Mosaicism between trophectoderm and inner cell mass

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Defining the actual incidence and prevalence of mosaicism in human blastocysts still remains a difficult task. The small amount of evidence generated by animal and human studies does not support the existence of mechanisms involved in developmental arrest, clonal depletion, or aneuploidy rescue for abnormal cells in euploid/aneuploid embryos during preimplantation development. However, studies in humans are mainly descriptive and lack functional evidence. Understanding the biological mechanisms that beset preimplantation differentiation holds the potential to reveal the role of aneuploidies and gene dosage imbalances in cell fate decision, providing important clues on the origin and evolution of embryonic mosaicism. The evidence on human blastocysts suggests that a mosaic euploid/aneuploid configuration is detected in around 5% of embryos. This figure supports the extremely low level of mosaicism reported in natural and IVF pregnancies. Similarly, the clinical management of patterns consistent with the presence of mosaicism in a trophectoderm biopsy during preimplantation genetic diagnosis cycles (PGD-A) is still a controversial issue. Despite the facts that some contemporary comprehensive chromosomal screening platforms can detect mosaic samples in cell mixture models with variable accuracy and many reproductive genetics laboratories are now routinely including embryonic mosaicism on their genetic reports, a diagnosis of certainty for mosaicism in PGD-A cycles is conceptually impracticable. Indeed, several technical and biological sources of errors clearly exist when trying to estimate mosaicism from a single trophectoderm biopsy in PGD-A cycles and must be understood to adequately guide patients during clinical care. (Fertil Steril® 2017;107:1098–106. ©2017 by American Society for Reproductive Medicine.)

Key Words: Chromosomal mosaicism, blastocyst, inner cell mass, trophectoderm, aneuploidies, preimplantation genetic screening

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The unbalanced transmission of chromosomes in human gametes and early preimplantation embryos causes aneuploidy, which is a major cause of infertility, pregnancy loss, and intellectual disability in humans (1). In the preimplantation and prenatal setting, chromosome abnormalities span a wide range of genomic imbalances, from polyploidy, to whole chromosome and large structural aneuploidies, down to submicroscopic deletions and duplications. Whole chromosome aneuploidies, monosomies and trisomies for the entire chromosomes, are the far more prevalent abnormalities and have been extensively investigated due to their high incidence in human conceptions and their clear association with clinical phenotypes and infertility. Undoubtedly, they represent the single most common form of aneuploidy and the primary cause for implantation failure in IVF cycles and miscarriages in human pregnancies. Another well-defined characteristic of human aneuploidies is their strict correlation with female age. As women age, oocytes become increasingly susceptible to chromosome segregation errors during the meiotic process. Extensive analysis of main autosomal trisomies in clinical pregnancies revealed that more than 90% had a meiotic origin. The majority are due to maternal errors, with >75% due to errors in meiosis I and <25% due to errors in meiosis II, while current estimates suggest that a minority (1%–2%) of the spermatozoa are afflicted (1, 2). Similar findings were observed in blastocyst-stage human embryos as well, where most of the aneuploidies appear to be meiotic in origin (3). The age-related processes that lead to the exponential increase in aneuploid conceptions are increasingly understood, and novel insights into the molecular mechanisms of chromosome segregation during female meiosis are being unraveled (3). Importantly, the fallibility of female meiosis is a panethnic and central biomedical subject (4) that has led to the introduction of several preimplantation/prenatal diagnostic programs worldwide to counteract the impact of aneuploidies in pregnancies, especially for women of advanced reproductive age. Indeed, there are no therapies available to counteract the age-related increase in aneuploidies. The only preventive interventions are fertility preservation (oocyte vitrification) at a young age and the adoption of diagnostic measures in the preimplantation (preimplantation genetic diagnosis-aneuploidy testing [PGD-A]) or prenatal period to prevent their complications.

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For what concerns the diagnosis of aneuploidies, it is important to outline that when the error occurs in the gametes, the embryo will show the extra or missing chromosome or chromosome region in all the cells. Embryos carrying a meiotic-derived chromosome abnormality are commonly referred to as uniform aneuploid. In this situation, where the chromosomal abnormality is uniformly present across all the cells of the preimplantation embryo, the evolution and implication for that conception can be predicted with enough accuracy. Indeed, whatever preimplantation or prenatal diagnostic approach is applied, the diagnosis will not be subjected to sample bias, meaning that the biopsy sample obtained from the fetus or its related tissues will be representative of the embryonic chromosomal constitution. The high relative contribution of numeric aneuploidies of meiotic origin in embryos and pregnancies resulted in the successful application of diagnostic programs either at the blastocyst and prenatal stage (5). PGD-A at the blastocyst stage has been proven to be an effective strategy to improve embryo selection, reducing miscarriage risk in IVF treatment (6).

Apart from uniform aneuploidies originating because of meiotic errors in both gametes, postzygotic errors in chromosome segregation can also occur and contribute to human aneuploidies and may be associated with developmental arrest or congenital abnormalities (7). Mitotic errors during the first cleavage divisions result in mosaicism within the preimplantation embryo and potentially in cell lines with different karyotypes. Although meiotic aneuploidies are uniformly present in all cells and can be accurately detected and managed in clinical diagnostic programs (1, 8), the embryonic fate and the clinical consequences of mosaic aneuploidies may depend on many variables. These include which chromosome is involved in the aneuploidy, when the error occurred during preimplantation development, what proportion of the embryo is aneuploid, and where abnormal cells are located within the embryo (9–11). As a consequence, the clinical implication of a mosaic aneuploidy can be seen as unique for each event and is difficult to interpret in the absence of well-defined genotype/phenotype associations. In this regard, the incidence and prevalence of chromosomal mosaicism in human blastocysts and its diagnosis in PGD-A cycles have recently been the subject of extensive investigation and debate. In particular, issues related to segregation and spatial allocation of aneuploid cells in a mosaic preimplantation embryo have recently raised concerns about the applicability and the effectiveness of PGD-A programs. Indeed, preimplantation embryos are normally assessed for genetic content by taking a small biopsy and testing the chromosomal constitution. For blastocyst-stage embryos, 5–10 randomly selected cells of the trophectoderm (TE) are commonly used to infer the chromosomal configuration of the inner cell mass (ICM). While for uniform aneuploidies this does not represent a limitation, in the context of a mosaic diploid/aneuploid embryo, the biopsied TE cells might not be representative of the actual chromosomal constitution of the ICM, causing misdiagnosis of the embryo's karyotype.

Despite the fact that chromosomal mosaicism is diagnosed in $<2\%$ of prenatal specimens and only a small proportion of them ($\approx 10\%$) is then confirmed in the fetus (12), estimates of preimplantation-stage mosaicism frequency are still inconsistent (13). Different approaches for data reporting and clinical management of mosaicism in PGD-A cycles have also been proposed (14–16). Due to the poor knowledge about the mechanisms of mitotic error and subsequent evolution of abnormal cells in preimplantation development, mosaicism has been also advocated as a major biological limitation to the success of blastocysts PGD-A programs in general (17). The main issues under debate are the TE representativeness of the ICM, the capability of contemporary comprehensive chromosome screening (CCS) technologies to quantify the ratio of normal/abnormal cells in a blastocyst biopsy, and how to attribute a clinical value to mosaic results.

Therefore, understanding the incidence and prevalence of mosaicism in blastocysts is essential and an area of intense scrutiny, with the objective to improve the diagnostic approaches and the treatment outcomes during medically assisted reproduction. The scope of this review is to communicate recent findings on the role of aneuploidies on preimplantation embryo development, provide a critical evaluation of existing data on the incidence and prevalence of chromosome mosaicism at the blastocyst stage, and propose a guideline on how these data may be appropriately managed in the PGD-A clinical setting.

THE IMPACT OF ANEUPLOIDIES ON PREIMPLANTATION EMBRYO DEVELOPMENT AND DIFFERENTIATION

Currently, a large amount of research is being carried out to investigate whether aneuploidy affects preimplantation development itself. Up to the moment of embryonic genome activation (EGA) at the 4- to 8-cell stage in humans (18), embryo development is under the control of maternally inherited mRNAs and proteins (subcortical maternal complex). Gene transcription is mostly inactive (19). It has been proposed that these early cell divisions are at higher risk for mitotic errors leading to mosaicism in cleavage-stage embryos (13, 20). Aneuploidies in blastomeres give rise to dosage imbalances in the expression of genes from the affected chromosomes (21), and the high progression failure occurring at the compaction stage during in vitro development has been explained by the negative effect of aneuploidies on cell differentiation when EGA takes place. A large body of research indeed suggests that mosaicism is lower in blastocysts than in cleavage-stage embryos (13, 20). Some of the differences in the magnitude of aneuploidy between cleavage and blastocyst stage are likely to reflect the lower diagnostic reliability of single-blastomere analysis compared with the multicell TE samples. It is thus likely that mosaicism has been overestimated in cleavage-stage embryo studies. Despite this, the existence of mechanism(s) that "correct" or "prevent" aneuploidy (referred to as "self-correction") during preimplantation development has been suggested (22, 23). One of the fundamental questions for the basic understanding of mosaicism in embryos relates to the characterization of whether chromosome perturbations and associated gene dosage imbalances might contribute to embryonic arrest or, for those surviving to the blastocyst stage, whether the abnormal cells can be selected against by apoptosis or lower mitotic progression or "corrected" by a second mitotic error. Insights