

Clinical management of mosaic results from preimplantation genetic testing for aneuploidy (PGT-A) of blastocysts: a committee opinion

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Since the advent of preimplantation genetic testing for aneuploidy (PGT-A) in the 1990s, substantial changes in test methodology and technology now allow the detection and reporting of intermediate chromosome copy number (commonly referred to as mosaicism) for aneuploidy in a trophoctoderm biopsy sample. Clinicians are grappling with how to interpret such findings and how to counsel patients about embryo transfer decision-making. This document reviews the available literature and outlines the various issues surrounding the reporting of intermediate copy number and consideration of storage or transfer of blastocysts with intermediate copy number results. This document does not endorse, nor does it suggest that PGT-A is appropriate for all cases of in vitro fertilization. (*Fertil Steril*® 2020;114:246–54. ©2020 by American Society for Reproductive Medicine.)

Key Words: Preimplantation genetic testing for aneuploidy, assisted reproductive technology, mosaicism, infertility, aneuploidy

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Mosaicism is defined as the presence of more than one chromosomally distinct cell line in a single sample originating from one individual—for example, the peripheral blood karyotype in an individual who is mosaic for Turner syndrome, 45,X/46,XX. It is important to recognize that the diagnosis of chromosomal mosaicism in a trophoctoderm biopsy is not made by direct witnessing of both euploid and aneuploid individual cells. Rather, the diagnosis is inferred from the presence of an intermediate chromosome copy number (between monosomy and disomy, or between disomy and trisomy) on a next-generation sequencing (NGS) profile. It is also important to recognize that, aside from mosaicism, other proposed explanations for intermediate copy number results include statistical

variation (test artifact/“noise”), amplification bias, contamination, mitotic state, variation in embryo biopsy technique, and embryology laboratory conditions (1–3). It is unknown to what extent a mosaic trophoctoderm biopsy reflects the true composition of the blastocyst and to what extent it predicts outcomes. Nonetheless, for ease of reading, in the remainder of this document the term “embryo with mosaic results” will be used to describe embryos with intermediate copy number results, and an embryo biopsy result interpreted as euploid after testing will be referred to as a “euploid embryo.”

Mosaicism has long been recognized as a phenomenon and potential limiting factor in the interpretation of preimplantation genetic testing for aneuploidy (PGT-A) (4). Since the

advent of PGT-A (formerly referred to as preimplantation genetic screening, or PGS), it has been recognized that preimplantation embryos have a high rate of true mosaicism (3, 5, 6), which is much higher than that reported in the prenatal and postnatal cytogenetic literature. Mosaicism was initially identified in clinical validation studies and cited as a contributing factor of PGT-A misdiagnosis related to biopsy sample size (7). Mosaicism rates reported by PGT-A laboratories have been influenced by the stage of embryo biopsy (cleavage vs. blastocyst) and the method of analysis—fluorescent in situ hybridization (FISH), array comparative genomic hybridization (aCGH), or single nucleotide polymorphism (SNP) microarray (3).

With more recent and sensitive assays such as NGS, it has become increasingly common to report identification and quantification of mosaicism within a trophoctoderm biopsy sample. The rate of mosaic diagnoses in clinical testing of trophoctoderm is estimated to

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be 3% to 20% depending on the specific NGS platform used, the cutoffs used to classify results as mosaic, technician and software interpretation, and individual PGT-A testing laboratory classification schemes (8, 9).

A PGT-A result showing an intermediate copy number therefore indicates that the biopsied embryo may be at risk of having mosaicism that is unable to be confirmed on clinical testing. Note that this document is intended for reference in a clinical setting when discussing mosaic results with patients; it is outside the scope of this document to comment in detail about potential laboratory variables that may contribute to a mosaic diagnosis.

Given that a mosaic result may not be representative of the chromosomal constitution of the remainder of the embryo as well as the previously described technical limitations, embryos diagnosed as mosaic based on trophoctoderm analysis may be: fully euploid, fully aneuploid, mosaic for a euploid and an aneuploid cell line, or mosaic for two or more different abnormal cell lines (8).

There is a paucity of outcome data regarding the health of pregnancies and children after transfer of embryos with mosaic results. Thus far, the limited outcomes reported after such transfers seem to be reassuring; embryos have either failed to implant or have miscarried, or they have resulted in a live birth with no apparent abnormal phenotype (10, 11). These preliminary outcomes have led the reproductive medicine community to a gradual but increasing acceptance of the transfer of embryos with mosaic results as a viable option for patients. Such transfers are performed in the hope that either the mosaic diagnosis is due to an analytical error (a false-positive result of a euploid biopsy), or an embryo that is in fact mosaic will “self-correct,” not be clinically impacted by the presence of some abnormal cells, fail to implant, or miscarry through natural means. In contrast, however, from a prenatal and postnatal perspective, mosaicism in a pregnancy or neonate has traditionally been cause for appreciable concern and follow-up management. This document will attempt to navigate both perspectives.

Although current outcome data are limited for transfer of an embryo with mosaic results, they are also somewhat encouraging. Thus, it is not unusual for clinicians to discuss the options of storage and transfer of embryos with mosaic PGT-A results. In an effort to provide some structure to the discussion about PGT-A mosaicism and how patients are counseled, several professional organizations have created statements for reference. The American Society for Reproductive Medicine (ASRM) released an Ethics Committee Opinion in 2017 providing general recommendations for handling positive results from PGT (12). The Human Fertilisation and Embryology Authority (HFEA) of the United Kingdom addresses this as well in the *Code of Practice* (13). The Preimplantation Genetic Diagnosis International Society (PGDIS) and Congress on Controversies in Preconception, Preimplantation and Prenatal Genetic Diagnosis (CoGen) also developed statements to provide guidance to clinicians who are faced with the incredibly complex task of interpreting such laboratory results and fostering patient decision-making (9, 14, 15).

CONSIDERATIONS FOR TRANSFER OF EMBRYOS DIAGNOSED AS MOSAIC

Data regarding outcomes associated with embryonic mosaicism are limited, as the routine reporting of mosaic PGT-A results is a relatively recent practice. Most of the published data regarding outcomes associated with chromosomal mosaicism are derived from testing performed prenatally or postnatally without prior PGT-A.

Categories of Perinatal Outcome Data Associated with Chromosomal Mosaicism

Three main risk categories have been delineated: confined placental mosaicism (CPM), true fetal mosaicism, and uniparental disomy (UPD).

Confined placental mosaicism (CPM). Chromosomal mosaicism detected by chorionic villus sampling (CVS) is confined to the placenta approximately 87% of the time (16). Although most pregnancies with CPM have normal outcomes, some studies have found higher incidences of pregnancy loss, fetal growth restriction, and other obstetric complications (17). The risk of such complications largely depends on whether the mosaicism is localized to the cytotrophoblast (type I CPM), mesenchymal core (type II CPM), or both cell types, and specific aneuploidies show preferential distribution among these tissues (17).

True fetal mosaicism. When detected by CVS, mosaicism is confirmed in fetal tissues approximately 13% of the time (16). Certain aneuploidies, including trisomies 21 and 18, and sex chromosome abnormalities, are more likely to be confirmed in the fetus after amniocentesis; others are identified less frequently in the fetus (16). In the presence of ultrasound anomalies, true fetal mosaicism poses a high risk for developmental and physical disabilities. However, in the absence of ultrasound findings, outcomes are far more difficult to predict, as phenotypes largely depend on the proportion of abnormal cells and distribution among various tissues in addition to the specific chromosomal abnormality (18). Postnatally, most identifiable chromosomal mosaicism is associated with physical and developmental anomalies. However, this finding is subject to ascertainment bias, as chromosomal analysis of infants is typically pursued only when congenital anomalies or dysmorphic features are present. It is thus important to recognize that mosaicism has also been identified in normal offspring (18, 19).

Uniparental disomy (UPD). When mosaicism is caused by a postzygotic trisomy or monosomy rescue event, the two remaining chromosomal copies may originate from the same parent, a phenomenon known as uniparental disomy. For most chromosomes, there is no apparent phenotypic effect related to UPD (18). However, UPD of chromosomes with imprinted regions—that is, those containing genes for which expression depends on parent of origin—has been associated with abnormal phenotypes. Specifically, regions of chromosomes 6, 7, 11, 14, and 15 are associated with known imprinting disorders while there is less consistent literature regarding UPD for chromosomes 2, 16, and 20 (18). Additionally, there are multiple documented cases of recessive

TABLE 1

Outcome studies after transfer of embryos with mosaic results.

Study	Embryos with mosaic results included, n ^a	Outcome	
		OP and LB	SAB and biochemical pregnancies
Prospective			
Greco et al. 2015 (11)	18	6 of 18 (33.3%) LB rate; confirmed normal chromosomes by CV karyotype.	2 of 18 (11.1%) biochemical pregnancy rate; 0 of 6 (0%) SAB rate.
Munné et al. 2017 (6)	143	58 of 143 (40.6%) OP rate; no LB or karyotype confirmation data.	18 of 76 (23.7%) SAB rate per clinical pregnancy with GS.
Spinella et al. 2018 (21)	78	24 of 78 (30.8%) LB rate; no karyotype confirmation data.	6 of 30 (20.0%) SAB rate per clinical pregnancy with GS.
Victor et al. 2019 (22)	100	30 of 100 (30.0%) combined OP and LB rate per embryo transferred; 8 of 11 amniocenteses with confirmed normal chromosomes; 3 of 11 with chromosomal abnormalities of unspecified clinical significance.	7 of 37 (18.9%) SAB rate per clinical pregnancy with GS.
Retrospective ^b			
Maxwell et al. 2016 (23)	18	6 of 38 (15.8%) LB from aCGH embryos deemed euploid retroactively found to be mosaic by NGS.	12 of 38 (31.6%) of SAB from aCGH embryos deemed euploid retroactively found to be mosaic by NGS.
Fragouli et al. 2017 (24)	44	12 of 44 (27.3%) LB rate.	5 of 17 (29.4%) SAB rate per clinical pregnancy.
Lledó et al. 2017 (25)	52	13 of 52 (25.0%) combined OP and LB rate, 10 LB reported to be healthy.	1 of 14 (7.1%) SAB rate.
Zhang et al. 2019 (10)	102	48 of 102 (47%) LB rate; 3 of 3 amniocenteses with confirmed normal chromosomes. "All infants were found to be healthy after a detailed physical examination performed by a local pediatrician after delivery."	8 of 67 (11.9%) biochemical pregnancy rate; 12 of 59 (20.3%) SAB rate per clinical pregnancy.

Note: aCGH = array comparative genomic hybridization; CV = chorionic villi; GS = gestational sac; LB = live birth; NGS = next-generation sequencing; OP = ongoing pregnancy; SAB = spontaneous abortion.

^a Embryos transferred may be represented in more than one data set. Consistently, studies have suggested that embryos with mosaic results tend to result in fewer ongoing pregnancies and more SABs compared with the rates generally seen with embryos deemed euploid. A limitation of these studies is that embryos with mosaic results are typically only transferred when there are no euploid embryos available (i.e., as a last resort). Although there is a paucity of literature on the subject, it is likely that many patients who elect to transfer embryos with mosaic results have been unable to produce euploid embryos or have had previous failed embryo transfers. Therefore, the population of patients who transferred embryos with mosaic results may contain more poor-prognosis patients than the population who transferred euploid embryos. This potential discrepancy in population characteristics has not been controlled for in any outcome study to date.

^b aCGH embryos deemed euploid retroactively found to be mosaic by NGS.

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monogenic disease attributed to UPD, which can occur if a pathogenic variant is located on the duplicated parental allele (20).

Outcomes After Transfer of an Embryo with Mosaic Results After PGT-A

The studies reporting on the outcomes to date after transfer of an embryo with mosaic results are summarized in [Table 1](#). As of mid-2019, there have been approximately 100 documented live births after transfer of an embryo with mosaic results. To date, no adverse events related to transfer of an embryo with mosaic PGT-A results have been documented in the literature, including pregnancy complications, abnormal prenatal or postnatal karyotypes of known clinical significance, congenital anomalies, or other health concerns.

However, these outcome data have several limitations. First, documented karyotype, chromosomal microarray (CMA), and UPD data from these infants have been largely absent. As abnormal phenotypes may not present immediately in the neonatal period, mosaicism may go unrecognized without these analyses. Second, there have not been any formal studies thus far to evaluate and document the health of newborns. Third, the lag time between the transfer of an embryo with mosaic results and the publication of outcome data can be substantial. Fourth, there have not been any longitudinal studies to assess long-term outcomes of children born from embryos with mosaic results. Finally, the number of live births reported so far is relatively small. Therefore, while reassuring, the available outcome data must be interpreted with caution because the risks associated with prenatally and postnatally detected mosaicism remain a possibility.

In the future, investigators reporting on outcomes from transfers of an embryo with mosaic results are encouraged to obtain both phenotypic information and documented chromosomal data on any resulting pregnancies.

Which Embryos with Mosaic Results are Acceptable to Transfer?

Recent attempts have been made to prioritize different types of mosaic PGT-A results with respect to their acceptability for embryo transfer so as to enable individualized patient counseling about potential success rates, risks, and outcomes as well as to assist with embryo selection decisions in situations whereby multiple embryos with mosaic results are under consideration for transfer.

Although initial statements issued by PGDIS in 2016 recommended the transfer of embryos with mosaic monosomies over those with mosaic trisomies, this statement was updated in 2019 and eliminated this particular recommendation. Currently a prioritization model based on the level of mosaicism and chromosome involved is suggested (14, 15). Autosomes were ranked according to their perceived viability in the aneuploid state, risk of placental dysfunction and fetal growth restriction, and risk of a known syndrome associated with UPD. Shortly after these initial recommendations were issued, CoGEN released a position statement with analogous recommendations (9) but acknowledged emerging data (6)

suggesting that mosaic monosomies and mosaic trisomies implanted at the same rate. Subsequently, another group applied data from a large cohort of prenatal samples and products of conception to suggest a similar but ultimately different hierarchy of risk based on the involvement of specific chromosomes and their association with persisting fetal aneuploidy, UPD syndromes, and spontaneous abortion (SAB) (26).

Despite these various ranking approaches, it may be premature to apply any for purposes of embryo-transfer decisions or for providing clinical recommendations to patients. The influence of mosaicism-related factors on clinical outcome data has been inconsistent; for example, some studies have found differences in live-birth rates depending on the level of mosaicism identified in a trophectoderm sample (21) or involvement of a full versus partial chromosome (24), but others have not found statistically significant differences when using the same classification system (6). Furthermore, while large data sets from prenatal and products of conception samples are undoubtedly valuable, it is problematic to assume that these data can be extrapolated to the preimplantation embryo because it is unknown whether fetoplacental and embryonic mosaicism are intricately related or whether they may arise from distinct mechanisms. The proposed categories, supporting evidence (or lack thereof), and relevant considerations are summarized in [Table 2](#).

Therefore, additional data are needed to determine whether these categories can be applied for clinical decision-making. In the interim, it is recommended that clinicians inform patients that there is currently no evidence-based method available to determine which embryos with mosaic results have the best chance of resulting in a successful pregnancy, or which may have the lowest risks of an undesired outcome. Studies reporting on transfers of an embryo with mosaic results should continue to provide detailed information about PGT-A results leading to specific outcomes to assist with the development of evidence-based clinical guidelines for transfer of an embryo with mosaic results in the future.

GENETIC COUNSELING Clinic Policy Development

The ASRM Ethics Committee Opinion “Transferring Embryos with Genetic Anomalies Detected in Preimplantation Testing” expresses support for providers both in transferring and declining to transfer embryos with “variable phenotypes creating uncertainty about outcomes,” including those diagnosed as mosaic, and encourages individualized decision-making (12). Each in vitro fertilization (IVF) program is strongly encouraged to develop its own internal policy addressing the transfer and storage of embryos diagnosed as mosaic (12). Such policies should be shared widely with patients before the initiation of an IVF/PGT-A cycle and at relevant touch points throughout the treatment process. A clinic policy template is provided in the [Supplemental Appendix](#) (available online) that addresses the nuances and complexities encountered by clinics ordering and receiving mosaic PGT-A results.

TABLE 2

Classifications and considerations for PGT-A mosaic results.

Category	Theory	Considerations
Percentage of mosaicism	A lower level of mosaicism (i.e., fewer aneuploid cells in a trophectoderm biopsy) is likely associated with a better outcome.	<p>Data regarding clinical outcomes associated with higher vs. lower proportion of aneuploid cells in a trophectoderm biopsy have been inconsistent. Some studies have found that lower-level mosaicism is associated with improved ongoing pregnancy rates (21); while others have not found a statistically significant difference (6, 22, 27).</p> <p>Data do not support an equal distribution of mosaicism throughout the trophectoderm (28), suggesting that mosaicism levels may be highly dependent on the site of biopsy.</p> <p>Prenatal and postnatal data do not support an association between the level of mosaicism and phenotypic outcome (18).</p>
Specific chromosome(s) involved	<p>Mosaicism involving certain chromosomes is more likely to: Result in a viable, ongoing pregnancy despite a persisting aneuploid cell line in the fetus. Pose a risk for UPD syndromes. Pose a risk for fetal growth restriction if aneuploid cells persist in the placenta.</p>	<p>While there are data regarding mosaicism identified prenatally or postnatally and associated risks/outcomes depending on the specific chromosome number involved (16), it is not known whether this mosaicism is mechanistically related to embryonic mosaicism nor how well such data can be extrapolated to potential risks of transfer of an embryo with mosaic results.</p> <p>Mosaic aneuploidies involving most chromosomes have been reported in pregnancies or live births with abnormal phenotypes (18, 19).</p>
Monosomy vs. trisomy	Monosomies of most chromosomes are not viable.	<p>Current PGT-A methodologies cannot distinguish a pure monosomy or trisomy cell line from mixed reciprocal monosomy/trisomy cell lines present in the same biopsy (8).</p> <p>No difference in pregnancy or SAB rates has been seen when comparing embryos mosaic for monosomies vs. trisomies (6, 22).</p> <p>While pure non-mosaic monosomies are not viable (with the exception of 45,X), live births with mosaic autosomal monosomies have been reported in the literature (29).</p>
Full chromosome vs. partial chromosome	Aneuploidies involving a full chromosome may have different chances of viability compared to those involving a chromosomal segment (deletion or duplication).	<p>Data regarding clinical outcomes from embryos mosaic for full vs. partial aneuploidies have been inconsistent. Some studies have found a higher ongoing pregnancy rate for partial chromosome mosaics (10, 22, 24); while others have not found a difference (6).</p> <p>There are currently no data supporting an increased chance of viability with persisting fetal mosaicism of a partial chromosome aneuploidy compared to a full chromosome aneuploidy.</p> <p>Data suggest that mosaicism reported for partial chromosome aneuploidies are more likely to represent false-positive results due to test artifact (1), suggesting that these embryos may have better implantation potential.</p> <p>Prenatal and postnatal literature suggests that, in general, the smaller the chromosome segment, the more likely it is to be compatible with life with an abnormal phenotype (30).</p> <p>Due to the limited resolution of PGT-A platforms, it is essential to recognize that deletions and duplications detected by PGT-A are generally much larger than those detected in ongoing pregnancies or live births.</p>
No. of chromosomes involved	Embryos diagnosed as mosaic for multiple chromosome aneuploidies may have lower chances of ongoing pregnancy.	There are some data indicating reduced pregnancy potential of embryos diagnosed as mosaic for three or more chromosomes (6) or segmental mosaic for two or more chromosomes (22); however, other studies did not find a significant difference between mosaicism involving one vs. two chromosomes (6, 25).

Note: PGT-A = preimplantation genetic testing for aneuploidy; SAB = spontaneous abortion; UPD = uniparental disomy.

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Pretest Counseling

Before pursuing any genetic testing, including PGT-A, patients should be informed of the risks, benefits, and limitations of the technology used (31). If the PGT-A platform used includes detection and reporting of mosaicism, the pretest counseling should include discussion of:

- The expected frequency of mosaic results (as quoted by the testing laboratory).
- The technical and clinical difficulties in interpreting mosaic PGT-A results.
- The limited outcome data available and potential challenges associated with making embryo-transfer decisions in the absence of clear risk information.
- The potential outcomes of chromosomal mosaicism, including congenital anomalies, fetal growth restriction, and other adverse perinatal outcomes such as fetal or neonatal demise.
- The clinic's policy regarding the transfer and storage of embryos with mosaic results (see the [Supplemental Appendix](#) for a sample policy). It should be noted that laboratory-based genetic counselors often cannot comment on individual clinic protocols.
- The option to decline PGT-A, (or pursue PGT-A without mosaicism reporting) to avoid uncertain results and the burden of decision-making regarding transfer or storage of embryos diagnosed as mosaic (32).

Posttest Counseling

Patients may consider transfer of embryos with mosaic results under several circumstances including: lack of euploid embryos after an IVF/PGT-A (with or without PGT-M/SR) cycle or prior use of all available euploid embryos. Occasionally, patients may also request transfer of an embryo with mosaic results in combination with one or more euploid embryos.

Due to the current lack of robust outcome data regarding embryos with mosaic results and bearing in mind the overarching goal of IVF is the birth of a healthy infant, a single embryo deemed euploid, if available, should be preferentially transferred. If no euploid embryos are available, patients should be counseled on the option of proceeding with another IVF/PGT-A cycle in the hopes of identifying a euploid embryo for transfer.

However, patients who wish to consider transfer of an embryo diagnosed as mosaic—and are supported by their physician in considering this option—should receive comprehensive genetic counseling regarding this diagnosis and its uncertainties. Such counseling should be provided before initiation of the embryo transfer cycle by a genetics specialist with a thorough understanding of mosaic PGT-A results as well as perinatal and pediatric outcome data (see www.nsgc.org for a directory of genetic counselors). Patients should be counseled that the clinical significance of mosaicism identified in embryonic trophoctoderm biopsies is largely unknown and that there are several possible explanations for mosaic PGT-A results (32). Counseling should include a discussion of available outcome studies of transfer of an embryo with mosaic results as outlined in [Table 1](#) and the difficulties in

stratifying risk based on the considerations outlined in [Table 2](#).

Perinatal and postnatal risks should also be reviewed, including the small but unknown risk of live birth with aneuploidy (in the full or mosaic state) or uniparental disomy, either of which could result in congenital anomalies to varying degrees. When the identified mosaic aneuploidy is associated with a known syndrome or phenotype, patients should be made aware of any corresponding clinical outcome information. Whether or not corresponding perinatal or pediatric data for a specific mosaic finding are available, patients should understand that a mosaic full or partial aneuploidy involving any chromosome could have an abnormal outcome and that this outcome could differ from prior cases. For patients who may find the transfer decision stressful or anxiety-provoking, providers should encourage supportive counseling or psychotherapy with a mental health professional.

Patients should also understand that uncertainties and counseling challenges may persist after embryo transfer and into the prenatal and postnatal diagnosis realms. These may occur due to the lack of available prenatal testing that can provide full reassurance and because prenatal providers often are not familiar with embryonic mosaicism. It should be recognized that pregnancy loss, fetal anomalies, pregnancy termination, or adverse postnatal outcomes can have substantial emotional and financial effects, and the time lost before a patient can pursue another IVF cycle or alternative reproductive options is particularly relevant for women of advanced age (32).

Before transfer of an embryo with mosaic results, counseling about the general benefits, risks, and limitations of prenatal screening and diagnostic testing should be provided. Gestational carriers into whom embryos with mosaic results may be transferred should also receive thorough pretransfer counseling and should understand any plans the intended parents may have for prenatal diagnostic testing and management of an affected pregnancy. Any patient who becomes pregnant after PGT-A (from a transfer of euploid or embryo with mosaic results) should be counseled about prenatal genetic testing options (33, 34).

Prenatal Screening

Prenatal screening includes the following tests:

- Maternal serum (biochemical) screening.
- Ultrasound, including nuchal translucency and fetal anatomy scan.
- Cell-free DNA (cfDNA), also known as noninvasive prenatal testing or screening (NIPT or NIPS).

Patients should be made aware that screening tests cannot diagnose chromosomal aneuploidy. In some cases, ultrasound and biochemical analytes may help identify congenital anomalies that may be associated with an aneuploid pregnancy; however, many aneuploidies (and mosaic aneuploidies in particular) do not result in visible ultrasound anomalies or skewed biochemical analytes and may be easily missed.

Cell-free DNA testing analyzes free-floating placental DNA present in maternal blood and may test for a select number of full and partial aneuploidies, or all aneuploidies within a specified chromosomal resolution, depending on the specific test used by the laboratory. If the chromosome or chromosomal segment of interest is in fact able to be assessed by the assay used, an aneuploidy may be detected. However, it is important to recognize that NIPT is not designed for the detection of mosaicism and may result in false-negative results. False-positive results may also occur because NIPT analyzes placental (and not fetal) DNA (35).

Prenatal Diagnostic Testing

Prenatal diagnostic testing includes the following tests:

- Chorionic villus sampling (CVS) (placental testing)
- Amniocentesis (fetal testing)

Chorionic villus sampling is typically performed between 10 and 13 weeks of gestation and involves karyotyping a placental biopsy sample. Amniocentesis is typically performed beginning at 16 weeks' gestation and involves sampling fetal epithelial cells isolated from amniotic fluid. Both tests are associated with a small risk of procedural-related miscarriage (36) and thus may be undesirable for some patients, but they are the only tests available that can diagnose chromosomal aneuploidy in a pregnancy.

Although CVS is an earlier option, there are limitations of analyzing cells that are placental in origin, similar to PGT-A which tests only trophoblast/placental DNA. Alternatively, although amniocentesis cannot be performed until later in gestation, it provides the major advantage of direct analysis of fetal cells. Both tests are limited by the sample obtained; that is, they will detect mosaicism if present in the sample, but mosaicism present at a lower level or in nonplacental or nonepithelial cells will be missed. Therefore, although amniocentesis offers the best representation of the chromosome complement within fetal tissues, patients must be made aware that mosaicism can escape detection.

If prenatal diagnostic testing is performed, additional analyses on prenatal samples should be considered depending on the specific PGT-A result, and at the discretion of the ordering provider. These may include:

- Chromosomal microarray, if a partial chromosome aneuploidy is involved.
- Uniparental disomy studies, depending on the chromosome involved (37).
- Additional cell counts with a traditional karyotype, in an effort to identify lower-level mosaicism.

Tracking of Outcomes

Programs performing transfers of embryos with mosaic results should document clinical outcomes including implantation and SAB rates; prenatal and postnatal genetic test results (e.g., karyotype, chromosomal microarray); and clinical information obtained by fetal ultrasound and/or physical examination. Laboratories performing PGT-A should also track

clinical outcomes because pooling data from multiple centers will provide more powerful data sets to generate meaningful conclusions. However, caution should be taken in extrapolating outcomes from one patient to another because embryos with the same types of mosaicism will not necessarily follow the same developmental paths (32). Providers should also recognize that the reliability of karyotyping is limited because the number of cells counted can preclude detection of low-level mosaicism; the need for actively dividing cells limits the detection of mosaicism to certain cell types; and results from one tissue cannot be extrapolated to other tissues (38).

Key Points for Counseling Regarding Mosaic PGT-A Results

- Clinicians should understand the prevalence of mosaic PGT-A results issued by their reference laboratory.
- Clinics should have a policy in place regarding the reporting and management of mosaic PGT-A results. The policy should be known to staff and shared with patients before PGT-A testing.
- Transfer of embryos deemed euploid should be prioritized before considering transfer of embryos with mosaic results.
- If no embryos deemed euploid are available for transfer, patients should be offered, with due consideration of their clinical situation, the option of another IVF cycle, with or without PGT-A.
- Patients considering transfer of embryos with mosaic results should consult with a clinical genetics specialist, such as a board-certified genetic counselor, who has specific knowledge of perinatal and pediatric outcomes associated with chromosomal mosaicism.
- Patient counseling should include a discussion of the various possible explanations for mosaic PGT-A results and potential outcomes.
- A decision regarding transfer of an embryo with mosaic results is optimally made with ample time for careful consideration of the risks, benefits, and alternatives associated with this option.
- The limited outcomes reported after transfer of an embryo with mosaic results seem to be reassuring; however, current data are limited and should be interpreted with caution:
 - Lower implantation rates and higher miscarriage rates have been reported after transfer of embryos with mosaic results compared with embryos deemed euploid; these outcomes may be due in part to biases in the patient populations studied.
 - A small number of apparently healthy live births have been reported in the literature after transfer of embryos with mosaic results.
- In the prenatal and pediatric populations, cytogenetic mosaicism involving nearly every chromosome in monosomic and trisomic form has been reported in association with congenital anomalies, fetal growth restriction (also known as intrauterine growth restriction), intellectual disabilities, and/or long-term health problems. When an embryo with mosaic results successfully implants, the chance for the occurrence of such an adverse outcome is currently

unknown. Nonetheless, until further data are available, patients should be counseled on these risks.

- The following parameters for risk stratification of embryos with mosaic results have been proposed:
 - By percentage of mosaicism
 - By specific chromosome(s) involved
 - By monosomy versus trisomy
 - Whether full chromosome versus partial chromosome is affected
 - By the number of chromosomes involved (single vs. double vs. complex aneuploidies)

However, no evidence-based classification system currently exists for prioritizing embryos based on these parameters. It remains to be determined whether prenatal or postnatal mosaicism data can be applied to predict outcomes for preimplantation embryos identified as mosaic.

- Prenatal genetic counseling is strongly recommended for any pregnancy resulting from the transfer of embryos with mosaic results and should include a discussion of the risks, benefits, and limitations of CVS and amniocentesis. If prenatal diagnostic testing is performed, additional analyses beyond routine karyotyping should be considered depending on the specific PGT-A result. At the discretion of the ordering provider, these may include:
 - Chromosomal microarray, if a partial chromosome aneuploidy is involved.
 - Uniparental disomy studies (UPD), depending on the chromosome involved (37).
 - Additional cell counts, in an effort to identify lower-level mosaicism.
- Postnatal evaluation by peripheral blood karyotype and/or microarray should be considered, particularly if prenatal diagnostic testing is declined. Referral to a pediatric specialist in genetics is recommended in the event of an abnormal physical or developmental phenotype.
- Large-scale outcome studies are needed to improve data available for patient counseling. Providers are encouraged to track and publish prenatal, perinatal, and pediatric outcomes following transfer of embryo(s) with mosaic PGT-A results.

CONCLUSION

It should be recognized that this document does not endorse nor does it suggest that PGT-A is appropriate for all cases of IVF.

In clinics where PGT-A is performed there should be a policy in place regarding the reporting of mosaicism and allowance for the storage or transfer of embryos diagnosed as mosaic. This policy should be shared with every patient considering PGT-A before the initiation of their IVF cycle.

Any patient considering transfer of an embryo diagnosed as mosaic should receive genetic counseling before transfer. If an ongoing pregnancy should result, further prenatal genetic counseling and discussion of prenatal diagnostic testing options should be offered. Additionally, if an abnormal post-

natal phenotype is observed, referral to pediatric genetics should be made.

Current data suggest that embryos deemed mosaic by PGT-A result in fewer ongoing pregnancies and more SABs compared with euploid embryos. However, those patients who have had embryos transferred with mosaic results to date may have included an overrepresentation of poor prognosis patients, which introduces a population bias into these comparisons. Therefore, these data should be interpreted with caution.

Several studies reporting live births after transfer of an embryo with mosaic results have been documented, and the resulting newborns appear to be healthy. Although this is encouraging, there is a lack of accompanying postnatal correlation of chromosomal studies, and no formal evaluations or longitudinal studies have been conducted. Therefore, these data should be interpreted with caution. The field would greatly benefit from an improved effort to collect and publish the results of laboratory and clinical genetic follow-up evaluations.

There is currently no evidence-based classification system for prioritizing embryos according to the type of mosaic result. It remains to be determined whether prenatal or postnatal mosaicism data can be applied to predict outcomes for preimplantation embryos identified as mosaic. Future studies should focus on providing detailed information correlating the specific mosaic result (i.e., type of aneuploidy and level of mosaicism) of the embryos transferred with clinical outcomes, and report on documented prenatal and postnatal chromosomal data (karyotype, CMA, UPD studies) in addition to phenotypic information whenever possible.

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REFERENCES

- Goodrich D, Xing T, Tao X, Lonczak A, Zhan Y, Landis J, et al. Evaluation of comprehensive chromosome screening platforms for the detection of mosaic segmental aneuploidy. *J Assist Reprod Genet* 2017;34:975–81.
- Capalbo A. Mosaicism between trophoctoderm and inner cell mass. *Fertil Steril* 2017;107:1098–106.
- Munné S, Grifo J, Wells D. Mosaicism: “survival of the fittest” versus “no embryo left behind.” *Fertil Steril* 2016;105:1146–9.
- Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril* 2018;109:429–36.
- Chow J, Yeung WS, Lau EY, Lee VC, Ng EH, Ho PC. Array comparative genomic hybridization analyses of all blastomeres of a cohort of embryos from young IVF patients revealed significant contribution of mitotic errors to embryo mosaicism at the cleavage stage. *Reprod Biol Endocrinol* 2014;12:105.
- Munné S, Blazek J, Large M, Martinez-Ortiz PA, Nisson H, Liu E, et al. Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing. *Fertil Steril* 2017;108:62–71.
- Gutierrez-Mateo C, Colls P, Sanchez-Garcia J, Escudero T, Prates R, Ketterson K, et al. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril* 2011;95:953–8.
- Scott R, Galliano D. The challenge of embryonic mosaicism in preimplantation genetic screening. *Fertil Steril* 2016;105:1150–2.
- CoGEN. COGEN position statement on chromosomal mosaicism detected in preimplantation blastocyst biopsies. 2016. Available at: <https://ivf-worldwide.com/cogen/general/cogen-statement.html>.
- Zhang L, Wei D, Zhu Y, Gao Y, Yan J, Chen ZJ. Rates of live birth after mosaic embryo transfer compared with euploid embryo transfer. *J Assist Reprod Genet* 2019;36:165–72.
- Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. *N Engl J Med* 2015;373:2089–90.
- Ethics Committee of the American Society for Reproductive Medicine. Transferring embryos with genetic anomalies detected in preimplantation testing: an ethics committee opinion. *Fertil Steril* 2017;107:1130–5.
- Human Fertilisation and Embryology Authority. Code of Practice. 9th ed. London: HFEA. Available at: <https://portal.hfea.gov.uk/media/1527/2019-12-16-code-of-practice-9th-edition-december-2019.pdf>.
- Preimplantation Genetic Diagnosis International Society. PGDIS position statement on chromosome mosaicism and preimplantation aneuploidy testing at the blastocyst stage. *PGDIS Newsletter*; July 19, 2016. Available at: http://www.pgdis.org/docs/newsletter_071816.html.
- Cram DS, Leigh D, Handyside A, Rechitsky L, Xu K, Harton G, et al. PGDIS position statement on the transfer of mosaic embryos 2019. *Reprod Biomed Online* 2019;39:e1–4.
- Grati FR, Malvestiti F, Branca L, Agrati C, Maggi F, Simoni G. Chromosomal mosaicism in the fetoplacental unit. *Best Pract Res Clin Obstet Gynaecol* 2017;42:39–52.
- Lestou VS, Kalousek DK. Confined placental mosaicism and intrauterine fetal growth. *Arch Dis Child Fetal Neonatal Ed* 1998;79:F223–6.
- Wallerstein R, Misra S, Dugar RB, Alem M, Mazzoni R, Garabedian MJ. Current knowledge of prenatal diagnosis of mosaic autosomal trisomy in amniocytes: karyotype/phenotype correlations. *Prenat Diagn* 2015;35:841–7.
- Hsu LY, Yu MT, Neu RL, Van Dyke DL, Benn PA, Bradshaw CL, et al. Rare trisomy mosaicism diagnosed in amniocytes, involving an autosome other than chromosomes 13, 18, 20, and 21: karyotype/phenotype correlations. *Prenat Diagn* 1997;17:201–42.
- Engel E. A fascination with chromosome rescue in uniparental disomy: mendelian recessive outlaws and imprinting copyrights infringements. *Eur J Hum Genet* 2006;14:1158–69.
- Spinella F, Fiorentino F, Biricik A, Bono S, Ruberti A, Cotroneo E, Baldi M, et al. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. *Fertil Steril* 2018;109:77–83.
- Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. *Fertil Steril* 2019;111:280–93.
- Maxwell SM, Colls P, Hodes-Wertz B, McCulloh DH, McCaffrey C, et al. Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. *Fertil Steril* 2016;106:1414–9.
- Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, Wells D. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. *Hum Genet* 2017;136:805–19.
- Lledó B, Morales R, Ortiz JA, Blanca H, Ten J, Llácer J, et al. Implantation potential of mosaic embryos. *Syst Biol Reprod Med* 2017;63:206–8.
- Grati FR, Gallazzi G, Branca L, Maggi F, Simoni G, Yaron Y. An evidence-based scoring system for prioritizing mosaic aneuploid embryos following preimplantation genetic screening. *Reprod Biomed Online* 2018;36:442–9.
- Kushnir VA, Darmon SK, Barad DH, Gleicher N. Degree of mosaicism in trophoctoderm does not predict pregnancy potential: a corrected analysis of pregnancy outcomes following transfer of embryos. *Reprod Biol Endocrinol* 2018;26:16:6.
- Popovic M, Dheedene A, Christodoulou C, Taelman J, Dhaenens L, Van Nieuwerburgh F, et al. Chromosomal mosaicism in human blastocysts: the ultimate challenge of preimplantation genetic testing? *Hum Reprod* 2018;33:1342–54.
- Bunnell ME, Wilkins-Haug L, Reiss R. Should embryos with autosomal monosomy by preimplantation genetic testing for aneuploidy be transferred? Implications for embryo selection from a systematic literature review of autosomal monosomy survivors. *Prenat Diagn* 2017;37:1273–80.
- Gardner RJ, Sutherland GR, Shaffer LG, editors. *Chromosome abnormalities and genetic counseling*. 4th ed. Oxford: Oxford University Press; 2012.
- American College of Gynecologists and Endocrinologists. Counseling about genetic testing and communication of genetic test results. *ACOG Bulletin* 693. *Obstet Gynecol* 2017;129:e96–101.
- Besser AG, Mounts EL. Counselling considerations for chromosomal mosaicism detected by preimplantation genetic screening. *Reprod Biomed Online* 2017;34:369–74.
- Practice Committee of Society for Assisted Reproductive Technology, Practice Committee of American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril* 2008;90(Suppl):S136–43.
- American College of Gynecologists and Endocrinologists. Screening for fetal aneuploidy. *ACOG Practice Bulletin* 163. *Obstet Gynecol* 2016;127:979–81.
- Choi H, Lau TK, Jiang FM, Chan MK, Zhang HY, Lo PS, et al. Fetal aneuploidy screening by maternal plasma DNA sequencing: ‘false positive’ due to confined placental mosaicism. *Prenat Diagn* 2013;33:198–200.
- Akolekar R, Beta J, Picciarelli G, Ogilvie C, D’Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015;45:16–26.
- Del Gaudio D, Shinawi M, Astbury C, et al. Diagnostic testing for uniparental disomy: a points to consider statement from the American College of Medical Genetics and Genomics (ACMG). *Genet Med* [published online ahead of print, 2020 Apr 16], <https://doi.org/10.1038/s41436-020-0782-9>.
- Spinner NB, Conlin LK. Mosaicism and clinical genetics. *Am J Med Genet C Semin Med Genet* 2014;166C:397–405.