; however, this analysis demonstrates the marked improvement in PGT technology that has been accomplished by targeted NGS. The improved precision of PGTseq allows the accurate diagnosis of meiotic and mitotic embryonic mechanisms alike. With its reduced technical error, it is clear the recurrence of mosaicism is quite rare, even more so than has been reported in other rebiopsy studies that used WGA (30). This study empowers confidence in the predictive ability of PGT-A by the PGTseq method in a single TE biopsy to reflect the rest of the embryo.

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REFERENCES

1. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001;2:280–91.

2. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod 1993;8:2185–91.
3. Schoolcraft WB, Fragouli E, Stevens J, Munné S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril 2010;94:1700–6.
4. Girardi L, Serdarogullari M, Patassini C, Poli M, Fabiani M, Caroseli S, et al. Incidence, Origin, and predictive model for the detection and clinical management of segmental aneuploidies in human embryos. Am J Hum Genet 2020;106:525–34.
5. Munné S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. Fertil Steril 2017;107:1085–91.
6. Babariya D, Fragouli E, Alfarawati S, Spath K, Wells D. The incidence and origin of segmental aneuploidy in human oocytes and preimplantation embryos. Hum Reprod 2017;32:2549–60.
7. Lai HH, Chuang TH, Wong LK, Lee MJ, Hsieh CL, Wang HL, et al. Identification of mosaic and segmental aneuploidies by next-generation sequencing in preimplantation genetic screening can improve clinical outcomes compared to array-comparative genomic hybridization. Mol Cytogenet 2017;10:14.
8. Gleicher N, Metzger J, Croft G, Kushnir VA, Albertini DF, Barad DH. A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use. Reprod Biol Endocrinol 2017;15:33–40.
9. McCoy RC, Demko ZP, Ryan A, Banjevic M, Hill M, Sigurjonsson S, et al. Evidence of selection against complex mitotic-origin aneuploidy during preimplantation development. PLoS Genet 2015;11:e1005601.
10. Tiegs AW, Tao X, Zhan Y, Whitehead C, Kim J, Hanson B, et al. A multicenter, prospective, blinded, nonselection study evaluating the predictive value of an aneuploid diagnosis using a targeted next-generation sequencing–based preimplantation genetic testing for aneuploidy assay and impact of biopsy. Fertil Steril 2021;115:627–37.
11. Capalbo A, Poli M, Rienzi L, Girardi L, Cimadomo D, Benini F, et al. A prospective double-blinded non-selection trial of reproductive outcomes and chromosomal normalcy of newborns derived from putative low/moderate-degree mosaic IVF embryos. medRxiv. In press.
12. Sachdev NM, McCulloh DH, Kramer Y, Keefe D, Grifo JA. The reproducibility of trophectoderm biopsies in euploid, aneuploid, and mosaic embryos using independently verified next-generation sequencing (NGS): a pilot study. J Assist Reprod Genet 2020;37:559–71.
13. Navratil R, Horak J, Hornak M, Kubicek D, Balcova M, Tauwinklova G, et al. Concordance of various chromosomal errors among different parts of the embryo and the value of re-biopsy in embryos with segmental aneuploidies. Mol Hum Reprod 2020;26:269–76.
14. Victor AR, Griffin DK, Brake AJ, Tyndall JC, Murphy AE, Lepkowsky LT, et al. Assessment of aneuploidy concordance between clinical trophectoderm biopsy and blastocyst. Hum Reprod 2019;34:181–92.
15. Jalas C, Seli E, Scott RT. Key metrics and processes for validating embryo diagnostics. Fertil Steril 2020;114:16–23.
16. Taylor TH, Gitlin SA, Patrick JL, Crain JL, Wilson JM, Griffin DK. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum Reprod Update 2014;20:571–81.
17. Vera-Rodriguez M, Rubio C. Assessing the true incidence of mosaicism in preimplantation embryos. Fertil Steril 2017;107:1107–12.
18. Vanneste E, Voet T, Caignec CL, Ampe M, Konings P, Melotte C, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med 2009;15:577–83.
19. Carvalho CM, Lupski JR. Mechanisms underlying structural variant formation in genomic disorders. Nat Rev Genet 2016;17:224–38.
20. Treff NR, Franasiak J. Detection of segmental aneuploidy and mosaicism in the human preimplantation embryo: technical considerations and limitations. Fertil Steril 2017;2013:624–30.
21. Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. Fertil Steril 2019;111:280–92.
22. Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, et al. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. Hum Genet 2017;136:805–19.
23. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril 2021;115:1212–24.
24. Tiegs AW, Tao X, Zhan Y, et al. Transfer outcomes of embryos with preimplantation genetic testing for aneuploidy (PGT-A) diagnoses of undetermined reproductive potential: results from a prospective, blinded, multi-center non-selection study. Fertil Steril 2020;114:e32.
25. Neal SA, Sun L, Jalas C, Morin SJ, Molinaro TA, Scott RT Jr. When next-generation sequencing-based preimplantation genetic testing for aneuploidy (PGT-A) yields an inconclusive report: diagnostic results and clinical outcomes after re biopsy. J Assist Reprod Genet 2019;36:2103–9.
26. Cobo A, Castello D, Vallejo B, Albert C, Santos JMdl, Remohi J. Outcome of cryotransfer of embryos developed from vitrified oocytes: double vitrification has no impact on delivery rates. Fertil Steril 2013;99:1623–30.
27. Bradley CK, Livingstone M, Traversa MV, McArthur SJ. Impact of multiple blastocyst biopsy and vitrification-warming procedures on pregnancy outcomes. Fertil Steril 2017;108:999–1006.
28. Taylor TH, Patrick JL, Gitlin SA, Wilson JM, Crain JL, Griffin DK. Outcomes of blastocysts biopsied and vitrified once versus those cryopreserved twice for euploid blastocyst transfer. Reprod Biomed Online 2014;29:59–64.
29. Marin D, Xu J, Treff NR. Preimplantation genetic testing for aneuploidy: a review of published blastocyst reanalysis concordance data. Prenat Diagn 2021;41:545–53.
30. Wu L, Jin L, Chen W, et al. The true incidence of chromosomal mosaicism after preimplantation genetic testing is much lower than that indicated by trophectoderm biopsy. Hum Reprod 2021;36:1691–701.