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Commentary

Counselling considerations for chromosomal mosaicism detected by preimplantation genetic screening

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ABSTRACT

The evolution of preimplantation genetic screening (PGS) for aneuploidy to blastocyst biopsy and more sensitive 24-chromosome screening techniques has resulted in a new diagnostic category of PGS results: those classified as mosaic. This diagnosis presents significant challenges for clinicians in developing policies regarding transfer and storage of such embryos, as well as in providing genetic counselling for patients prior to and following PGS. Given the high frequency of mosaic PGS results and the wide range of possible associated outcomes, there is an urgent need to understand how to appropriately counsel patients regarding such embryos. This is the first commentary to thoroughly address pre- and post-test genetic counselling recommendations, as well as considerations regarding prenatal screening and diagnosis. Current data on mosaic PGS results are summarized along with embryo selection considerations and potential outcomes of embryos diagnosed as mosaic.

Background

Preimplantation genetic screening (PGS) has evolved to become a routine part of many IVF cycles, enabling selection of euploid embryos for transfer. Implementation of the most recent PGS technologies has been shown to improve pregnancy rates per transfer in randomized controlled trials, meta-analyses, and case-controlled prospective studies. The increased resolution of PGS technologies has facilitated the identification of chromosomal mosaicism in preimplantation embryos (Fragouli et al., 2011; Munne et al., 2010). Mosaicism is the presence of two or more genetically distinct cell lines and may occur with regard to a variety of genetic changes including chromosomal aberrations, single-nucleotide variations or small insertions/deletions. Such changes can either go unnoticed or underlie genetic disease.

Chromosomal mosaicism may refer to the presence of two or more different abnormal cell lines (e.g. aneuploid/aneuploid), or a normal and an abnormal cell line (e.g. euploid/aneuploid). In contrast to an aneuploidy present in all cells of an embryo, which typically occurs via meiotic nondisjunction and is associated with increasing maternal age, mosaic aneuploidy may occur by three mechanisms. It is presumed that the majority of cases result from an initially euploid zygote that undergoes nondisjunction postzygotically, resulting in trisomic and monosomic cell lines. Other cases result from anaphase lag (failure of a chromosome to be incorporated into the newly-formed cell), resulting in the formation of a monosomic cell. Alternatively, an initially aneuploid embryo can undergo postzygotic loss (also by nondisjunction or anaphase lag) or duplication of a whole chromosome ('aneusomic rescue'). The specific method by which mosaicism arises can result in distinctly different outcomes.

Chromosomal mosaicism in pregnancies and livebirths has been reported for various types of cytogenetic aberrations including trisomies, monosomies, deletions, duplications and other rare alterations. Prenatally, placental mosaicism is identified in 1–2% of chorionic villus samples (CVS) while approximately 0.2% of amniocentesis samples,

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which directly examine fetal tissues, exhibit mosaic findings. The variety of clinical outcomes in these situations presents significant counselling challenges (Spinner and Conlin, 2014).

The emerging classification of embryos as mosaic can be attributed to two phenomena. First, the evolution from blastomere biopsy of cleavage stage embryos to trophectoderm (TE) biopsy of blastocysts has allowed for the examination of multiple biopsied cells (a necessity for recognition of mosaicism) instead of just a single cell. Second, genetic technologies for detecting chromosomal copy number variations in embryos have evolved from the use of fluorescent in-situ hybridization (FISH) to comprehensive 24-chromosome screening methods including quantitative polymerase chain reaction (qPCR), single nucleotide polymorphism (SNP) arrays, array comparative genomic hybridization (aCGH) and, most recently, next-generation sequencing (NGS). aCGH and NGS in particular are sensitive enough to detect low level mosaicism in an embryo biopsy, with early estimates demonstrating that such technologies may be capable of detecting mosaicism levels as low as 20% (Greco et al., 2015; Mamas et al., 2012). While the actual rate of mosaicism in blastocysts is not well-defined, when NGS is performed, preliminary data suggest that 10–30% of blastocyst TE biopsies may be diagnosed as mosaic (Fiorentino et al., 2014; Fragouli et al., 2015; Munne et al., 2016).

Additionally, PGS laboratories differ in their approaches to mosaicism detection, thresholds used, classification and reporting structure. Discrimination between euploid and aneuploid samples relies on threshold values determined by statistical averages, and embryos are diagnosed as mosaic when the result falls into an 'intermediate' range between the threshold values (Scott and Galliano, 2016). Therefore, it is possible that some biopsies contain only a single cell line (euploid or aneuploid) but fall into the borderline ('mosaic') value between normal and abnormal due to overlap between mosaic and euploid (or mosaic and aneuploid) statistical ranges (Scott and Galliano, 2016). The thresholds and ranges can vary depending on the bioinformatics used by the testing laboratory. When the euploid and aneuploid ranges are narrow, biopsies diagnosed as euploid are less likely to be false-negatives (i.e. more likely to be entirely euploid) and biopsies diagnosed as aneuploid are less likely to be false-positives (i.e. more likely to be entirely aneuploid). This is consistent with early data, which has shown that a narrower euploid range is associated with improved implantation and reduced miscarriage. However, narrower euploid and aneuploid ranges mean a wider intermediate ('mosaic') range, and therefore, a greater number of embryos are given an uncertain diagnosis. Alternatively, wider euploid and aneuploid ranges decrease the percentage of results falling into the mosaic range; however, a higher frequency of false-negative or false-positive results may occur.

Clinical significance of chromosomal mosaicism

The clinical significance of chromosomal mosaicism diagnosed by PGS is not well delineated. First, embryos may have robust mechanisms of self-correction, as suggested by data showing rates of placental mosaicism to be similar between infertile and fertile women by the time of CVS (Huang et al., 2009). Second, TE cells may not always represent the cells of the inner cell mass, and other embryonic tissues may be comprised of cell lines that differ from the biopsied cells. Finally, the distribution of abnormal cells in an embryo can vary depending on the timing of mutational events and the degree of proliferation of aneuploid versus euploid cells (Spinner and Conlin, 2014). Therefore, embryos deemed mosaic by PGS have the potential to develop into a fetus that is chromosomally normal, chromosomally abnormal, or mosaic to a lesser, greater, or similar degree to that predicted by the biopsy results (Greco et al., 2015). A summary of the possible explanations for mosaic PGS results and associated risks is provided in **Table 1**.

There is sparse data regarding the transfer of embryos diagnosed as mosaic. In the only prospective study published to date, 6/18 transferred embryos with mosaic results involving different chromosomes resulted in apparently healthy live births (Greco et al., 2015). While normal karyotype studies were documented post-natally from chorionic villi, it is not known whether mosaicism persisted throughout embryonic development, and no additional follow-up on these babies was made available. While some would encourage cautious optimism regarding long-term outcomes, data is exceptionally limited at the current time.

Preliminary data suggests that embryos identified as mosaic may have a reduced chance of implantation when compared with euploid controls (Fragouli et al., 2015). Other early data sets suggest that embryonic mosaicism may play a significant role in pregnancy loss after IVF (Grifo et al., 2015), and cytogenetic and array-based analysis of miscarriages following spontaneously-conceived pregnancies commonly reveal chromosomal mosaicism (Robberecht et al., 2009).

Table 1 – Potential explanations and associated risks for mosaic results following preimplantation genetic screening (PGS).

Explanation	Embryo composition	Risk assessment
Fully euploid biopsy falling into mosaic result range	Likely euploid	Low risk
Fully aneuploid biopsy falling into mosaic result range	Likely aneuploid	High risk of failed implantation, miscarriage or aneuploidy syndrome depending on chromosome involved
True mosaic (euploid/aneuploid) biopsy	Mosaic TE, euploid ICM	Low risk of poor outcome; however, possible risk of CPM (including IUGR) depending on chromosome involved
	Mosaic TE, aneuploid ICM	High risk of failed implantation, miscarriage, or aneuploidy syndrome depending on chromosome involved
	Mosaic TE, mosaic ICM	Largely unknown risk; dependent on chromosome involved, proportion of aneuploid cells, affected tissue types
Mosaic for two reciprocal aneuploid cells lines (i.e. monosomic/trisomic for same chromosome) OR for two or more different aneuploid cell lines, with no euploid cells	Likely aneuploid or mosaic for multiple aneuploidies, with no euploid cells	High risk of failed implantation, miscarriage, or aneuploidy syndrome depending on chromosome(s) involved

CPM, confined placental mosaicism; ICM, inner cell mass; IUGR, intrauterine growth restriction; TE, trophectoderm.

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