Current experience concerning mosaic embryos diagnosed during preimplantation genetic screening

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The concept of embryos containing multiple cell lines (mosaicism) is not new, but much attention has been paid to this concept recently owing to recent advances in molecular techniques to analyze human embryos. Mosaicism in embryos has been known and reported for some time, originally in early cleavage-stage embryos diagnosed with the use of fluorescence in situ hybridization (FISH). However, the early data have come under attack owing to the limited ability of FISH to reliably detect the actual copy number count of chromosomes as well as potential ascertainment bias of those early studies, which were all performed on already analyzed embryos found to be aneuploid. More recent molecular techniques for analyzing embryos have allowed scientists to really begin to understand mosaic embryos, and to now transfer and follow this class of embryo. Indeed, it could be said that three classes of embryos now exist after preimplantation genetic screening: euploid, aneuploid, and mosaic aneuploid. This paper attempts to bring to light the latest data on mosaic embryos and to understand how clinicians and others will deal with this issue today and in the future. Finally, an attempt is made to look to other fields of genetics to understand how this important issue can be dealt with as a group much better than any one individual group may be able to. (Fertil Steril® 2017;107:1113–9. ©2017 by American Society for Reproductive Medicine.)

Key Words: Mosaicism, preimplantation genetic screening, comprehensive chromosome screening, trophectoderm, inner cell mass

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BACKGROUND ON MOSAIC EMBRYOS

Preimplantation chromosomal mosaicism is a phenomenon characterized by the presence of a mixture of chromosomally different cell lines in an embryo (1–3). Such a phenomenon is relatively common in human preimplantation embryos (4) and may occur because of a variety of genetic changes, including chromosomal aberrations, single-nucleotide variations, and small insertions/deletions. 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 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 via a variety of mechanisms, including anaphase lag, mitotic nondisjunction, inadvertent chromosome demolition, and premature cell division before DNA duplication (3, 5–9).

The percentage of abnormal cells within a euploid/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 (1). In addition, mosaicism may be confined to a certain area of the developing embryo, this is especially true for embryos at the blastocyst stage, where cells in the trophectoderm (TE) may exhibit mosaicism and the inner cell mass (ICM) is left unaffected. In addition, cells in one section of the TE may be affected by mosaicism while the rest of the cells in the developing blastocyst are left untouched.

The impact of mosaicism on implantation and the developmental potential of embryos is not fully known, although it has been shown that some euploid/aneuploid mosaic embryos hold the potential to implant, resulting in either mosaic pregnancies (the majority of which will miscarry) (10) or in chromosomally normal pregnancies that can result in the birth of healthy babies (11). Other embryos diagnosed as mosaic during preimplantation genetic screening (PGS) may not implant or may be lost during the implantation or early pregnancy development stages (11).

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Several mechanisms have been proposed to explain the situation where embryos diagnosed as mosaic at PGS may "correct" the detected aneuploidy. These include preferential growth of the euploid cells or preferential allocation of the normal cells to the ICM (12–14). Trisomic cell populations may self-correct by losing the extra chromosome via anaphase lag or nondisjunction (15); however, this explanation is less likely, given the low rate of detection of uniparental disomy among blastocysts (16). Experimental and clinical studies (4, 17) suggest that aneuploid cells have a growth disadvantage or are eliminated by processes such as apoptosis, leading to a decline in their numbers as development progresses, ultimately resulting in a normal fetus.

A recently published mouse study shed additional light onto the embryo's ability to self-correct an inherent mosaic state of mixed euploid and aneuploid cells (18). In that study, Bolton et al. demonstrated that the fate of aneuploid cells in early embryos depends on lineage: Aneuploid cells in the fetal lineage (i.e., ICM) are eliminated by apoptosis, whereas those in the placental lineage (i.e., TE) demonstrate severe proliferative defects.

Uniformly abnormal embryos are able to implant and elicit a maternal decidual reaction, but undergo early postimplantation resorption. In contrast, mosaic embryos that contain >30% of normal euploid cells have greater developmental potential (18). Based on this model, embryos with a low proportion of aneuploid cells would have greater development potential compared with those with a higher rate of mosaicism. The developmental potential of such embryos might also be related to the specific chromosome involved in mosaicism. However, in the paper by Bolton et al., the mosaicism model was generated with a drug introducing massive chromosome abnormalities for multiple chromosome (chaotic configuration), and it remains to be determined if mosaicism for one or a few chromosomes may result in similar effects on cell survival.

Most of our knowledge on mosaic embryos is derived from studies performed at the blastocyst stage. Early data on mosaicism at the cleavage stage is probably tainted with ascertainment bias of re-biopsying embyros already diagnosed as aneuploid. Data comparing mosaicism between the TE and ICM are very limited. A small study by Fragouli et al. (13) showed 100% concordance between TE and ICM. Another study, based on single-nucleotide polymorphism (SNP) array with 51 embryos, showed 96.1% concordance between TE and ICM (19). Finally, a study by Capalbo et al. (20) showed an overall rate of mosacism of 15.7%; however, only 2.9% of the embryos represented diploid/aneuploid error.

At this time, no valid method exists to analyze the ICM from a blastocyst. Current methods biopsy the trophectoderm and use this diagnosis as a surrogate for the ploidy status of the entire embryo, including the ICM. The main purpose of comprehensive chromosome screening (CCS) is to identify aneuploidy in human blastocysts and accurately predict the chromosome complement of the ICM.

Ideally, to investigate the concordance rate between the ICM and TE, we would disaggregate the whole embryo into single cells and classify and analyze them based on where they were located, i.e., ICM versus TE. However, the challenge with this approach is the lack of a robust method to isolate single cells from a blastocyst-stage embryo.

A particular class of abnormalities that can be diagnosed during PGS are segmental aneuploidies, which affect a small part of a chromosome and can be found as either a gain (duplication) or a loss (deletion) of DNA material. A key point here is our understanding of the mechanisms involved in DNA replication. Just before a cell begins mitosis, DNA replication, or the S phase, begins. This process takes ~11–16 hours from beginning to end. The S phase of DNA replication does not begin and end at a specific time or place in the genome; it begins and ends at different starting and ending points all over the genome. Therefore, any cell analyzed during S phase may be found to have many gains and losses of chromosome material that are not going to affect the embryo and are not necessarily indicative of a mosaic embryo. When analyzing a biopsy for PGS, the cellular stage of the cell may possibly play a role in the embryo diagnosis. In a recent paper by Ramos et al. (21), they showed that a majority (73.3%) of segmental imbalances were due to chromosome instability during cell division.

In addition to cell-stage differences and other issues noted above, inaccurate predictions of mosaicism may originate from the methodology used to assess the embryo, different methods of whole-genome amplification, different versions of the analysis software, and different protocols and thresholds for mosaic embryo calls. For example, most of the SNP array–based platforms use multiple-displacement amplification (22, 23), whereas Sureplex amplification (Rubicon) has been used with array comparative genomic hybridization (aCGH) (24, 25) and next-generation sequencing (NGS) (26–29). It is almost impossible to distinguish between amplification artefacts and real biologic events owing to the low level of actual mosaicism, particularly on segmental imbalances that may significantly effect the overall IVF outcome. Recent data by Fiorentino et al. (30) showed that after transferring 18 mosaic (range of 35%–50%) embryos, six of them resulted in normal live births. A paper by Maxwell et al. (31) reported that 38 patients who had frozen euploid embryo transfers after aCGH were mosaic (31.6%) according to NGS.

All current methods for analysis of preimplantation embryos use some software to help analyze the hundreds to millions of data points generated during PGS. Most, if not all, of the current software packages are designed to determine if a sample is aneuploid or euploid. However, depending on the technology, there are as many as a million data points to interpret per sample, which will always lead to some "gray area" for the software, which is most likely where mosaicism lives. Therefore, analyzing and interpreting a profile from any embryo requires extensive training and comprehensive embryology and genetic knowledge.

Based on our internal data, ~95% of the PGS profiles are called correctly with very high confidence by the software provided by the manufacturer. Although these software packages help us to determine and make correct calls, there are still ~5% gray-area/challenging calls owing to embryo mosaicism or noisy data that will be subjective to an analyst.