Reflections on preimplantation genetic testing for aneuploidy and mosaicism: how did we get here, and what does it mean clinically?

Preimplantation genetic testing for aneuploidy (PGT-A) has become a mainstay therapy to evaluate embryo ploidy in IVF. PGT began in the 1990s with sexing human preimplantation embryos, polar body analysis, and fluorescent in situ hybridization for Mendelian diseases and aneuploidy testing and later for chromosomal translocations. More advanced genetic technologies emerged in 2009 with microarrays using comparative genomic hybridization (aCGH) and in 2013 with next-generation sequencing (NGS). With these technologies, we can reliably identify the presence of all 24 chromosomes in the small numbers of cells obtained from trophectoderm biopsies of blastocysts. Over 90% of IVF centers do trophectoderm biopsies of blastocyst embryos for PGT-A. With this approach, one euploid embryo can be transferred, resulting in significantly improved pregnancy rates (70%–80%) with low multiple gestation rates and low miscarriage rates. In addition, this newer technology (aCGH or NGS) has the ability to reliably detect different patterns of chromosomal abnormalities including whole chromosome aneuploidies (i.e., trisomy 21), segmental aneuploidies (i.e., cri du chat syndrome, 5p deletion), mosaicism, and segmental mosaicism. Thus, the genetic report on PGT-A has become more complex. Now we have to contend with mosaics and even different types of mosaics. How do we counsel our patients about these chromosomal anomalies? Is there a clinical difference in outcomes? Segmental mosaicism detection is the newest advancement in the PGT field. In this current issue of *Fertility and Sterility*, Zore et al. (1) demonstrate interesting findings when transferring segmental mosaic embryos regarding outcomes (including live birth rates and spontaneous abortion rates). Let's take a step back and define the types of mosaicism.

What Does This All Mean?

Mosaic embryos can be further classified into three categories: whole chromosomal mosaicism, segmental mosaicism, and complex mosaicism.

Whole chromosomal mosaic embryos most commonly occur from postzygotic errors, where some cells have a normal number of chromosomes and other cells have a different, abnormal, number of chromosomes. Therefore, a single embryo contains cells with two different populations of chromosomes. These cells may have a normal number of chromosomes, an extra chromosome (trisomy), or a missing chromosome (monosomy).

Segmental mosaicism is the term used to describe a partial chromosomal deletion or duplication in a cell. In this case, the number of chromosomes does not change. In addition, there are two different types of segmental chromosome abnormalities: segmental aneuploidy and segmental mosaicism. If the segmental abnormality is present in all the biopsied cells, it is defined as segmental aneuploidy. However, if this finding is present in only a proportion of the biopsied cells, then it is defined as segmental mosaicism. The size of the chromosome segment is at least 10 MB, and segmental errors are most commonly seen in larger chromosomes. Interestingly, unlike whole chromosomal mosaicism, no clear trends have been identified with segmental mosaicism and maternal age. Rates of segmental mosaicism vary widely, ranging from 6% to 32% in blastocyst biopsy (2, 3).

Complex mosaic embryo is defined as an embryo that contains three or more whole chromosomal abnormalities.

How Do We Counsel Our Patients About These Chromosomal Anomalies?

Counseling patients regarding euploidy and aneuploidy is largely centered on data that have shown shorter time to pregnancy intervals, lower rates of spontaneous abortion, higher implantation rates, and reduced recurrent IVF failure with the transfer of a euploid embryo. Most embryos are classified as normal (euploid) or abnormal—and these results are easy to communicate when counseling couples. However, mosaicism is different since we have a paucity of data regarding the clinical outcomes. Even identifying mosaicism relies on the technology (aCGH or NGS) to identify the chromosome copy number per cell. Per the Preimplantation Genetic Diagnosis International Society (http://www.pgdis.org/docs/newsletter_071816.html), PGT-A results with $<$20% mosaicism should be reported as euploid, those with $>$80% should be reported as aneuploid, and those 20%–80% should be reported as mosaic. Even so, mosaicism poses a significant clinical quandary. What do we do with these mosaic embryos? Transfer them or discard them? Does mosaicism impact pregnancy rates, miscarriage rates, and live birth rates? Does it increase birth defect rates? Does mosaicism resolve over time? To answer these questions we need to look at pregnancy outcomes resulting from the transfer of only mosaic embryos (and not combined with euploid embryos).

Much of what we currently know about reproductive outcomes of mosaic embryos is based on studies of whole chromosome mosaics containing chromosomal duplications (trisomies) and/or deletions (monosomies). Mosaic embryos have been reported to result in live births, initially by Greco in 2015. Using array CGH on the trophectoderm biopsies, they reported 4.8% (n = 181) mosaic rate in embryos, and there were 18 women with only mosaic embryos available for transfer, which resulted in six live births (30%) (3). Fragouli et al. (3) studied archived trophectoderm biopsies with NGS and reported reproductive outcomes separately for mosaic and euploid embryos as follows: implantation 30.1% versus 55.8%, miscarriage rate 55.6% versus 17.2%, and ongoing pregnancy 15.4% versus 46.2%, respectively (P=.003). Munné et al. (4) reported the largest series to date of 29,195 blastocyst biopsied embryos from multiple centers using NGS at Reprogenetics (Table 3 of reference 3) and reported 42.87% euploid, 19.70% aneuploid, 11.91% complex abnormal, 0.84% triploid, and 24.68% various forms of mosaicism. Munné et al. (4) also reported reproductive outcomes

with mosaic embryos and euploid embryos separately (implantation rates, mosaic 53% vs. 70% euploid embryos; miscarriage rates, mosaic 25% vs. 10% euploid). Although these reproductive outcomes for mosaics are worse than for euploids, these results are still significantly better than no transfer. So how do we counsel these patients?

Is There a Clinical Difference in Outcomes Between the Different Types of Mosaic Embryos?

All types of mosaic embryos are not created equal. Fragouli et al. (3) reported on a cohort with a large proportion of segmental mosaics, 32% (n = 14), and were therefore able to separate outcomes by mosaic type. Segmental mosaic embryos were shown to have similar reproductive outcomes as euploid embryos, with a live birth rate of 57.1% and no difference in the rates of spontaneous abortion (3). Whole chromosome mosaics were found in 68% (n = 30), and they had a significantly reduced live birth rate of 13.3% and spontaneous loss rate of 16%. These data are confounded by the fact that 40% of those classified as whole chromosome mosaics were found to actually include both whole chromosome mosaics and segmental mosaics. Complex mosaics had the lowest reproductive potential, with a live birth rate of 6.25% (3). In the Munné et al. (4) series, mosaics were subdivided by type and the investigators reported ongoing implantation rates: complex mosaics (10%) and other forms of mosaicism performed better, with similar implantation rates (41%, whole chromosome and segmental mosaics separately).

While there seems to be consensus that complex mosaics have significantly reduced reproductive potential, there exists discrepancy regarding reproductive outcomes of segmental mosaics.

The study by Zore et al. (1) addresses segmental mosaicism in an interesting way—after the transfer! In this retrospective cohort study, 327 subjects underwent 377 frozen single "euploid" ETs. Embryos were initially reported as euploid or aneuploid by high-density aCGH and the euploid embryos were transferred. Only after the embryos were transferred did the genetic lab disclose which embryos had segmental mosaicism, and these investigators followed these subjects until delivery. A total of 20 embryos out of the 377 were found to have segmental mosaicism (5.3%). The authors assessed the outcome data from these segmental mosaics. When compared with euploid embryos, segmental mosaic embryos had a lower live birth rate (30% vs. 53.8%) and increased rate of spontaneous abortion (40% vs. 18.2%). This study clearly reports reduced reproductive potential for these segmental mosaics in comparison with the euploid embryos, without confounding the data with mixed classes of mosaic embryos.

This study has the limitations of small numbers and of using high-density aCGH. In comparison with NGS, aCGH has a lower resolution for the detection of mosaicism (>40%). The Preimplantation Genetic Diagnosis International Society released revised recommendations in 2016 (http://www.pgdis.org/docs/newsletter_071816.html), encouraging clinics to shift to NGS technology since it detects mosaicism at a lower level (20%). Furthermore, Lai et al. (5) showed differences in detecting segmental mosaicism by these different techniques in prospective parallel screening of 45 blastocysts, which identified chromosomal mosaicism in 10.7% with NGS and 3.9% with aCGH and segmental aneuploidy of 10.7% with NGS and 6.7% with aCGH. The authors recognize that this is a limitation with aCGH technology in that 20% of segmental mosaicism may be artifact since only over 40% of mosaicism is reliably detectable by the aCGH technique. The most significant limitation in this study and most others is the lack of correlation with prenatal testing with amniocenteses or karyotype after delivery. All patients should still be encouraged to have prenatal testing after PGT-A. We are still learning about these genetic mechanisms and the embryo responses. It is not clear what level of self-correction occurs, whether other biologic mechanisms are involved in affecting outcomes (apoptosis, slower growing or dividing mosaic cells compared with euploid cells, or preferential allocation of cells to the trophectoderm compared with the inner cell mass), or whether these genetic findings will persist in the resulting child or impact the child's life.

Therefore, it remains challenging to counsel these patients regarding PGT-A with mosaicism since we do not know the clinical impact of the different forms of mosaicism. More studies like Zore et al. (1) are needed. If we could expand our current Centers for Disease Control and Prevention/Society for Assisted Reproductive Technology registry of IVF with additional data fields on mosaic embryos, this may help us understand these genetic findings better.

The genetic technology for PGT-A has revolutionized the practice of IVF. These technologies are likely to improve in sensitivity and resolution over time. However, the technology has outpaced our clinical understanding of its outcomes regarding mosaicism, which presents as a clinical challenge in managing patients. There are many ongoing studies that will hopefully clarify the clinical impact of chromosome mosaicism on outcomes in the near future. Until then, patients should continue to be counseled on an individual basis, offered appropriate genetic counseling, encouraged to follow up with an amniocentesis, and be informed that we still do not have enough information regarding long-term outcomes of mosaic embryos, particularly those with segmental mosaicism.

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