PGT-A COMBINING COPY NUMBER VARIATION AND ALLELIC BALANCE CONFIRMS WHOLE CHROMOSOME AND SEGMENTAL MOSAICISM IN PREIMPLANTATION EMBRYOS

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Background: Mosaic embryos present a challenging clinical decision-point. Numerous studies support reduced reproductive success following mosaic transfer while cases of viable and healthy live births have also been observed from mosaic embryos (1). PGT-A results indicate a mosaic occurrence rate of 5-25% (2) with differences in analysis methods, sample variability, and data interpretation contributing to the discrepancy. This testing and classification uncertainty blurs true clinical outcomes and makes reliable assessment of transfer options difficult. Most PGT-A platforms are limited to detecting mosaicism by either allelic balance from single nucleotide polymorphisms (SNPs) *or* normalized copy number variation (CNV) across the genome. A method combining CNV and SNP data could reduce misclassification and provide more confident PGT-A results for clinicians when considering the transfer of mosaic embryos.

Objective: The primary objective of this study was to establish the utility of a PGT-A method leveraging both copy number variation and allelic balance for detecting whole chromosome and segmental mosaic aneuploidy. A second objective was to survey a large cohort of preimplantation embryos for the distribution of mosaic length, degree and complexity.

Materials and Methods: Approximately 45K de-identified samples submitted for PGT-A were used in this study. Trophectoderm biopsies were amplified using primary template-directed amplification (PTA, 2), target-enriched and sequenced to obtain deep, high-density genome-wide data. A platform-specific bioinformatics pipeline was then used to identify and confirm mosaic regions based on *both* normalized CNV and allelic balance.

Results: The method generated a large volume of SNP data for each sample, averaging 108K quality-filtered SNPs per genome (~33 SNPs/mb) at an average depth of 81 reads/SNP. Initial CNV analysis identified a subset of embryos with a single whole chromosome (1277) or segmental (1930) mosaic region. In both scenarios, mosaicism was confirmed by allelic balance in 65% of regions. Whole chromosome mosaicism was altered to euploid or full aneuploid in 17% and 18% of regions respectively, and 25% and 10% of segmental mosaic regions, respectively. Overall, 7.7% of embryos were classified as mosaic (50.0% Euploid, 40.7% Aneuploid, 1.6% Unclassified), with 5.0% and 2.7% showing high and low-level mosaicism, respectively. Complex mosaic aneuploidy (3+ regions) was observed in approximately 0.6% of samples. No difference was observed in baseline adjusted noise across sample classifications.

Conclusions: This study showed good relative concordance between SNP-based and coverage-based methods in identifying mosaic regions while highlighting the utility of a combined pipeline to generate higher confidence and reduce misclassification. The uniform noise level among all classified samples confirms that mosaicism is unlikely a platform artifact or the result of sample variability. This study suggests a relatively low but significant mosaic occurrence rate and emphasizes the need for accurate detection to further clarify clinical outcomes of mosaic transfer.

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References: 1. Viotti et al., Fertil. Steril., 2021, May;115(5):1212-12224. 2. Xiao, M et al., J Mol Diagn. 2021 Jun;23(6):710-718. 2. Gonzalez-Pena, V. et al., PNAS. 2021, Vol. 118: No. 24