Assessing the true incidence of mosaicism in preimplantation embryos

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Modern technologies applied to the field of preimplantation genetic diagnosis for aneuploidy screening (PGD-A) have improved the ability to identify the presence of mosaicism. Consequently, new questions can now be addressed regarding the potential impact of embryo mosaicism on diagnosis accuracy and the feasibility of considering mosaic embryos for transfer. The frequency of chromosomal mosaicism in products of conception (POCs) of early miscarriages has been reported to be low. Mosaic embryos with an aneuploid inner cell mass are typically lost during the first trimester owing to spontaneous miscarriages. Most of the mosaics in established pregnancies would derive from placental mosaicism or placental aneuploidy, and mosaic embryos with aneuploid inner cell mass should be lost mainly due to first-trimester spontaneous miscarriages. The well described clinical outcomes of live births from mosaic embryos suggest a wide spectrum of phenotypes, from healthy to severely impaired. Therefore, there is a need to balance the risks of discarding a possibly viable embryo with that of transferring an embryo that may ultimately have a lower implantation potential. (Fertil Steril® 2017;107: 1107–12. ©2017 by American Society for Reproductive Medicine.)

Key Words: Mosaicism, aneuploidy, blastocyst, embryo, preimplantation genetic diagnosis

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Preimplantation genetic diagnosis for aneuploidy screening (PGD-A) was introduced in the 2000s for the purpose of improving live birth rates and became popular at some large assisted reproduction centers. But after the publication of several randomized clinical trials (RCTs), controversies were raised regarding the usefulness of PGD-A, mainly owing to technologic limitations that allowed the analysis of only a small number of chromosomes. More recently, new diagnostic technologies, such as array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS), which interrogate all 46 chromosomes, have become available. Three pilot RCTs that tested trophectoderm (TE) biopsy and aCGH on patients with a good prognosis for live birth showed significant improvements in ongoing pregnancy rates and have changed the view of the PGD-A field (1–3). However, owing to the ability of the new technologies to better discriminate the copy number for each chromosome, the possibility of identifying the presence of embryonic mosaicism has also increased. It is now possible to consider the potential impact of embryo mosaicism on diagnosis accuracy and whether mosaic embryos should be used for transfer.

DEFINING, TYPING, AND DETECTING MOSAICISM

Despite originating from the same zygote, not all embryonic cells share identical chromosomal complements. Mitotic errors during embryo development can result in chromosomally distinct cell populations; these are termed mosaic embryos. Mosaicism can occur as early as the 2-cell stage, although detection at the blastocyst stage is more common because more TE cells can be simultaneously analyzed.

At the blastocyst stage, four different types of mosaic embryos have been described depending on the cell lineage affected (4). A "total mosaic" embryo is observed when aneuploid and euploid cells are found indistinctly in the inner cell mass (ICM) and TE (Fig. 1). Alternatively, the mosaic population may be confined exclusively to one of these cell populations, thus generating "ICM mosaicism" or "TE mosaicism" (Fig. 1). Finally, having all cells in the ICM being aneuploid and those of the TE being euploid (or vice versa) confers "ICM/TE mosaicism" (Fig. 1).

Many factors contribute to the difficulty in diagnosing mosaicism. For example, ICM/TE and ICM mosaicism can not be detected with the use of a TE biopsy (Fig. 1). Even in embryos with TE mosaicism, detection will vary by biopsy location according to the tissue distribution of chromosomally distinct euploid and aneuploid cells (Fig. 1). Similarly, the percentage of mosaicism in the TE cells biopsied can not be extrapolated to the whole embryo. Therefore, the information from a biopsy should be considered to be

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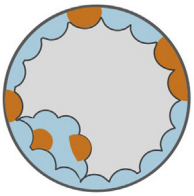
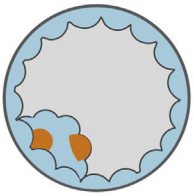
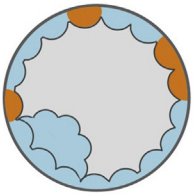
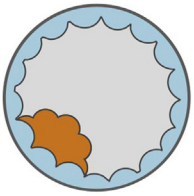
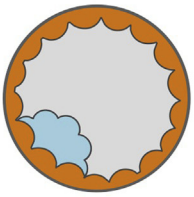
FIGURE 1

Mosaicism type	Possible TE biopsy	Diagnoses accuracy
Total Mosaic 	Euploid	Misdiagnosis
	Mosaic	Accurate
	Aneuploid	Misdiagnosis
ICM Mosaic 	Euploid	Misdiagnosis (Mosaicism never detectable)
TE Mosaic 	Euploid	Misdiagnosis
	Mosaic	Accurate
	Aneuploid	Misdiagnosis
ICM/TE Mosaic Type I 	Euploid	Misdiagnosis (Mosaicism never detectable)
ICM/TE Mosaic Type II 	Aneuploid	Misdiagnosis (Mosaicism never detectable)

Types of blastocyst mosaicism and options of trophectoderm (TE) biopsy. There are several types of blastocyst mosaicism according to the cell lineage affected. When the TE cell population includes aneuploid and euploid cells ("Total Mosaic" or "TE Mosaic"), the biopsy could include both cell lineages or just euploid or aneuploid cells. According to the biopsy location, the diagnoses will be more or less accurate. When the mosaicism is confined to the inner cell mass ("ICM Mosaic"), the TE biopsy will be always fully euploid, as the TE is, and will not represent the whole cell population in the embryo, giving a misdiagnosis. Similarly, when the ICM and TE are chromosomally distinct (ICM/TE Mosaic"), the trophectoderm biopsy will always show the contrary diagnoses, aneuploid versus euploid, to the one observed in the ICM.

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relevant only to the biopsy itself. The concordance between the TE biopsy and the whole embryo can be empowered by additional factors, such as mosaicism type and degree, biopsy location, the number of cells biopsied, and experimental quality related to sample manipulation. Thus, in general, an embryo diagnosed as mosaic is truly only at risk of being mosaic.

METHODS TO ASSESS THE INCIDENCE OF MOSAICISM IN PREIMPLANTATION EMBRYOS AND BLASTOCYSTS

Mosaicism was initially predicted more than 25 years ago, from analysis of two blastomeres that were assessed for preimplantation genetic screening (PGD-A) with the use of fluorescence in situ hybridization (FISH) (5). Subsequently, multiple FISH studies analyzing single cells of whole blastocysts have confirmed mosaicism in day-3 biopsies with nonconcordance rates (when genotypes of analyzed cells do not match one another) ranging from 18% to 46% (6–9). Similarly, studies analyzing cells from cleavage-stage embryos showed rates of aneuploid/euploid mosaicism from 39% to 46% (10, 11). These high nonconcordance rates on day 3 suggested very high mosaicism in the cleavage-stage embryo, arguing for the use of TE biopsy as a more reliable option for PGD-A than embryo biopsy (12). Nonetheless, some studies claimed that technical limitations of nucleus fixation and FISH interpretation could result in overestimation of abnormalities in day-3 biopsies (13, 14).

More recently, aCGH for PGD-A has allowed the simultaneous analysis of the 23 chromosome pairs. Studies comparing aCGH results from day-3 biopsies with FISH reanalysis showed false positive rates of 2%–3% (15, 16), which is much lower than earlier day-3 FISH studies (6–9). Similarly, using aCGH, a blinded study comparing day-3 and day-5 biopsies with whole-blastocyst analysis confirmed high concordance rates independently from the day of analysis, with 98% for day-3 and 96.6% for day-5 biopsies (17). These results also confirmed studies that compared FISH with single-nucleotide polymorphism arrays on day-3 embryo biopsies, which concluded that the FISH technique was a poorer predictor of aneuploidies (18, 19). These later studies support the hypothesis that the high nonconcordance rates between day-3 and day-5 blastocysts reported from FISH analyses may have originated from the technique itself rather than mosaicism.

Novel applications of modern analytic methods, such as aCGH and NGS, on TE biopsies have aided assessment of mosaicism. Several studies have used cell line mixture models to estimate the sensitivity and specificity of these methods for detecting mosaicism, estimating levels of detection to be 40%–50% for aneuploid cells with the use of aCGH and 20% with the use of NGS (20–24). As described above, the results of a TE biopsy may not be representative of the entire embryo, the unbiopsied TE cells, or the ICM. Some studies have attempted to determine how frequently a TE biopsy correctly represents the embryo mosaicism (22, 25). Analysis of two to three biopsies in the same embryo showed concordance rates as high as 95%–100% (22, 25). In addition, these studies also analyzed the ICM of the same embryos to estimate the discordance frequencies between cell lineages. The TE and ICM showed discordant mosaicism rates of ~3%–4% (22, 25). Therefore, TE biopsy is considered to be a good method to accurately diagnose blastocyst mosaicism.

IMPACT OF MOSAICISM IN PREIMPLANTATION GENETIC DIAGNOSIS ACCURACY AND EFFICIENCY

Techniques such as NGS now enable the diagnosis of mosaicism in a group of TE cells, presenting for the first time the possibility to decide or decline to transfer mosaic embryos and to follow up such decisions with clinical outcome results. It should be noted, however, that studies addressing the clinical outcome after transfer of mosaic embryos remain scarce.

To estimate the real incidence and possible consequences of mosaic transfer, the diagnosis of a mosaic blastocyst by means of NGS with the use of a TE biopsy should be evaluated at three levels: blastocyst quality and number of analyzed cells, implantation, and miscarriage rates and the health of the baby.

Blastocyst Quality and Number of Analyzed Cells

For a blastocyst, the analytic criterion standard would be to assess all of its cells individually with the use of NGS or aCGH. However, to our knowledge, that has not been performed, most likely owing to high cost. Another approach would be to analyze multiple TE biopsies. A recent NGS study of two to five TE biopsies in embryos with reported mosaic aneuploidy could not confirm the same mosaic pattern in 43% of the aneuploidies (20). Another study analyzed two to four TE biopsies and ICM from 43 embryos originally diagnosed as mosaic and, similarly, showed that 28% of the ICM samplesand 34% of the TE samples were fully euploid (26). Finally, we analyzed whole-blastocyst embryos with a mosaic segmental aneuploidy at the single-cell level with the use of FISH (27). We found that in nine out of ten segmental aneuploidies with a mosaic pattern detected by NGS, the mosaicism was confirmed after the single-cell analysis of the whole blastocyst (27). In summary, we could state that for embryos diagnosed as mosaic at the blastocyst stage, 50%–90% of them are reconfirmed as mosaic depending of the type of secondary analysis performed: ICM biopsy, TE biopsy, or whole-blastocyst analysis.

Implantation

Two studies have been published in which mosaic embryos were transferred to the patients (28, 29). Both studies showed similar implantation rates, ranging from 38% to 45% (28, 29). One of the studies showed that the implantation rate was significantly lower compared with the control group (29). Therefore, it is important to note that implantation of mosaic embryos is not equivalent to implantation of euploid embryos, because we can not infer the real mosaicism degree of the embryo analyzed by means of the TE biopsy.

Miscarriage Rates and Health of the Baby

Assessing miscarriage rates is a critical aspect for understanding the consequences of mosaic transfer. One study showed that 25% (n = 8) of implanted embryos after mosaic transfer ended in a biochemical pregnancy; the remainder resulted in a healthy baby. This percentage is similar to the of percentage mosaic embryos initially transferred (33.3%) (28). In a second study, 12% of transferred mosaic embryos ended in miscarriage and 26% in ongoing pregnancies; delivery information and infant follow-ups were not available (29).

In addition, it is important to analyze mosaicism frequency in POCs after transfer of euploid blastocysts, in case misdiagnoses due to mosaicisms have been made. Werner et al. estimated that the clinically recognizable diagnosis error rate per ongoing pregnancy was 0.13%, with only four POCs available for study. That study described evidence of mosaicism in all of the POCs, suggesting mosaicism as the origin of the misdiagnoses (30). In contrast, a recent study analyzing 20 POCs from PGD-A cycles found mosaicism in only 15% of the samples (20). Because these studies showed contrasting results, it is clear that more research is needed to understand the error rate in the diagnosis of euploid embryos that may be mosaic.

INCIDENCE OF MOSAICISM IN MISCARRIAGES AND PRENATAL DIAGNOSIS OF SPONTANEOUS AND IVF PREGNANCIES

Although a high frequency of mosaicism has been reported in preimplantation human embryos, classic cytogenetic studies of miscarriage POCs have reported only a 5%–6% incidence of mosaicism (31–33). These numbers indicate that most of the mosaicism found in established pregnancies is placental mosaicism or aneuploidy. Furthermore, most mosaic embryos with aneuploid ICM are likely lost by the first trimester through spontaneous miscarriages (34). However, many POC studies have been performed with either the fetus or extraembryonic tissues (most often from chorionic villi). Few have assessed the incidence of fetoplacental mosaicism, which is an unexpected discordant chromosomal status between the fetus and placenta. Hysteroembryoscopy has been described as an ideal tool to assess the true rate of fetoplacental discordances/mosaicism in first-trimester miscarriages, with the use of either karyotyping (35) or molecular techniques (36). With the use of hysteroembryoscopy and molecular analysis with the use of aCGH or NGS, our group has described an incidence of 2% fetoplacental discrepancy in 46 analyzed POCs (37).

Recently, Segawa et al. reported on a large series of IVF miscarriages after single-blastocyst transfer, with the use of POC analysis (38). In 1,030 POC cases, 19.4% displayed a normal karyotype, whereas 80.6% were identified as aneuploid. The aneuploid cases stratified as 62.3% trisomy, 7.8% double trisomy, 0.5% triple or quadruple trisomy, 1.3% monosomy 21, 3.2% monosomy X, 0.1% 47,XXY, 1.0% polyploidy, 1.0% mixed, 2.4% structural anomalies, and only 1.1% aneuploid mosaicism.

Disparities between trophoblast and fetal cells occur in 1%–2% of viable pregnancies studied by chorionic villus sampling (CVS) karyotyping (39). In most, chromosomal abnormalities (most often trisomy) are confined to the placenta and may be associated with a poor perinatal outcome and miscarriages. In confined placental mosaicism (CPM), the chromosomal abnormality can be confined to the trophoblast (type I), the chorionic stroma (type II), or both cell lineages (type III) while the fetal cells remain normal. In rare cases, the placental karyotype is normal and fetal cells show an abnormal karyotype (40). In patients undergoing intracytoplasmic sperm injection, CVS studies revealed that CPM increased to 5.88%. CPM may be associated with a negative pregnancy outcome, including congenital abnormalities and intrauterine growth restriction (IUGR). Complications most often occur when mosaicism persists to term and affects a large proportion of cells (41).

Chromosome 16 is frequently implicated in mosaicism in ongoing pregnancies and live births. Langlois et al. found that the majority of trisomy 16 mosaic cases diagnosed by CVS had a good postnatal outcome. In contrast, mosaicism 16 diagnosed in amniotic fluid was associated with major congenital anomalies and an increased risk of developmental delay (42). Yong et al. found that 66% of prenatally diagnosed trisomy 16 mosaicism pregnancies resulted in live birth (43). Of those, 45% exhibited one or more malformations. In cases assessed by means of direct CVS, the proportion of trisomic cells correlated with more serious birth defects (i.e., higher risk of malformation as well as more severe IUGR). In cases assessed by means of cultured CVS, the proportion of trisomy correlated only with more severe IUGR. Similarly, trisomy assessed by means of amniocentesis of amniotic fluid correlated with both IUGR and malformation; in contrast, trisomy detected in amniotic mesenchyme correlated only with IUGR. The authors concluded that the levels of trisomy in different fetoplacental tissues are significant predictors, particularly in mosaic trisomy 16 pregnancies.

For trisomy 20, the proportion of trisomic cells in amniotic fluid correlates with different outcomes (44). Indeed, typical outcomes were reported when low levels of trisomy (<12%) were detected. In contrast, developmental delay and oligohydramnios were observed in two cases with high proportions of trisomic cells in amniotic fluid (96% and 58%). These findings support work from previously published cases finding that the proportion of trisomic cells correlates with outcome. When <40% trisomic cells were detected, only 4% had abnormal outcomes. Curiously, significantly higher levels of trisomy were observed in male fetuses compared with female fetuses.

CLINICAL IMPACT IN LIVE BIRTHS AND ETHICAL CONCERNS

The frequency of mosaicism in live births is undetermined, because chromosomal analysis in live births, children, and adults are mostly performed only when there is a clinical indication or a strong suspicion for a chromosomal disorder. In fact, the clinical manifestations are represented by a spectrum of phenotypes, and their relationship with different syndromes has been widely described. For example, a higher incidence of chromosomal mosaicism in individuals with

major psychiatric disorders and autoimmune diseases has been reported. The incidence of mosaicism in several diseases has been described as follows: 3%–18% in chromosomal syndromes; 3%–5% in mental retardation and/or multiple congenital malformation; 16% in autism; schizophrenia with mosaic aneuploidy of chromosomes 1, 18, and X in cells of the schizophrenia brain; mosaic X chromosome aneuploidy in blood lymphocytes; monosomy of chromosome X in systemic sclerosis (6.2% of cells); autoimmune thyroid disease (4.3% of cells); and Alzheimer disease (>10% in brain cells) (45).

Furthermore, complex chromosomal abnormalities have been implicated in mosaic aneuploidies of live births. Warburton et al. reported an adult with severe microcephaly and mental retardation where karyotype analysis of lymphocytes, skin fibroblasts, bone marrow, and lymphoblasts showed ≥10% of cells with trisomy on many different chromosomes (46). In that example, trisomies were the most observed, with the exception of chromosomes 5, 10, 13, 14, and 17. The existence of the predominant mosaic trisomy in four specific tissues and in repeated cultures over a 3-year period suggested that the mosaicism was due to a genetic abnormality that resulted in mitotic instability. When this subject was compared with six similar cases, including two pairs of siblings, clinical and cytogenetic differences among the patients made it unclear whether the cases actually represented the same condition. The term "mosaic variegated aneuploidy with microcephaly" was suggested as a descriptive term for this syndrome.

Other trisomies provide additional insight regarding the impact of mosaicism on live births. Mosaic trisomy 18 displays a wide phenotypic spectrum, ranging from near normal to early death. Significant discrepancies between the levels of trisomic cells in skin fibroblasts and lymphocytes may lead to misdiagnosis (47). Though rare, individuals with mosaic trisomy 15 display similar features, including IUGR, craniofacial abnormalities and facial dimorphisms, cardiac disease, hypopigmentation, abnormal cerebral vasculature, and dysplastic kidneys and other organ anomalies (48). Some evidence even suggests that nearly all individuals are mosaic for trisomy 21 in some tissues. The understanding of tissue-specific trisomy 21 mosaicisms may have important ramifications for understanding the pathogenesis, prognosis, and treatment of medical problems related to this trisomy (49).

Explaining the wide phenotypic variance of mosaicism is not simple. One explanation uses skewed X-chromosome inactivation (XCI), in which one X chromosome is preferentially inactivated (rather than randomly inactivated). Skewed XCI is often found in the diploid fetal tissues of individuals with mosaic trisomy that originated from a "trisomic zygote rescue" event. The idea is that, at the time of XCI, the presence of a high proportion of trisomic cells in the embryo leads to their subsequent elimination by selection. Skewed XCI in such cases is proposed to result in poor fetal outcomes because not all trisomic cells are eliminated from the fetal tissues (50). Another reported adverse risk is uniparental disomy for chromosomes 14 and 15 (51, 52) and hidden mosaicism for trisomy 16 (53).

CONCLUSION

Reported data suggest that ~60% of embryos diagnosed as mosaic in a TE biopsy would be mosaic in the entire embryo. Therefore, 40% would be expected to implant and 25%–30% would result in continuing pregnancies and healthy babies. Alternate outcomes could be caused by either a low degree of mosaicism or misdiagnosis due to experimental noise. Therefore, it is important to note that an increase in detection of mosaicism may result in a decrease in specificity (21). More published clinical outcome data, including studies with larger numbers of embryos and more focus on the consequences of transferring mosaic embryos, such as monitoring success rates and/or genetic analysis of miscarriages, will help to explain the wide variance in live birth phenotypes from mosaicism. There is also a need for more obstetrical and neonatal outcome data to help balance the risks of discarding a possibly viable embryo versus transferring one with lower implantation potential (54).

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