Diagnosis and clinical management of embryonic mosaicism

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Embryonic mosaicism occurs when two or more cell populations with different genotypes are present within the same embryo. New diagnostic techniques for preimplantation genetic screening (PGS), such as next-generation sequencing, have led to increased reporting of mosaicism. The interpretation of mosaicism is complicated because the transfer of some mosaic embryos has resulted in live births. Mosaic embryos may represent a third category between normal (euploidy) and abnormal (aneuploidy). This category of mosaic embryos may be characterized by decreased implantation and pregnancy potential as well as increased risk of genetic abnormalities and adverse pregnancy outcomes. Euploid embryos should be preferentially transferred over mosaic embryos. Genetic counseling is necessary before the transfer of a mosaic embryo is considered. Certain types of mosaic embryos should be preferentially transferred over others. Transfer of embryos with mosaic trisomies 2, 7, 13, 14, 15, 16, 18, and 21 may pose the most risk of having a child affected with a trisomy syndrome; however, the transfer of embryos with mosaic monosomies or other mosaic trisomies are not devoid of risk. Patients must be counseled about the risk of undetected monosomies or trisomies within a biopsy specimen as well as the risk of intrauterine fetal demise or uniparental disomy with the transfer of mosaic embryos. Until more data are available, patients should be encouraged to undergo another cycle to obtain euploid embryos, when possible, rather than transferring a mosaic embryo. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)

Key Words: Preimplantation genetic screening, next-generation sequencing, embryonic mosaicism

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Mosaicism within an embryo is defined as the presence of two or more cell populations with different genotypes. Early studies demonstrated mosaicism within preimplantation human embryos at the cleavage stage with the use of fluorescence in situ hybridization (FISH) of sex chromosomes (1). Embryonic mosaicism was found to result from mitotic errors occurring after fertilization, occasionally in the first cleavage but more commonly in the second or third cleavage (2). Mosaic embryos may be classified as aneuploid mosaic, where two different aneuploid genotypes exist and 100% of the cells within the embryo are abnormal, or diploid-aneuploid mosaic, where one population of the cells is euploid and the other is aneuploid. The percentage of abnormal cells within a diploid-aneuploid mosaic embryo is influenced by the cleavage stage in which the chromosomal segregation error occurs. For example, errors occurring at the time of the second cleavage may result in a greater proportion of abnormal cells than errors occurring during the third cleavage (2).

The early embryo is prone to errors of mitosis because of inactivation of the genome at fertilization. Oocyte mRNA is degraded, and genome stability is dependent on oocyte cytoplasmic transciptomes during the first three cell divisions. Embryonic genome activation does not occur until after the third cleavage stage, and some genes important for cell division are not expressed until the blastocyst stage (3). Mosaicism may develop within a diploid embryo for a variety of reasons, including anaphase lag, mitotic nondisjunction, inadvertent chromosome demolition, and premature cell division before DNA duplication (4, 5). For this reason, the detection of mosaicism among cleavage-stage blastomere biopsies is high (6). Mosaic cleavage-stage embryos left in extended culture have been shown to self-correct to euploid blastocysts in nearly 50% of cases (7). Several mechanisms may be involved in the correction of aneuploidy, including increased apoptosis of aneuploid cells, decreased division of aneuploid cells in relation to euploid cells, or preferential development of euploid cells within the inner cell mass (ICM) (8). Trisomic cell populations may self-correct by losing the extra chromosome via anaphase lag or nondisjunction (9); however, this explanation is less likely, given the low rate of detection of uniparental disomy among blastocysts (10).

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DETECTION OF MOSAICISM AND INTERPRETATION OF MOSAIC RESULTS

The rate of mosaicism within preimplantation embryos not only varies based on the stage of the embryo, but also with the chromosomal detection technique used. Preimplantation genetic screening (PGS) was initially performed with the use of FISH from a single blastomere biopsy. FISH uses fluorescent microscopy to visualize fluorescent probes hybridized, most commonly, to five chromosomes (X, Y, 13, 18, and 21). Aneuploidy detection by means of FISH is limited when probes for more than ten chromosomes are used in one sample. Mosaicism of the remaining autosomes, therefore, could not be detected. Additionally, studies on cleavage-stage embryo mosaicism are limited to discarded embryos because of the risk of embryo damage from the requirement for multiple blastomere biopsies (2). Comprehensive chromosome screening (CCS) with the use of whole genome amplification and comparative genomic hybridization (CGH) to assess all 24 chromosomes emerged as a superior method for the assessment of mosaicism. Findings confirmed that high levels of mosaicism (up to 75%) are seen in cleavage-stage embryos (6).

Given the considerable findings of mosaicism in cleavage embryos, trophectoderm (TE) biopsy of blastocysts with the use of CCS has become widely used in clinical practices worldwide. Blastocyst biopsies contain approximately four to ten TE cells (11), allowing for the detection of mosaicism in a single biopsy. Numerous studies have demonstrated the utility of array CGH (aCGH) for use in PGS (12, 13). It uses whole genome amplification to amplify embryonic DNA and reference DNA, followed by fluorescent labeling of each with two distinct colors. DNA probes, approximately 4,000 DNA markers spaced throughout the genome, are spread out on the microarray. Both sets of DNA then compete for hybridization on the microarray. Computer software analyzes the fluorescent intensities of the hybridized DNA, and calculates the copy number of reference DNA compared with embryonic DNA (14). Array CGH is used to detect whole chromosome aneuploidy, but it is not validated to detect structural chromosomal aberrations in the genome (15).

The rate of mosaicism among blastocysts with the use of aCGH is estimated to be 4.8%–32% (16–18) and may vary based on the aCGH protocols used. The ability of aCGH to detect mosaicism is dependent on the percentage of aneuploid cells within the TE biopsy specimen. Mamas et al. (2012) investigated the detection rate of aCGH on known mixtures of euploid and aneuploid (trisomic) cells. Array CGH was able to pick up mosaicism when >50% of cells were abnormal (defined as log2 ratio >0.3). Confidence intervals of log2 ratios, however, were shown to span from the upper limits of normal (euploid) to abnormal (aneuploid), demonstrating the difficulty in interpreting borderline values (19).

Another study performed by Capalbo et al. (2013) evaluated the concordance of aneuploidy results between aCGH-screened embryos and FISH reanalysis of blastocyst TE biopsy and ICM samples (17). They found that ~2% of embryos studied were diploid-aneuploid mosaic with >40% normal cell lines according to aCGH and FISH. Array CGH failed to detect diploid-aneuploid mosaicism when <25% of cells in the TE biopsy specimens were abnormal. Array CGH accurately detected all cases of mosaicism when >40% of TE biopsy samples were aneuploid. With medium-grade mosaicism (25%–40% abnormal cells), aCGH correctly identified three cases and misdiagnosed two cases. Concordance for all chromosomes was 97% (68/70 blastocysts) between TE and ICM biopsies with the use of aCGH and 100% for chromosomal complement on a per-embryo basis. The distribution of abnormal cells within the tested embryos was uniform, which was consistent with previous findings (17, 20).

Next-generation sequencing (NGS) has emerged as a new technique for PGS with the advantages of high accuracy with increased throughput and decreased cost compared with aCGH (21, 22). Multiple DNA samples may be analyzed at the same time and reports generated within 13–16 hours. The two most common platforms used for PGS are the MiSeq from Illumina and the Personal Genome Machine from Thermo-Fischer Scientific. Whole genome amplification is first performed. DNA is then lysed into fragments, and fragments are fused with an adapter and a barcode. For the MiSeq platform, a bridge polymerase chain reaction (PCR) step is performed, followed by optics-based sequencing by synthesis. After quality assurance metrics are performed, data are then analyzed with the use of BlueFuse software (Illumina). The MiSeq platform is designed to identify whole chromosome aneuploidy and mitochondrial copy number. Illumina's VeriSeq genome analysis on the MiSeq platform is designed to detect whole chromosome aneuploidy and mosaicism of $\geq$50%. The Personal Genome Machine, conversely, involves an emulsion PCR step followed by detection of hydrogen ion release by DNA polymerase during sequencing by DNA synthesis. A sensor detects the change in pH due to the release of hydrogen ions. The Torrent Browser software performs quality assurance metrics, and then data are analyzed with the use of the Ion Reporter Software. The Personal Genome Machine is designed to detect whole chromosome aneuploidy, deletions, or duplications down to a resolution of 800 kb to 1 Mb, mosaicism of $\geq$20%, and mitochondrial copy number. Both NGS platforms can be used to detect single gene mutations (15).

NGS may have a greater ability to detect mosaicism in multicellular samples, owing to its increased dynamic range in comparison to aCGH (23). A randomized blinded study comparing NGS and quantitative PCR for the detection of mosaicism with the use of mixed model aneuploidy cell lines showed that NGS is able to detect mosaicism when as few as 17% of the cells are aneuploid with 100% specificity across variable proportions of aneuploid cell mixtures. The application of custom analysis criteria, however, significantly increased the sensitivity of detecting aneuploid cell lines, but simultaneously increased the false positive rate from 0% to 33% (24). Differences in analysis criteria between laboratories may explain the different reporting rates of mosaicism in blastocyst biopsies.

With increased reporting of mosaicism with the use of NGS, the question of whether a single TE biopsy is indicative of the chromosomal complement of the entire embryo has