# Clinical management of mosaic results from preimplantation genetic testing for aneuploidy of blastocysts: a committee opinion

Practice Committees of the American Society for Reproductive Medicine and the Genetic Counseling Professional Group

American Society for Reproductive Medicine, Washington, DC

This revised document incorporates a growing number of published studies about mosaic embryo transfer and provides current evidence-based considerations for the clinical management of embryos with mosaic results on preimplantation genetic testing for aneuploidy. This document replaces the document titled "Clinical management of mosaic results from preimplantation genetic testing for aneuploidy (PGT-A) of blastocysts: a committee opinion," published in 2020 (Fertil Steril 2020;114:246–54). (Fertil Steril® 2023;120:973–82. ©2023 by American Society for Reproductive Medicine.)

**El resumen está disponible en Español al final del artículo.**

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

The value of preimplantation genetic testing for aneuploidy (PGT-A) as a universal screening test for all patients undergoing in vitro fertilization (IVF) has not been established (1). Indeed, 2 randomized controlled trials have shown no benefit of PGT-A in improving live birth (LB) rates, particularly in women <38 years of age (2, 3). Nonetheless, the use of PGT-A has continued to increase in the US. In particular, the significance of suspected chromosomal mosaicism in embryos has been a widely discussed and controversial topic since the first known LBs from these embryos were documented in 2015 (4). Although previous interpretations of mosaic results and patient counseling relied heavily on prenatal and pediatric literature about mosaicism, a growing body of evidence suggests that these data may not apply to preimplantation embryos. This document aims to provide a balanced discussion, review the most recent data about embryonic mosaicism, and provide evidence-based guidance to providers facing decisions about mosaicism reporting and counseling their patients about the possibility of mosaic embryo transfer (MET).

## OVERVIEW OF MOSAICISM REPORTING IN PGT-A

Traditionally, in medical genetics, mosaicism is defined as the presence of more than one chromosomally distinct cell line in one individual. In humans, any variation from 46 chromosomes is considered aneuploid. Mosaicism is diagnosed in an individual or prenatally when the presence of cells with normal and abnormal chromosome complements is observed after a standard cytogenetic karyotype, for example, on a blood or amniotic fluid sample. In contrast, next-generation sequencing (NGS), the most common method of analysis in PGT-A, uses a bioinformatics algorithm to measure the amount of DNA represented by each chromosome compared with a normal reference. Therefore, the diagnosis of chromosomal mosaicism in a trophectoderm biopsy is not determined using the visual observation of distinct euploid and aneuploid individual cells. Instead, it is inferred from collectively analyzing the DNA extracted and amplified from a group of cells and observing an intermediate chromosome copy number on an NGS profile (Fig. 1).

An intermediate copy number between 2 and 3 (disomy and trisomy ranges) may be interpreted as mosaic trisomy, whereas an intermediate copy number between 1 and 2 (monosomy and disomy ranges) may be interpreted as mosaic monosomy. It is important to recognize that, aside from true

Received August 25, 2023; accepted August 29, 2023; published online September 9, 2023.
Correspondence: Practice Committee of the American Society for Reproductive Medicine, 1209 Montgomery Highway, Birmingham, Alabama 35216 (E-mail: asrm@asrm.org).

Fertility and Sterility® Vol. 120, No. 5, November 2023 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2023.08.969

mosaicism, there are several other proposed contributors to and explanations for intermediate copy number results, including statistical variation (test artifact and "noise"), DNA amplification bias, contamination, mitotic state, variation in embryo biopsy technique, and embryology laboratory conditions (5–8). A PGT-A result showing an intermediate copy number, therefore, infers that the biopsied sample is mosaic, and such embryos may, in fact, be euploid, aneuploid, mosaic for a euploid and an aneuploid cell line, or mosaic for 2 or more different abnormal cell lines (9). Because of the inability to confirm a diagnosis of true mosaicism in such embryos, it has been proposed that the term "mosaic" be extinguished altogether (10).

True embryonic mosaicism has long been recognized as a potential limiting factor in the interpretation of PGT-A (1) and as a contributing factor in misdiagnosis related to biopsy sample size (11, 12). Suspected mosaicism has typically gone undetected or unreported with prior methods of PGT-A, such as fluorescent in situ hybridization, which tested single cells, and array comparative genomic hybridization, as well as the single nucleotide polymorphism microarray (currently in use). With more recent and sensitive assays, such as NGS, it has become increasingly common to identify and report results consistent with an intermediate copy number.

The frequency and clinical relevance of mosaicism have been the subject of much debate (7, 13, 14). The rate of mosaic results (without concurrent nonmosaic aneuploidy) in the clinical testing of trophectoderm is between 2% and >20% (9, 15), depending on multiple factors (Table 1) (16, 17). Unique test methodologies, assays, reporting practices, and philosophies all contribute to the rate of mosaic results reported by each specific PGT-A laboratory. Because of this, the same biopsy could be resulted as "high-level mosaic" at one laboratory and an "aneuploid" or "euploid" at another. Examining the issue of analytical and clinical validation of PGT-A and mosaic results (i.e., how exactly one arrives at an accurate PGT-A diagnosis that is predictive of the embryo's clinical outcome) is beyond the scope of this document but it is an important topic that deserves further consideration (18). Similarly, the question of whether reporting of mosaic results increases IVF success rates by allowing the deprioritization of embryos with lower reproductive potential or, conversely, leads to unnecessary risk counseling and patient anxiety without clinical benefit is a critical ongoing debate (19, 20).

In an effort to provide guidance about PGT-A mosaicism, several professional organizations have created statements to guide clinicians who are faced with the complex task of interpreting such laboratory results and fostering informed patient decision-making. The American Society for Reproductive Medicine released an Ethics Committee Opinion in 2017, providing general recommendations for handling "anomalous" positive results from preimplantation genetic testing (PGT) (21). The Human Fertilisation and Embryology Authority of the United Kingdom also addresses this in the Code of Practice (22). The Preimplantation Genetic Diagnosis International Society and Congress on Controversies in Preconception, Preimplantation, and Prenatal Genetic Diagnosis also developed statements (15, 23, 24).

## REVIEW OF OUTCOME DATA AFTER TRANSFER OF EMBRYOS WITH MOSAIC PGT-A RESULTS

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. There have now been over 2,700 documented embryos transferred with mosaic results (8).

### Reproductive potential of embryos with mosaic results

Initial studies found MET to be associated with reduced embryo implantation and sustained pregnancy, as well as increased (SAB) (14, 25–30), compared with euploid embryo transfer (ET). The largest MET study to date was published in 2021 and retrospectively reported on outcomes associated with 1,000 mosaic embryos that had been tested by similar PGT-A assays and transferred at 5 different clinics and compared these outcomes to those of euploid embryos (31). Mosaic embryos had significantly lower implantation rates, even when controlling for differences in embryo morphology. Mosaic ETs also led to more than twice the rate of SABs compared with euploid ETs. A limitation of retrospective outcome studies is that embryos with mosaic results are typically only transferred when there are no euploid embryos available. Therefore, the population of patients that underwent MET likely contained more poor-prognosis patients (i.e., those unable to produce any or as many euploid embryos or with a history of failed euploid ETs) than the population that transferred euploid embryos. Furthermore, given the known overlap between mosaic and aneuploid copy number ranges, it is likely that the embryos represented in MET studies contain some aneuploid embryos. These 2 factors alone could explain the poorer outcomes.

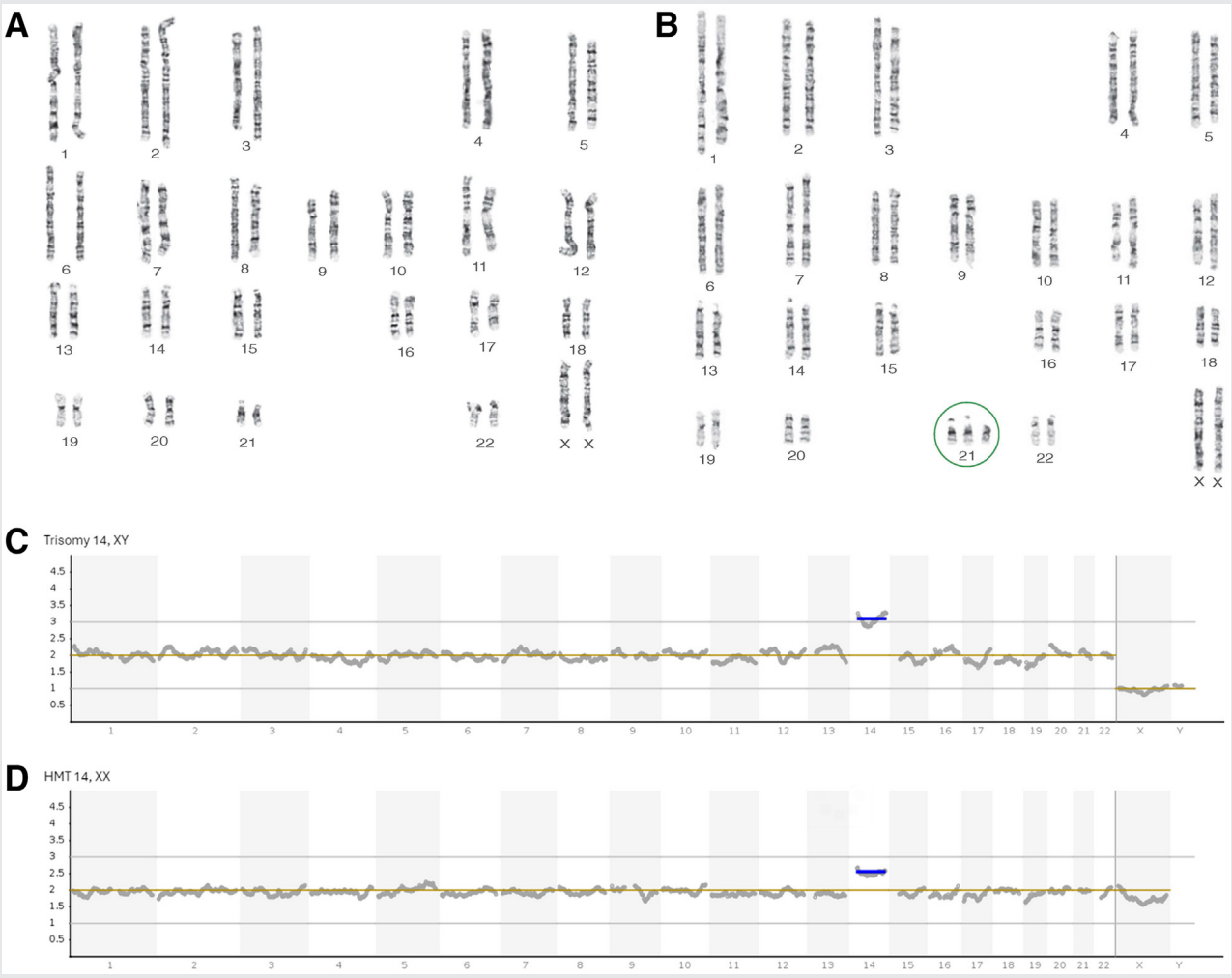
Subsequently, a prospective nonselection study was conducted in which embryos were classified as euploid when the copy number was determined to be between 1.5 and 1.8 or 2.2 and 2.5 (i.e., under 50% predicted mosaicism for monosomy or trisomy) (20). Intermediate copy numbers below 50% were only revealed after transfer outcomes were known, and the investigators did not find any difference in ongoing pregnancy or SAB rates compared with embryos with <20% mosaicism (copy numbers 1.8–2.2) or "true" euploidy. The investigators, therefore, concluded that putative mosaicism levels under 50% do not impact early embryonic development when using their particular PGT-A assay in an unselected population free of ascertainment bias.

Given the different findings among MET outcome studies to date, more data are needed to clarify whether certain mosaic findings are clinically relevant to an embryo's reproductive potential. In addition, given the variations in laboratory mosaic result calling and rates, it is unknown whether clinical outcome data associated with using one laboratory's PGT-A assay can be extrapolated to another.

### Prenatal and neonatal outcomes of embryos with mosaic results

In the general population, mosaicism identified in a pregnancy or neonate is associated with an increased risk of an adverse

## FIGURE 1




(A) Normal karyotype; (B) Trisomy 21 karyotype; (C) Three copy (trisomy) chromosome 21 NGS profile; and (D) Intermediate 2–3 copy number chromosome 21 next-generation sequencing profile. Images are used with permission from NextGen Genetics.

*Goldstein. Practice Committees of the American Society for Reproductive Medicine and the Genetic Counselors Professional Group. Mosaic results. Fertil Steril 2023.*

outcome and, therefore, may be cause for concern. Mosaic aneuploidies involving nearly every chromosome have been associated with abnormal phenotypes in pregnancies and LBs, regardless of the method of conception (32, 33). In contrast, mosaicism identified in the preimplantation embryo has thus far not been definitively associated with a significantly increased risk of an adverse fetal or neonatal outcome.

A review of 25 published studies about MET was published in 2021 and found that <1% of 2,759 embryos transferred resulted in an ongoing aneuploid pregnancy related to the original PGT-A result (8). As more data from METs continues to accumulate, it is expected that additional cases of fetal or neonatal confirmation of the mosaic PGT-A result will be discovered.

At the time of this writing, there have been 3 published case reports of fetal aneuploidy resulting from an embryo identified as mosaic for the same chromosome. In the first case, extremely low-level mosaicism was detected prenatally in a normal-appearing fetus after testing performed only because of the mosaic PGT-A finding. Although the resulting newborn appeared phenotypically normal, the low-level mosaicism was detected also in peripheral blood, and the investigators acknowledged that because of no additional tissues were studied, the long-term significance of the low-level mosaicism is unknown (34). In the second case, a fetal nonmosaic duplication identified prenatally was in a similar chromosomal location to a mosaic duplication that had been reported on the embryo's PGT-A; the resulting newborn was reported to have an apparently isolated coarctation of the aorta (35). In the third case, a complex structural aneuploidy and uniparental disomy (UPD) involving the same chromosome reported as mosaic on PGT-A were detected postnatally after an investigation of newborn feeding difficulties (36). It is important to highlight that the neonatal aneuploidy did not appear to be mosaic in the latter 2 cases. Although discordance between the trophectoderm and inner cell mass and/or the possibility of complex aneusomy rescue mechanisms (such as chromosome shattering) may explain these results,

**TABLE 1**

**Factors influencing the interpretation and reporting of mosaic results.**

| Factor | Example |
|---|---|
| Technology | • DNA amplification method (whole genome vs. targeted)<br>• Assay and platform<br>• Method of analytical validation<br>• Custom laboratory protocols and after-market modifications |
| Results interpretation | • Software (e.g., algorithm-assisted)<br>• Subjective (technician-dependent) |
| Reporting protocol | • Thresholds and cutoffs for distinguishing mosaic from euploid or aneuploid results; allowance for clinicians to request masking of mosaic results (and whether the masked intermediate copy number is reported as euploid or aneuploid)<br>• Selective reporting (e.g., not reporting mosaicism for certain whole chromosomes or when in combination with other mosaic and aneuploid chromosomes; reporting mosaic segmental findings as aneuploid) |
| Clinical and embryology factors | • Undetermined (under investigation); proposed variables include type of culture media, pH, temperature, osmolality, and oxygen concentration; laboratory techniques; method of insemination; and laser use or handling of biopsied cells (16, 17). |

*Goldstein. Practice Committees of the American Society for Reproductive Medicine and the Genetic Counselors Professional Group. Mosaic results. Fertil Steril 2023.*

an alternative and perhaps simpler explanation is that the initial mosaic PGT-A results were misdiagnoses of embryos with nonmosaic (meiotic) aneuploidy (8, 37, 38).

**Uniparental disomy** On the basis of experience with prenatal and postnatal cases, concerns have been raised regarding the possibility of an increased risk for UPD in pregnancies resulting from mosaic embryos (23, 39, 40). Although rare, when fetal mosaicism is caused by a postzygotic trisomy or monosomy rescue event, the 2 remaining chromosomal copies may originate from the same parent, resulting in UPD. Although there is no apparent phenotypic effect for most chromosomes related to UPD (32), those chromosomes with imprinted regions containing genes for which expression depends on the parent of origin have been associated with abnormal phenotypes. Specifically, regions of chromosomes 6, 7, 11, 14, 15, and 20 are associated with known imprinting disorders, although there is less consistent literature regarding UPD for other chromosomes (40). Additionally, there are documented cases of recessive monogenic disease attributed to UPD, which can occur when a pathogenic variant is located on the duplicated parental allele (41). However, the largest data set determining the parental origin of chromosomes for neonates conceived from mosaic embryos found that all 38 infants were confirmed to have genome-wide biparental inheritance, with no cases of UPD identified (32). A single case report described an abnormal phenotype potentially related to the occurrence of UPD following MET (36). The estimated prevalence of UPD is 1 in 2,000 to 5,000 births (40); because of its overall rarity and because UPD testing is not performed routinely as part of prenatal care, it is unknown whether the risk of a clinically significant UPD is increased after MET.

**Confined placental mosaicism** A theoretical concern about confined placental mosaicism (CPM) resulting in fetal growth restriction or other obstetric complications after MET has been raised (15, 23, 42). However, a case-control study to assess the birth weight and length of gestation of 162 newborns from each MET and euploid ET was conducted in 2020, and no significant differences were observed (43). Placental data from MET pregnancies are lacking and would be needed to determine whether CPM prevalence is indeed higher and whether CPM presence is associated with an increased risk of adverse outcomes. In the absence of such data, increased fetal growth restriction surveillance after MET may not be warranted.

## Pediatric and long-term outcomes of embryos with mosaic results

To date, MET studies have focused on prenatal and newborn outcomes; no longitudinal studies have been performed to assess long-term outcomes beyond the neonatal period. Nonetheless, in its current application, it is outside of the scope of PGT-A to predict an individual's health status or reduce medical burdens after delivery. Because PGT-A can only assess aneuploidy status, which is associated primarily with the viability (or nonviability) of an embryo, current evidence does not support its use to predict long-term health issues aside from those related to aneuploidies.

## Risk assessment and ranking of mosaic result embryos

Attempts have been made to prioritize embryos with different types of mosaic PGT-A results concerning their success rate and perceived risk (15, 23, 24, 42, 31). Despite these approaches, the influence of mosaicism-related factors on clinical outcome data has been inconsistent, and a universally applicable, evidence-based approach has not been developed. As discussed previously, the diagnosis and clinical significance of a mosaic result frequently differ across laboratories (e.g., 45% aneuploidy may be considered euploid, low-level mosaic, or high-level mosaic, depending on the laboratory). Furthermore, although large data sets from prenatal and products of conception samples may be referenced, it should not be assumed that these data can be extrapolated to the preimplantation embryo because fetoplacental and embryonic mosaicism may not be mechanistically related. Factors that may be used in evaluating and comparing MET outcomes and risks are summarized in Table 2 (5, 8, 9, 14, 25–27, 39–

**TABLE 2**

**Potential factors to evaluate MET outcomes and risks (2, 5, 12, 23-25, 27, 28, 29, 36, 42, 43)**

| Risk | Considerations |
|---|---|
| Percentage of mosaicism | • Although mosaicism levels may differ based on the site of biopsy (42) and cutoffs are determined in part by the test assay and validation strategy, most studies have found a lower percentage of mosaicism to be associated with a higher implantation and ongoing pregnancy rate (24, 29).<br>• It is unknown whether mosaicism levels impact risks to an ongoing pregnancy. However, higher-level mosaicism may have greater overlap with the full (nonmosaic) aneuploid copy number range (5, 36) and therefore be more likely to represent a misdiagnosis of a true aneuploid embryo. |
| Specific chromosome(s) involved | • There is no known correlation between specific mosaic chromosomes and the reproductive outcome (success rate or risk to fetus/neonate).<br>• Although it may be intuitive to assign higher risk to mosaic aneuploidies involving certain chromosomes (e.g. 13, 18, 21, commonly referred to as "viable aneuploidies" in the nonmosaic state), to date a limited number of such METs have not resulted in any fetal confirmations. |
| Monosomy vs. trisomy | • No differences in pregnancy or SAB rates have been observed when comparing embryos mosaic for monosomies vs. trisomies (12, 25).<br>• 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); therefore, an embryo with mosaic monosomy may also have an undetectable mosaic trisomy cell line. |
| Full chromosome vs. partial (segmental) chromosome | • Segmental mosaic aneuploidy may be more likely than whole chromosome mosaic aneuploidy to represent a false-positive result due to test artifact (2).<br>• Segmental mosaic aneuploidy detected in trophectoderm is less likely to show concordance with the inner cell mass, compared with whole chromosome aneuploidy (43).<br>• Most prospective MET studies have found higher ongoing pregnancy rates for embryos with segmental mosaic aneuploidy as compared with whole chromosome mosaics (23, 25, 27).<br>• There is currently not enough data to determine whether embryos with segmental vs whole chromosome mosaic aneuploidy have different risks of resulting in persisting fetal aneuploidy. Due to differences in resolution, deletions and duplications detected by PGT-A are generally much larger than those detected in ongoing pregnancies or live births. |
| Number of chromosomes involved | • Current prospective MET data indicate reduced pregnancy potential of embryos reported as mosaic for 3 or more chromosomes as compared with those with one or 2 mosaic findings (12, 29). A significant difference between mosaicism involving one vs. 2 chromosomes (12, 28, 29) has not been observed. |

*Goldstein. Practice Committees of the American Society for Reproductive Medicine and the Genetic Counselors Professional Group. Mosaic results. Fertil Steril 2023.*

31, 38, 44, 45). Additional data are needed to determine whether these categories can reliably be applied to clinical decision-making.

The question of whether euploid embryos should universally be transferred preferentially to mosaic embryos has been raised (46). Although outcome data do not necessarily support this recommendation, to reduce the patient burden of uncertainty presented by MET, it may be reasonable to prioritize euploid embryos over mosaic embryos, depending on patient preference. Differences in morphology may also be considered as part of this decision (43).

Undoubtedly, more pregnancy and neonatal outcome data will be published in the future, adding to our understanding of reproductive potential and the risks associated with the transfer of embryos with mosaic results.

## PRENATAL TESTING AFTER MET

### Prenatal Screening

Prenatal screening includes the following tests:

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

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

Cell-free DNA testing analyzes 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. It should be noted that NIPT is not designed to detect mosaicism and may result in false-negative results. False-positive results may occur also because of the limitations of this technology (47). Additionally, NIPT analyzes placental (and not fetal) DNA (48). Therefore, CPM could potentially obfuscate results. For these reasons, a prenatal diagnosis is recommended to confirm a positive NIPT result.

### Prenatal diagnostic testing

Prenatal diagnostic testing includes the following tests:

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

Chorionic villus sampling (CVS) is typically performed between 10 and 13 weeks of gestation and involves analyzing a placental biopsy sample. Amniocentesis is typically performed beginning at 16 weeks of gestation and involves sampling fetal epithelial cells isolated from amniotic fluid. Both tests are associated with a small risk of procedural-related

miscarriage (49) 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 to analyzing cells that are placental in origin, similar to PGT-A, which tests only trophectoderm and placental cells. 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 when it is present in the sample, but mosaicism will be missed when present at a lower level or in nonplacental or nonepithelial cells. Therefore, although amniocentesis offers the best representation of the chromosome complement within fetal tissues, patients must be made aware that mosaicism can escape detection.

Analyses on prenatal testing samples beyond a standard karyotype may be considered, depending on the specific PGT-A result and at the discretion of the ordering provider. These may include:

- Chromosomal microarray, when partial chromosome aneuploidy is involved
- Additional cell counts with a traditional karyotype or fluorescent in situ hybridization to identify lower-level mosaicism
- Uniparental disomy studies, depending on the chromosome involved (40, 50)

Currently, there is a lack of data to inform evidence-based recommendations for prenatal testing after MET (19). One consideration is whether pregnancies from embryos with mosaic results are at increased risk of general fetal anomalies compared with embryos with euploid results. Another point of discussion is the risk of precise fetal aneuploidy related to the mosaic PGT-A result, which is likely very low on the basis of the currently available data but should not be dismissed. The American College of Obstetricians and Gynecologists recommends that prenatal diagnosis be offered to all pregnant people (51), including pregnancies conceived from IVF with PGT-A, regardless of the statistical risk for fetal aneuploidy. Whether prenatal diagnosis is specifically recommended after MET and which tests are indicated is a matter of debate. Although some publications unequivocally recommend prenatal diagnosis, others have advised that the options of screening and diagnosis be presented along with the benefits and limitations specific to MET and a discussion of the patient's goals (15, 35, 39, 46). The American College of Medical Genetics and Genomics recommends prenatal UPD testing after METs involving certain chromosomes (40). Given that prenatal diagnostic procedures introduce a small but real risk of pregnancy loss and complications, and each additional analysis ordered increases the cost and chance for uncertain results (50), questions remain about whether these risks are outweighed by the benefits of gaining more clarity about the pregnancy (19). Genetic counseling is strongly recommended for any patient pregnant after the transfer of a mosaic-result embryo and should include a discussion of the risks, benefits, and limitations of prenatal testing options.

### Investigation of abnormal genetic results after conception from a mosaic embryo

Because genetic results can be highly nuanced, any finding identified by prenatal or product of conception (POC) testing warrants review by a genetic counselor. A chromosomal finding may be related or unrelated to the original PGT-A result, and this distinction is essential when reporting on MET outcome data. For example, when an embryo with reported mosaic trisomy 13 is transferred and the resulting POC is identified to have trisomy 13, then these results are considered related. In contrast, when the POC is identified as having trisomy 8, these results are highly unlikely to be related. Unrelated results may include also small copy number variants (deletions or duplications) below the resolution of PGT-A, which are identified in approximately 2.5% of prenatal samples and 4.4% of POCs (52, 53).

Mosaic aneuploidy detected in an ongoing pregnancy can be difficult to interpret. Additionally, caution should be taken in extrapolating outcomes from one embryo to another because embryos with the same types of mosaicism will not necessarily follow the same developmental paths (39). In the presence of ultrasound anomalies, true fetal mosaicism poses an increased 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 their distribution among various tissues in addition to the specific chromosomal abnormality (32). Postnatally, chromosomal mosaicism is frequently associated with physical and developmental anomalies. However, these findings are subject to ascertainment bias, and mosaicism has been identified also in individuals without phenotypic anomalies (32, 33). Therefore, PGT laboratories and IVF programs should document clinical outcomes after MET, including implantation and SAB rates; prenatal and postnatal genetic test results (e.g., karyotype, chromosomal microarray); and phenotypic information obtained by fetal ultrasound and postnatal physical examination.

## CLINICAL POLICY DEVELOPMENT AND GENETIC COUNSELING

### Clinic policy development: reporting of mosaic results

Most PGT-A laboratories offer ordering practitioners different choices regarding the reporting of mosaic results. Putting the onus on the provider to decide how results should be categorized is uncommon in other areas of medicine, and the clinical implications of these decisions are essential for providers and patients to understand. For both mosaic reporting and nonmosaic reporting, the cutoffs used to categorize results can differ dramatically between laboratories (and even within a laboratory for certain chromosome findings) on the basis of laboratory-selected thresholds and the preferences of the ordering provider. When mosaic results are not reported, it is crucial that the provider understand whether intermediate copy number findings are reported as "euploid and normal"

or as "aneuploid and abnormal" on the basis of the laboratory's reporting thresholds.

Providers are encouraged to use current data to make evidence-based clinical decisions regarding whether to opt in or out of mosaicism reporting. For example, some data show that categorizing 20%–50% mosaic results as aneuploid could lead to decreased LB rates per cycle because of fewer embryos available to transfer (20). Clinics should additionally consider the impact of the 21st Century Cures Act, which enables patients to have on-demand access to their medical records, including laboratory test results (54). Whether patients have the right to access unreported or "raw" (uninterpreted) data from the laboratory remains unclear and controversial (55). In addition, clinics should determine how to manage PGT laboratory changes to mosaicism reporting that may alter categorization or lead to reinterpretation of previously reported results when requested.

## Clinical policy development: transfer, storage, and disposition of mosaic embryos

Each IVF program should develop its own internal policies addressing the transfer, storage, and disposition of embryos reported as mosaic (39). These policies define whether or not the clinic supports MET and address any requirements and restrictions around this allowance, including:

- Genetic counseling
- Consent form(s)
- Waiting periods (e.g., in the event that a preferred embryo does not survive warming on the day of planned ET)
- Whether certain types of mosaic embryos are not permitted to be transferred
- Allowance for multiple ETs (e.g., multiple mosaic embryos, mosaic with an euploid embryo, and mosaic with an untested embryo)
- Use of mosaic embryos before euploid or untested embryos
- Use of a compensated or compassionate gestational carrier
- Transport of mosaic embryos to or from another clinic or storage facility

Such policies should be shared widely with staff and patients before initiating a PGT-A cycle and at additional relevant touchpoints in the treatment process, such as during the review of PGT-A results and at annual embryo storage billing.

## Genetic counseling: pretest education

Before pursuing any genetic testing, including PGT-A, patients should be informed of the risks, benefits, and limitations of the technology used (51) and the implications of the results. Pretest education and informed consent about PGT-A should include discussion of the following:

- The purpose, scope, and limitations of PGT-A (1)
- The expected frequency of each type of result, including mosaic results (as quoted by the testing laboratory)
- The potential for receiving information that may be unclear or difficult to interpret in the context of ET and storage decisions (39)
- The clinic's policy regarding the transfer and storage of embryos with different types of results
- The option to decline PGT-A

## Genetic counseling: pretransfer

A variety of circumstances may lead a patient to consider transferring an embryo with mosaic results. Before the initiation of the ET cycle, patients should be offered genetic counseling by a board-certified genetic counselor specializing in PGT. The discussion should include the following points:

- There are several possible explanations for mosaic PGT-A results; for example, trophectoderm mosaicism with or without inner cell mass mosaicism and test artifacts.
- There is currently no evidence-based method to predict the risk of an adverse outcome or rank mosaic embryos for transfer on the basis of the factors outlined in Table 2.
- Studies have suggested that some types of mosaic-result embryos may have reduced implantation potential and an increased risk of spontaneous abortion compared with euploid embryos.
- Ongoing pregnancy and delivery data is largely reassuring; for cases where prenatal test results have been available, $<1\%$ have been confirmed in the fetus or neonate (8).
- Prenatal genetic counseling is recommended to discuss the benefits, risks, and limitations of prenatal screening and diagnosis.
- When fetal aneuploidy is confirmed prenatally, there may be a significant risk of adverse outcomes; however, the magnitude of the risk may be unclear.
- Consultation with a mental health professional may benefit patients in their MET decision-making.

## SUMMARY

- 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.
- Thus far, outcomes reported in the literature suggest that mosaic results may impact early pregnancy potential and embryo viability. Lower implantation and higher miscarriage rates after the transfer of embryos with certain mosaic results compared with those deemed euploid have been observed in many studies; these outcomes may be due in part to biases in the patient populations studied.
- On the basis of currently available data, fetal aneuploidy related to the mosaic PGT-A result is likely very low ($<1\%$).
- Although categories of mosaic result types may be useful for assessing reproductive potential and prioritizing ET, it is unclear whether they can be used to predict prenatal and postnatal risks accurately.

## CONCLUSIONS

- 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.

- Clinicians should understand the prevalence and reporting structure (including the implications of "masking") of mosaic PGT-A results issued by their reference laboratory.
- Patients considering the transfer of embryos with mosaic results should be offered a consultation with a board-certified genetic counselor specializing in PGT and mosaic results.
- As with all pregnancies, genetic counseling and prenatal testing should be offered to patients who conceive after MET in accordance with the American College of Obstetricians and Gynecologists and the American College of Medical Genetics and Genomics guidelines.
- As with all neonates, referral to a pediatric specialist in genetics is recommended in the event of an abnormal physical or developmental phenotype (56).
- Providers and PGT laboratories are encouraged to track and publish prenatal, perinatal, and pediatric outcomes after the transfer of embryos with mosaic PGT-A results to further improve patient counseling.

**Acknowledgments:** This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine have approved this report.

This document was reviewed by ASRM members, and their input was considered in the preparation of the final document. The following members of the ASRM Practice Committee participated in the development of this document: Alan Penzias, M.D.; Paula Amato, M.D.; Jacob Anderson; Kristin Bendikson, M.D.; Clarisa Gracia, M.D., M.S.C.E.; Tommaso Falcone, M.D.; Rebecca Flyckt, M.D.; Karl Hansen, M.D., Ph.D.; Micah Hill, D.O.; Sangita Jindal, Ph.D.; Suleena Kalra, M.D., M.S.C.E.; Tarun Jain, M.D.; Bruce Pier, M.D.; Michael Thomas, M.D.; Richard Reindollar, M.D.; Jared Robins, M.D.; Chevis N. Shannon, Dr.Ph., M.B.A., M.P.H.; Anne Steiner, M.D., M.P.H.; Cigdem Tanrikut, M.D.; and Belinda Yauger, M.D. The Practice Committee acknowledges the special contribution of Andria Besser, M.S., C.G.C.; Lauri Black, M.S., C.G.C.; Amy Jordan, M.S., C.G.C.; and Emily Mounts, M.S., C.G.C. in the preparation of this document. All Committee members disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients. Members of the Committee who were found to have conflicts of interest based on the relationships disclosed did not participate in the discussion or development of this document.

## REFERENCES

1. 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(3):429–36.
2. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, et al. Live birth with or without preimplantation genetic testing for aneuploidy. N Engl J Med 2021;385:2047–58.
3. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril 2019;112(6):1071–9.e7.
4. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med 2015;373:2089–90.
5. 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.
6. Capalbo A, Rienzi L. Mosaicism between trophectoderm and inner cell mass. Fertil Steril 2017;107:1098–106.
7. Munné S, Grifo J, Wells D. Mosaicism: "survival of the fittest" versus "no embryo left behind" Fertil Steril 2016;105:1146–9.
8. Treff NR, Marin D. The "mosaic" embryo: misconceptions and misinterpretations in preimplantation genetic testing for aneuploidy. Fertil Steril 2021;116:1205–11.
9. Scott RT, Galliano D. The challenge of embryonic mosaicism in preimplantation genetic screening. Fertil Steril 2016;105:1150–2.
10. Paulson RJ, Treff NR. Isn't it time to stop calling preimplantation embryos "mosaic"? F S Rep 2020;1:164–5.
11. Gutiérrez-Mateo C, Colls P, Sánchez-García 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.
12. Friedenthal J, Maxwell SM, Tiegs AW, Besser AG, McCaffrey C, Munné S, et al. Clinical error rates of next generation sequencing and array comparative genomic hybridization with single thawed euploid embryo transfer. Eur J Med Genet 2020;63:103852.
13. Chow JF, 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.
14. 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.e8.
15. CoGEN. COGEN position statement on chromosomal mosaicism detected in preimplantation blastocyst biopsies. Available at: https://ivf-worldwide.com/cogen/general/cogen-statement.html. Accessed October 3, 2023.
16. Swain JE. Controversies in ART: can the IVF laboratory influence preimplantation embryo aneuploidy? Reprod Biomed Online 2019;39:599–607.
17. Huang QX, Wang ZH, Huang WJ, Mao LH, Lin CL, Chen GY, et al. Factors influencing mosaicism: a retrospective analysis. Reprod Biomed Online 2022;45:491–500.
18. Jalas C, Seli E, Scott RT Jr. Key metrics and processes for validating embryo diagnostics. Fertil Steril 2020;114:16–23.
19. Besser AG, Mounts EL, Grifo JA. Evidence-based management of preimplantation chromosomal mosaicism: lessons from the clinic. Fertil Steril 2021;116:1220–4.
20. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet 2021;108:2238–47.
21. 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.

22. Human Fertilisation and Embryology Authority. Code of practice. 9th ed. London: Human Fertilisation and Embryology Authority; 2021. Available at: https://portalhfea.gov.uk/media/it1n3vpo/2022-07-01-code-of-practice-2021.pdf. Accessed September 21, 2023.
23. Preimplantation genetic diagnosis International Society. PGDIS position statement on chromosome mosaicism and preimplantation aneuploidy testing at the blastocyst stage; 2016. PGDIS Newsletter. Available at: http://www.pgdis.org/docs/newsletter_071816.html. Accessed October 3, 2023.
24. 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(Suppl 1):e1–4.
25. 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.
26. Spinella F, Fiorentino F, Biricik A, Bono S, Ruberti A, Cotroneo E, et al. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. Fertil Steril 2018;109:77–83.
27. 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.
28. Maxwell SM, Colls P, Hodes-Wertz B, McCulloh DH, McCaffrey C, Wells D, 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.e5.
29. Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, et al. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. Hum Genet 2017;136:805–19.
30. 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.
31. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril 2021;115:1212–24.
32. 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.
33. 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.
34. Kahraman S, Cetinkaya M, Yuksel B, Yesil M, Pirkevi Cetinkaya C. The birth of a baby with mosaicism resulting from a known mosaic embryo transfer: a case report. Hum Reprod 2020;35:727–33.
35. Yang M, Rito T, Metzger J, Naftaly J, Soman R, Hu J, et al. Depletion of aneuploid cells in human embryos and gastruloids. Nat Cell Biol 2021;23:314–21, Erratum in: Nat Cell Biol 2021;23:314–321.
36. Schlade-Bartusiak K, Strong E, Zhu O, Mackie J, Salema D, Volodarsky M, et al. Mosaic embryo transfer—first report of a live born with non-mosaic partial aneuploidy and uniparental disomy 15. F&S Reports 2022;3:192–7.
37. Handyside AH, McCollin A, Summers MC, Ottolini CS. Copy number analysis of meiotic and postzygotic mitotic aneuploidies in trophectoderm cells biopsied at the blastocyst stage and arrested embryos. Prenat Diagn 2021;41:525–35.
38. García-Pascual CM, Navarro-Sánchez L, Navarro R, Martínez L, Jiménez J, Rodrigo L, et al. Optimized NGS approach for detection of aneuploidies and mosaicism in PGT-A and imbalances in PGT-SR. Genes (Basel) 2020;11:724.
39. Besser AG, Mounts EL. Counselling considerations for chromosomal mosaicism detected by preimplantation genetic screening. Reprod Biomed Online 2017;34:369–74.
40. Del Gaudio D, Shinawi M, Astbury C, Tayeh MK, Deak KL, Raca G, et al. Diagnostic testing for uniparental disomy: a points to consider statement from the American College of Medical Genetics and Genomics (ACMG). Genet Med 2020;22:1133–41.
41. 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.
42. 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.
43. Viotti M, Victor A, Barnes F, Zouves C, Besser AG, Grifo JA, et al. New insights from one thousand mosaic embryo transfers: features of mosaicism dictating rates of implantation, spontaneous abortion, and neonate health. Fertil Steril 2020;114:e1–2.
44. 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.
45. Kim J, Tao X, Cheng M, Steward A, Guo V, Zhan Y, et al. The concordance rates of an initial trophectoderm biopsy with the rest of the embryo using PGTseq, a targeted next-generation sequencing platform for preimplantation genetic testing-aneuploidy. Fertil Steril 2022;117:315–23.
46. Leigh D, Cram DS, Rechitsky S, Handyside A, Wells D, Munne S, et al. PGDIS position statement on the transfer of mosaic embryos 2021. Reprod Biomed Online 2022;45:19–25.
47. Strom CM, Anderson B, Tsao D, Zhang K, Liu Y, Livingston K, et al. Improving the positive predictive value of non-invasive prenatal screening (NIPS). PLOS ONE 2017;12:e0167130.
48. 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.
49. 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.
50. Mounts EL, Besser AG. Lack of evidence to support recommendation for prenatal uniparental disomy (UPD) analysis following mosaic embryo transfer. Genet Med 2021;23:230–1.
51. Screening for Fetal Chromosomal Abnormalities. Obstetrics & Gynecology 2020;136:e48–69.
52. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012;367:2175–84.
53. Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, Demko Z, et al. Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. Obstet Gynecol 2014;124:202–9.
54. The Office of the National Coordinator for Health Information Technology, 21st century Cures Act. Available at: https://www.healthit.gov/topic/oncs-cures-act-final-rule. Accessed September 21, 2023.
55. ACLU. Our genes, our rights. Available at: https://www.aclu.org/cases/our-genes-our-data-patients-right-access-their-own-genetic-information. Accessed October 3, 2023.
56. Pletcher BA, Toriello HV, Noblin SJ, Seaver LH, Driscoll DA, Bennett RL, et al. Indications for genetic referral: a guide for healthcare providers. Genet Med 2007;9:385–9.

**Manejo clínico de los resultados de mosaico en las pruebas genéticas preimplantacionales para aneuploidía de blastocistos: opinión del comité**

Este documento revisado incorpora un número creciente de estudios publicados sobre la transferencia de embriones mosaico y proporciona consideraciones actuales basadas en la evidencia para el manejo clínico de embriones con resultado de mosaico en las pruebas genéticas preimplantacionales para aneuploidía. Este documento sustituye al documento titulado: "Manejo clínico de resultados mosaico en las pruebas genéticas preimplantacionales para aneuploidía (PGT-A) de blastocistos: una opinión de comité", publicado en 2020.