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REVIEW

PGDIS position statement on the transfer of mosaic embryos 2021

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KEY MESSAGE

Since the 2019 PGDIS position statement on mosaicism, uncertainty has remained about the implications of mosaic embryo transfer. Recently published information is reviewed and updated recommendations are provided for test laboratories, clinics, clinicians and genetic counsellors on the transfer of mosaic embryos, replacing previous 2016 and 2019 PGDIS position statements.

ABSTRACT

Chromosome testing strategies, such as preimplantation genetic testing for aneuploidy (PGT-A), improve initial IVF outcomes by avoiding unwitting transfer of aneuploid embryos in morphology-based selection practices. Newer technologies have revealed that some embryos may appear to have intermediate whole chromosome (or parts of a chromosome termed segmental) copy number results suggesting trophectoderm mosaicism. An embryo with a trophectoderm mosaic-range result may be the only option for transfer for some patients. Recent data suggest that such mosaic embryos can be transferred without added risk of abnormal birth outcomes but may be associated with increased implantation failure and miscarriage rates, with higher values of mosaicism appearing to be less favourable for producing good outcomes. In this Position Statement, we provide guidance to laboratories, clinics, clinicians and counsellors to assist in discussions on the utility and transfer of mosaic embryos.

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INTRODUCTION

As a society, the Preimplantation Genetic Diagnosis International Society (PGDIS) promotes the implementation of quality processes at all stages of embryo analysis, including technical competency in carrying out any preimplantation genetic testing (PGT) process as well as appropriate interpretation of all testing results. Since the release of the previous PGDIS Position Statement 2019 (www.pgdis.org), uncertainty has remained about the implications of mosaic embryo transfer; consequently, many clinics are avoiding the transfer of such embryos. This document is the final Report of PGDIS Expert Consultation on Mosaic Embryo Transfer, 2021, using the most recent information available, and provides an updated summary to testing laboratories, clinics, clinicians and genetic counsellors on the transfer of mosaic embryos, replacing previous 2016 (https://pgdis.org/docs/newsletter_052417.html) and 2019 (*Cram et al., 2019*) documents issued on behalf of PGDIS.

Chromosome testing strategies, such as preimplantation genetic testing (PGT-A), are designed to improve an IVF embryo transfer outcome by avoiding the unwitting selection of aneuploid embryos that occurs in morphology-based approaches. Newer testing technologies have revealed that some embryos may appear to have intermediate whole chromosome (or parts of a chromosome termed segmental) copy number results suggestive of trophectoderm mosaicism. An embryo with a trophectoderm intermediate copy number (or mosaic-range) result (referred herein as 'mosaic embryos') may be the only option for transfer for some patients. Data are still limited on the outcomes after a mosaic embryo transfer, but compiled information with follow-up exists for over 2500 cases and no increase in abnormal live birth was indicated (*Treff et al., 2021*).

BACKGROUND

Approximately one-half of early pregnancy losses are associated with uniform chromosome imbalances; however, an analysis of products of conception in miscarriages suggested that 10% were a result of a mosaic autosomal trisomy (*Chen et al., 2017*). *Wang et al. (2013)* also showed that some abnormal liveborn infants were a result of feto–placental mosaicism. Presumably, these miscarriages were, at some stage of development, a mosaic pregnancy, potentially at the early embryonic stage. Mosaicism detected in trophectoderm biopsies can, therefore, have theoretical clinical implications for the fetus, placenta, or both, in any pregnancy, including effects on placental function, liveborn disease syndromes, or both (*Grati et al., 2018*).

The primary purpose of PGT-A is to improve an IVF transfer outcome by reducing the number and effect of aneuploid embryo transfers inherent in morphology-based embryo choices (*Forman et al., 2013*). Biopsy is not a tool to improve an embryo but is currently the only proven approach to identify the genetics of an embryo. Although it is biologically logical, the transfer of an aneuploid embryo was only recently demonstrated to have significantly decreased rates for implantation, continuing pregnancy and live birth compared with euploid embryos (*Tiegs et al., 2021*; *Wang et al., 2021*).

Early studies designed to identify and avoid transfer of aneuploid embryos failed to demonstrate any advantage of PGT-A (previously referred to as preimplantation genetic screening) over simple morphology selection when carried out using cleavage stage biopsy and limited fluorescence in-situ hybridization analysis. Comprehensive chromosome analysis (*Fragouli et al., 2008*), in conjunction with biopsy of several trophectoderm cells, however, altered the aneuploid discovery rate of chromosome aberrations. This approach is now considered optimal for the evaluation of embryo chromosomal status. Earlier developmental stage biopsy, with its greater reported potential for reduction in embryo outcomes (*Scott et al., 2013*), has been discontinued in most clinics. Analysis of more than one cell in a single assay, however, introduces the possibility of whole chromosome (or partial/segmental chromosome) intermediate copy number results.

OVERVIEW OF NEW KNOWLEDGE

Incidence of mosaic embryos

Chromosome mosaicism has been observed commonly, although usually in only a minority of embryos. Sensitive technologies, such as array comparative genomic hybridization and methods based on next-generation sequencing (NGS) can variably distinguish uniform aneuploidies (affecting all cells in the biopsy) from mosaic aneuploidies (affecting only some of the cells in the biopsy). At the blastocyst stage, the incidence of reported mosaicism using NGS methods is highly variable among clinics, ranging from as low as 2% to as high as 40% with most clinics reporting between 5 and 15% depending on the age group being investigated (*Munne et al., 2016*; *Fragouli et al., 2019*; *Rodrigo et al., 2020*). A consistent high incidence of mosaic embryos in a selected clinic may be related to a predominant patient age group (although several studies suggest no age-related incidence), clinical treatment, specific embryology practices, or all (*Ruttanajit et al., 2016*; *Fragouli et al., 2019*), whereas a high level of apparent mosaicism across all referral clinics in a single testing facility may be indicative of poorer testing laboratory practices. In both cases, a review of clinical, testing laboratory practices, or both, may be warranted. Clinics sending biopsies for PGT-A to an outside testing laboratory should request the laboratory to disclose their overall embryo mosaic rates as well as any cut-off ranges used in their determinations. This will assist clinics in assessing their own performances and the analytical capabilities of any referred testing laboratory.

Transfer outcomes from mosaic embryos

Since the first published study reporting successful pregnancies after transfer of known mosaic embryos (*Greco et al., 2015*), other groups have also reported outcomes involving larger numbers of mosaic embryos (*Munne et al., 2017*; *Victor et al., 2019a*; *Zhang et al., 2019*; *Zore et al., 2019*; *Capalbo et al., 2021*; *Viotti et al., 2021*).

In a recent study, *Capalbo et al. (2021)* proposed that lower range (defined as <50%) mosaic embryos can be transferred without added risk of poorer outcomes compared with euploid embryos whereas other published research (*Fragouli et al., 2017*; *Lledo et al., 2017*; *Munne et al., 2017*; *Lin et al., 2020*; *Viotti et al., 2021*) have suggested that mosaic embryo transfers may be associated with increased implantation failure and miscarriage rates with higher values of mosaicism appearing to be

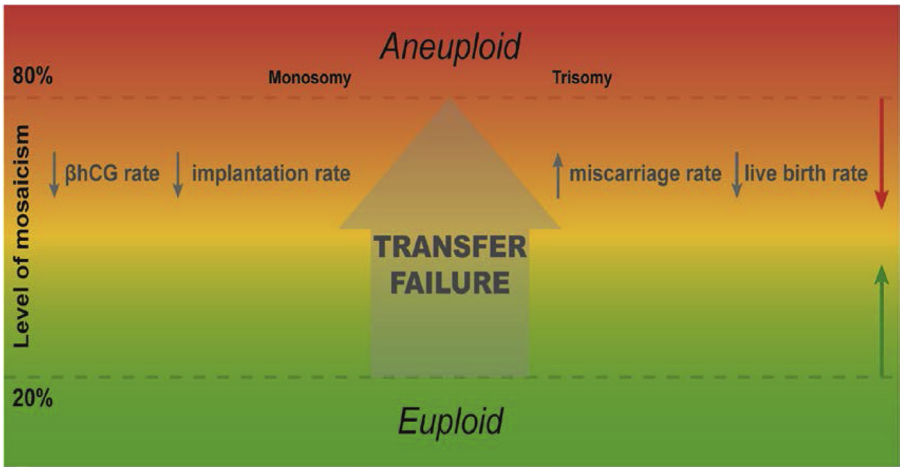

FIGURE 1 Relationships between level of mosaicism and transfer outcome of mosaic embryo. Arrows on the rightside show increasing mosaicism in euploid (green) or aneuploid (red) embryos.

even less favourable for producing good outcomes for the patient (FIGURE 1). Several controlled studies have reported this decline in transfer outcomes, with the largest study being a retrospective analysis (*Viotti et al., 2021*). The results suggest that mosaic embryos seem to have poorer transfer outcomes.

Other recent studies have revealed that mosaic whole chromosome or mosaic segmental embryos do give rise to healthy pregnancies but suggest that such transfers may be associated with reduced implantation rates (*Wang et al., 2021*), higher miscarriage rates (*Lin et al., 2020*; *Viotti et al., 2021*), or both, although final birth rates may be similar to euploid embryo transfers (*Capalbo et al., 2021*; *Tiegs et al., 2021*; *Wang et al., 2021*).

In the collective transfer data summarized by *Treff and Marin (2021)*, it is evident that a high proportion of mosaic embryos have a significant level of developmental competence and should not be disregarded in terms of suitability for transfer, as discussed previously in the PGDIS 2019 Position Statement (*Cram et al., 2019*). In general, good success rates were reported after the transfer of lower range mosaic embryos, whereas putative mosaic embryos that appeared to have higher levels of abnormal cells in the trophectoderm biopsy specimen were less likely to achieve a viable pregnancy.

At present, nearly all prenatal diagnoses of established pregnancies after a mosaic embryo transfer have revealed normal euploid fetuses, with all live births reported to date showing no evidence of chromosome-based syndromes. Currently, a number of published reports of a mosaic embryo transfer giving rise to an affected child have been published (*Mounts et al., 2019*). Although adverse outcomes from mosaic embryo transfers have not been directly reported to date, recent studies have indicated that one in 320 syndromic infants undergoing clinical exome sequencing in a large US-based testing centre (*Scuffins et al., 2021*) are the result of a uniparental disomy (UPD) typically involving an imprinted chromosome. It has also been noted that in the general population, around one in 2000 live born infants have a UPD chromosome pattern (*Nakka et al., 2019*) which, given the different possible segregation modes of trisomy/monosomy rescue, suggest as many as approximately one in 660 pregnancies have a chromosomal imbalance early in their development. These UPD patterns are nearly two-thirds meiotic in origin, whereas the remaining one-third may be either monosomy rescue events or mitotic non-disjunction followed by a rescue event. All of these live born infants would presumably have displayed a mosaic status during early embryo development, or at a very early fetal development, albeit less likely. These events demonstrate that mosaicism is a genuine biological phenomenon and can potentially result in an outcome of clinical relevance.

More recently, the total analysis of embryos donated to research has revealed additional information on the chromosomal constitution of mosaic blastocysts (*Popovic et al., 2018*; *Capalbo et al., 2021*; *Viotti et al., 2021*). Initial euploid and aneuploid results tend to be confirmed in subsequent embryo biopsies. In general, a high-level mosaicism in the initial biopsy often shows a full aneuploidy in subsequent trophectoderm biopsy and inner cell mass (*Capalbo et al., 2021*). If a lower level of mosaicism was followed in subsequent trophectoderm and inner cell mass biopsies, many embryos were uniformly euploid (*Victor et al., 2019b*; *Ou et al., 2020*; *Capalbo et al., 2021*; *Marin et al., 2021*).

How does this affect aneuploidy testing in clinical practice?

Although most (>85%) trophectoderm biopsy results are either uniform euploid for all chromosomes or full aneuploid involving one or more chromosomes, a small proportion of embryo biopsies may show intermediate copy number changes for one or more chromosomes. The risk of an abnormal birth from a mosaic embryo seems to be relatively low (*Viotti et al., 2021*), but transfer failures (*Wang et al., 2021*) and miscarriages may be higher than euploid transfers (*Lledo et al., 2017*; *Lin et al., 2020*).

FOR THE TESTING LABORATORY

Circumstantial evidence suggests that NGS and associated data analysis pipelines used to measure chromosome copy number may, at times, incorrectly indicate mosaicism (*Fragouli et al., 2019*; *Marin et al., 2021*; *Treff et al., 2021*).

Theoretically, mosaicism estimates could be exaggerated by the following: variations in the biopsy technique

(*Ruttanajit et al., 2016*; *Xiong et al., 2021*); poor sample handling and transport; sub-optimal DNA amplification and library construction; and choice of algorithms used for normalizing the chromosome mapping bins.

Further comments

For technical reasons, only an analysis platform that can reproducibly estimate chromosome copy number should be used for any reporting of putative mosaic levels in the trophectoderm biopsy sample. Testing laboratories can carry out their own baseline control experiments for both euploid and aneuploid amplified DNA products from a range of samples. Such experiments may be repeated at regular intervals, to be defined within each laboratory, to ensure mosaicism detection does not alter. Studies from different groups suggest a typical cut-off value for euploid assignment is less than 20% and for aneuploidy assignment greater than 80%. These values essentially represent analytical noise bands and may show some variation based on the specific technology or algorithms used (*Maxwell et al., 2016*; *Fragouli et al., 2017*; *Munne et al., 2017*; *Spinella et al., 2018*). Embryo chromosome deviations less than 20% may be reported as euploid, whereas embryo deviation values greater than 80% may be reported as aneuploid. Profiles with chromosome values outside these ranges are considered to indicate potential or putative mosaicism. Some groups use less stringent euploid and aneuploid cut-off values, e.g. 30%/70% (*Garcia-Pascual et al., 2020*), resulting in lower reported numbers of intermediate copy number embryos but they are, therefore, logically accepting higher analytical noise levels with any implications for similar overlap in the mosaic range. Groups that report high transfer outcomes with mosaic embryos may, in fact, be reflecting higher false rates of mosaic calls (*Treff et al., 2021*) associated with read counts used, different testing platforms, algorithms or both, used in analysis (*Garcia-Pascual et al., 2020*; *Navratil et al., 2020*; *Rodrigo et al., 2020*; *Capalbo et al., 2021*; *Zhou et al., 2021*). Irrespective of cut-off values used for determining the euploid and aneuploid calls, these lower and upper mosaic limit points should be reported by the testing laboratory to the referring clinician to facilitate any transfer discussions with their patient.

Given the nature of the biology underlying the genesis and propagation of mosaicism, a trophectoderm biopsy indicating putative mosaicism may not accurately reflect the rest of the embryo (*Lin et al., 2020*). Any value cited should be considered a reference point only for reporting purposes and for facilitating any further or subsequent discussions.

It is also inherently difficult to assign an averaged, single value to what may be a relatively broad data spread along a single chromosome in addition to any analytical errors for euploid and aneuploid ranges. The implicit understanding of a euploid (or aneuploid) embryo having a noise band of 20% (or 30%) must, therefore, result in all mosaic averages being similarly ascribed such a noise band. Technically, this means that any estimated value may be higher or lower than the average value. It can be suggested, therefore, that reports using a single internal mosaic-range cut-off are not logical because, if there is a noise band for the euploids and aneuploids, similar uncertainties must apply to any cut-off value. Specifically, a single, fixed point internally separating low from high mosaicism is not logical (*Treff and Marin, 2021*), and should be considered as a reference point for counselling purposes only. Any putative mosaicism identified as present will lie within a range and not be a single discreet value. From reports on the transfer of mosaic embryos, those with higher values seem to be less successful in transfer outcomes than those with lower values (FIGURE 1).

Although it is understood that commercial imperatives may be involved, testing laboratories should not classify mosaic embryos as fully aneuploid as this may reduce patient cycle potential. This includes embryos with multiple chromosomes in the mosaic range. This may mean a 'no result' assignment is most appropriate (*Marin et al., 2021*).

Testing laboratories should refrain from classifying a mosaic embryo as not suitable for transfer as this may restrict subsequent clinical treatment options. Laboratory report formats should be updated to include reporting of mosaic results, apparent % mosaicism, any cut-off values used in ascribing euploid, mosaic and aneuploid status, as well as any chromosome abnormality identified. A chromosome result profile that indicates apparent mosaicism for any embryo should also be provided on request for the purpose of genetic counsellors or clinicians explaining the PGT-A results to patients.

FOR THE IVF CLINIC

To minimize the effect of the process on the remaining embryo while still giving a robust, balanced amplification, it is recommended that only five to 10 trophectoderm cells be biopsied; care should be taken at all times to ensure minimum effect on the embryo. Damage to the cells during biopsy, as well as washing or tube loading should be minimized to reduce amplification bias and improve the likelihood of yielding a DNA product reflecting the original embryonic cells. If a consistently high incidence of mosaicism is identified in embryo cohorts within a given clinic, consideration should be given to investigating both the embryology and overall PGT-A practice to assist the identification of any possible underlying problems.

FOR THE GENETIC COUNSELLOR OR CLINICAL SUPPORT GROUP

The wide variety and quality of published research, and information available on social media forums, has confused the understanding of the usefulness of mosaic embryos in IVF treatment. Poorly informed debate in the scientific literature, popular press and on social media has led to uncertainty among clinics and patient support and advocacy groups. Preimplantation genetic testing for aneuploidy is a process designed to improve the outcome of any specific transfer by identifying those embryos that have a chromosome constitution most likely to lead to a successful transfer outcome.

Pregnancy failure with aneuploid embryos is undisputed and, similarly, the necessity for a balanced chromosome set for a successful pregnancy is also undisputed. At this point in time, the best approach for examining the constitutional chromosome set is to remove a small sample from the blastocyst-stage embryo. As with any analysis involving more than one cell, the possibility of identifying chromosomal differences within those cells exists. If two different chromosome complements exist among the cells, the chromosomes will show an intermediate copy number profile,

i.e. neither two (termed euploid), nor one or three (termed aneuploid). The apparent presence of an intermediate level is commonly referred to as a mosaic (mixed) state. As only a small piece is removed from the embryo, a mosaic state may be limited to the region biopsied or may be present throughout other parts of the embryo.

Any individual embryo cohort may have no embryo identified as euploid or may have one or more embryos classified as mosaic. This Position Statement is devised to assist in the decision-making process when faced with the option of a mosaic embryo transfer. The most recent reports summarizing outcomes after mosaic embryo transfers (*Treff et al., 2021*; *Viotti et al., 2021*) suggests that the risks of any subsequent births being chromosomally abnormal are low.

It is understood that different opportunities and constraints are faced by each individual patient; therefore, the decision-making process needs to be fully discussed with an appropriate professional. It should be kept in mind that mosaic embryos have always existed and been used in the IVF process without prior identification as such. It should also be recognized that even some of the euploid embryos selected for transfer could prove mosaic if biopsied in another region of the trophectoderm (*Friedenthal et al., 2020*).

Given the nature of mosaicism and the way in which it arises during early embryonic development, it is obvious that a single biopsy specimen, tentatively characterized as mosaic, does not prove that the surrounding trophectoderm or the rest of the embryo is also mosaic. Increasing level of mosaicism may be less favourable to good outcomes (FIGURE 1); however, for both technical reasons (analysis platform, amplification variations, analysis algorithms) and biological reasons (localized mosaicism versus uniform mosaicism), no precise cut-off values for transfer considerations should be adhered to (*Lin et al., 2020*; *Marin et al., 2021*; *Treff et al., 2021*).

RECOMMENDATIONS FOR THE CLINICIAN

Although laboratories deliver reports for individual embryos, clinicians should have some proficiency in understanding an embryo chromosome result profile as they may be called upon by patients to explain transfer opportunities. A chromosomal profile can usually be presented as a simple, pictorial representation of an embryo's relative chromosome copy number.

Recommendations for the clinician include the following: (1) patients should continue to be advised that any genetic test based on sampling one or small number of cells biopsied from preimplantation embryos cannot be 100% accurate because of a combination of technical and biological factors, including cell mosaicism; (2) patient information and consent forms for aneuploidy testing should be modified to include the possibility of mosaic results; and (3) in general, transfer of blastocysts with a normal euploid result should be prioritized over those with mosaic results unless other indications, such as patient preference, are raised.

Further stimulation cycles can incur financial, medical and emotional burdens, especially given uncertainties about any embryos with possible implantation potential remaining in storage. Use of a mosaic embryo, however, is not without some possible increased risk of negative outcome compared with that of a euploid embryo. Therefore, any proposal for transfer of a mosaic blastocyst should be offered only after appropriate consideration and consultation on these potential risks. Clinicians should also consider and discuss with the patient the alternative option of a further PGT-A cycle to increase the chance of identifying a euploid blastocyst for transfer. As this is a discussion based on clinical findings in addition to individual patient circumstances, it is best managed by a professional familiar with both aspects.

PRENATAL OPTIONS

Prenatal diagnosis of any pregnancy established after IVF and PGT is recommended by PGDIS. This is consistent with current recommendations (*ACOG, 2020*) that state prenatal diagnosis should be discussed and made available for every pregnancy, regardless of method of conception or prior genetic testing.

Non-invasive testing

Non-invasive prenatal testing (NIPT) and screening is a convenient test, with early gestational timing and high sensitivity and specificity. As such, it has become standard practice in many countries. These screening technologies vary widely, with some tests only able to investigate a low number of specific chromosomes, whereas others offer the potential for a genome-wide screen. For any early pregnancy investigations considered for specifically investigating a mosaic embryo transfer, preference should be given to 24-chromosome NIPT methodology that includes the mosaic chromosomes in question (*Benn et al., 2019*), the simple five chromosome NIPT tests (21, 18, 13, X and Y) available in many countries may not be appropriate for some of these specific investigations. Previous studies using NIPT based on whole genome sequencing show that this approach can detect cases of low-level placental mosaicism (*Canick et al., 2013*; *Wang et al., 2013*; *Hartwig et al., 2017*), which are likely a consequence of mitotic errors in cells located solely within the placenta (confined placental mosaicism) or possibly originate from a true, mosaic early embryo. It is important to note, however, that some segmental changes detected by PGT-A may be below the limits of detection of NIPT and so the referrer must be made aware that the segment must be within detection limits if they are to consider the use of such an approach. The referrer should also understand that non-invasive testing can only assess placental chromosome status, which does not always reflect the remaining structures or the fetus.

Invasive testing

Amniocentesis analysis from gestational week 14 onwards is the most representative of the chromosomal complement of the fetus. Earlier gestational stage chorion villus sampling may be considered but, as with NIPT, it may only reveal placental chromosome constitution, which could differ from the actual fetal chromosome set. Non-directive counselling on all the options should be offered in all cases.

SUGGESTED RECOMMENDATIONS TO ASSIST IN THE PRIORITIZATION OF MOSAIC EMBRYOS CONSIDERED FOR TRANSFER

On the basis of new knowledge gained from recent embryo analysis and transfer studies, the following is a guide to assist the clinician (or a genetic counsellor if available) when a mosaic embryo is being considered for transfer: (1) embryos with

higher-level mosaicism may be associated with less favourable outcomes compared with lower-level mosaicism. Currently, experience on higher grade mosaic embryo transfers is limited (*Lin et al., 2020*). Relative percentage of mosaicism seems to be a better predictor of outcome than the specific chromosomes involved, and thus should be included in reporting and patient discussion; (2) a decision to transfer a mosaic embryo can be prioritized either on the level of mosaicism or type of mosaicism (whole chromosome versus segmental changes).

If there is a choice between the transfer of two embryos with similar levels of mosaicism, given that higher morphological grade embryos tend to give better transfer outcomes (*Capalbo et al., 2014*) preference of an embryo may be considered based on embryo morphology or alternatively on the nature of the variation (whole chromosome mosaic embryo transfers (*Capalbo et al., 2021*; *Tiegs et al., 2021*) are reported to give implantation outcomes more similar to euploid embryos than segmental (*Tiegs et al., 2021*) or mosaic segmental chromosome embryo transfers (*Zore et al., 2019*)).

CONCLUSION

Recent developments in genomic technologies for PGT have allowed a more complete spectrum of chromosome abnormalities to be identified, including full chromosome and segmental mosaicism, areas in which current knowledge of the outcomes is incomplete and still evolving. Historical IVF outcomes, in which transfer of mosaic embryos was inevitable, have not indicated increased risks for live born chromosome disorders compared with natural pregnancies. Transfer of mosaic embryos seems to be a relatively safe option for couples, with low or minimal risk of negative outcomes for the birth beyond the background risk for any pregnancy. Non-invasive prenatal testing has been shown to be capable of detecting many rare (non-live born) trisomies (*Scott et al., 2018*), which gives an opportunity of non-invasive follow-up of the original trophectoderm mosaicism result. A traditional invasive test is also available but at a later gestational time.

At the research level, chromosome analysis of donated mosaic embryos continues to shed light on the significance of the initial biopsy assessments and gives valuable information about the genetic constitution of putative mosaic embryos (*Capalbo et al., 2021*). Similarly, detailed chromosome investigations of the placenta after birth would add valuable information on the nature and extent of any mosaicism observed in the original transferred mosaic embryo. As further information evolves, this Position Statement will be updated accordingly.

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