The distribution of high and low-level mosaicism and maternal age: lessons and hesitations

The goal of embryo diagnostics is to determine whether or not a given blastocyst has reproductive and developmental potential. Today, >40% of in vitro fertilization cycles in the United States use preimplantation genetic testing for aneuploidy (PGT-A) to minimize the risk of genetically abnormal pregnancy or pregnancy failure. Next-generation sequencing (NGS)-based platforms for PGT-A have become an integral component of current assisted reproductive technology protocols. The advent of the NGS technology has allowed enhancement in throughput, efficiency, and the capability to perform highly detailed analyses; however, it provides challenges with new diagnoses of unknown clinical significance. Embryonic aneuploidy errors arising from a mitotic origin resulting in different cell lineages are believed to be one of the etiologies of mosaicism. In cases where mosaicism is diagnosed, this additional information provides a challenge to those responsible for the interpretation of these results. Embryos deemed mosaic do not seem to have the same "all or nothing" effect as euploid vs. aneuploid embryos. The question of whether mosaic embryos should be transferred continues to be highly controversial because the developmental and phenotypic outcomes of mosaicism remain poorly understood.

The effect of age on whole chromosomal aneuploidy has been well established. In a large retrospective study evaluating comprehensive chromosomal screening of >15,000 trophectoderm biopsies, both younger and older age groups showed higher rates of aneuploidy as well as higher complexity of aneuploid errors with increased frequency of multiple chromosomal errors seen per embryo in both age groups (1). The STAR multicenter randomized control trial interestingly demonstrated a significant increase in ongoing pregnancy rate per embryo transfer in the PGT-A group in the subgroup of women aged 35–40 years compared with the embryo morphology group (2), highlighting the role of embryonic diagnostics in at-risk age groups at both extremes of age. Knowing this about whole chromosomal aneuploidy, how should providers move forward without the clarity of a definitive aneuploid diagnosis?

In their study to determine how mosaicism varies across patient-specific variables and clinics, Armstrong et al. (3) demonstrated that mosaicism is overall higher in patients aged ≥35 years; however, the complexity of errors increases with advancing reproductive age. Although this latter finding complements previous data demonstrating similar trends in whole chromosomal aneuploidy, it is important to consider true signal vs. technical noise. Moreover, it is important to highlight that the investigators failed to account for embryos that were both aneuploid and mosaic. Given that older women are more likely to have aneuploid embryos, this may have obscured the true rate of mosaicism in embryos from older patients.

The investigators also reference significant variations in lab thresholds for defining an embryo with mosaic results. It is also imperative to consider variations in the technique when evaluating this multicenter study, which raises the question of differences in trophectoderm biopsy protocols and embryologists' technical and embryo grading experience. Furthermore, it is important to consider whether the PGT-A assay has undergone validation with clinical samples. Even studies investigating the dissimilarity in euploid blastocyst rate and live birth rate have demonstrated significant variation in the outcomes between four PGT-A laboratories (4). Although the study of Armstrong et al. (3) limits the performer of PGT-A to one single entity, further studies are needed to compare the accuracy of NGS mosaic calls across multiple independent laboratories that are responsible for their own validation.

As for the findings regarding subsequent cycles, the investigators provide a clinical counseling point for patients who may be concerned about an increased risk of mosaicism in future cycles. It is also proposed that the clinical significance of mosaicism is dependent on the maternal age, reflecting an increased hesitation to reassure women of advanced reproductive ages on mosaic results. The investigators report a 51.8% increase in available embryos if low- and high-level mosaics in the >42-year-old age group were to be considered for use, which excludes the mosaic complex abnormal group. The decision for clinicians to deem one of these embryos suitable for transfer, particularly in the high-level group, should be considered with caution because the level of mosaicism is reported on a sliding scale. Although previous literature demonstrates poorer pregnancy outcomes in more complex high-level mosaics compared with segmental mosaics, further studies may elucidate the association between copy number count percentage and pregnancy outcomes. Ultimately, there are now data to suggest that the transfer of low and medium-grade mosaic embryos can result in similar live birth rates without an increased risk of miscarriage (5). Although the study by Armstrong et al. (3) adds to the body of knowledge surrounding the prevalence of mosaic embryos, a careful scrutinization of the data might lead one to ask if we should even care.

Biologic explanation regarding the nature of mosaicism starts with understanding where the prevalence lies. In light of Armstrong et al.'s (3) findings of higher complexity mosaicism with increasing maternal age, the question still lies of what is our threshold to use one of the embryos for transfer? Is there utility in rebiopsy before making that decision in cases of complex results, particularly in age groups where the availability of autologous embryos is limited? Furthering our knowledge surrounding these clinical scenarios across the female age spectrum can aid in the development of clinical guidelines surrounding mosaic embryo transfer.

Stephanie Willson, M.D.
Thomas Molinaro, M.D.
IVIRMA New Jersey, Basking Ridge, New Jersey

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REFERENCES

1. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014;101:656–63.e1.
2. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril 2019;112:1071–9.e7.
3. Armstrong A, Kroener L, Miller J, Nguyen A, Kwan L, Quinn M. The nature of embryonic mosaicism across female age spectrum: an analysis of 21,345 preimplantation genetic testing for aneuploidy cycles. F&S Reports 2023;4:256–61.
4. Bardos J, Kwal J, Caswell W, Jahandideh S, Stratton M, Tucker M, et al. Reproductive genetics laboratory may impact euploid blastocyst and live birth rates: a comparison of 4 national laboratories' PGT-A results from vitrified donor oocytes. Fertil Steril 2023;119:29–35.
5. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet 2021;108:2238–47.