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REVIEW

The mosaic embryo: what it means for the doctor and the patient

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ABSTRACT

INTRODUCTION: Mosaic embryos are embryos that on preimplantation genetic analysis are found to be composed of euploid and aneuploid cells. Although most of these embryos do not implant when transferred into the uterus following IVF treatment, some may implant and are capable of giving rise to babies.
EVIDENCE ACQUISITION: There is currently an increasing number of reports of live births following the transfer of mosaic embryos. Compared to euploid, mosaic embryos have lower implantation rates and higher rates of miscarriage, and occasionally aneuploid component persists. However, their outcome is better than that obtained after the transfer of embryos consisting entirely of aneuploid cells. After implantation, the ability to develop into a full-term pregnancy is influenced by the amount and type of chromosomal mosaicism present in a mosaic embryo. Nowadays many experts in the reproductive field consider mosaic transfers as an option when no euploid embryos are available. Genetic counseling is an important part of educating patients about the likelihood of having a pregnancy with healthy baby but also on the risk that mosaicism persisting, resulting in liveborn with chromosomal abnormality. Each situation needs to be assessed on a case-by-case basis and counseled accordingly.
EVIDENCE SYNTHESIS: So far, the transfers of 2155 mosaic embryos have been documented and 440 live births resulting in healthy babies have been reported. In addition, in the literature to date, there are 6 cases in which embryonic mosaicism persisted.
CONCLUSIONS: In conclusion, the available data indicate that mosaic embryos have the potential to implant and develop into healthy babies, albeit with lower success rates than euploids. Further clinical outcomes should be collected to better establish a refined ranking of embryos to transfer.

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Introduction

Successful in-vitro fertilization (IVF) is based in part on a thoughtful selection of viable embryos from a cohort obtained following ovarian stimulation. Historically, the main method to grade embryos was based on their morphological aspect.[1] Although excellent standardization systems for an assessment of embryo morphology have been developed,[2] the practice remains

subjective, and morphology alone has been shown to be a limited predictor of implantation[3] It was soon understood that even embryos with highest morphologic scores often harbor chromosomal aneuploidy, especially embryos derived from women of advanced reproductive age.[4] In parallel, it was demonstrated that undetected aneuploidy might increase the risk of first trimester pregnancy loss.[5] It became apparent that a technique to assess the numerical chromosome constitution of embryos to deselect those with structural and copy number abnormalities could be an interesting prospect.[4] This methodology, called preimplantation genetic testing for aneuploidies (PGT-A), involves chromosomal analysis starting with the collection of a small amount of cells from the embryo. The rationale for PGT-A use is based on the assumption that transferring euploid embryos, in place of selection of the most viable embryo for transfer based only on morphology, could improve clinical outcomes of IVF treatments by increasing the implantation rate and decreasing the risk of miscarriage.[6, 7] With the accumulation of knowledge and the development of genetic analysis technologies, it has been discovered that there are embryos consisting entirely of euploid or aneuploid cells and others consisting of a combination of euploid and aneuploid cells (*i.e.* mosaic embryos).[8, 9] Therefore, IVF clinics have adopted a classification system that also takes mosaic embryos into account.[10] The aim of such categorization was to obtain an enhanced ranking system to permit selection of the embryo with best likelihood of a positive clinical outcome.[11, 12] Indeed, the category of mosaic embryos is characterised by decreased implantation and pregnancy potential compared to euploid embryos, as well as increased risk of genetic abnormalities and adverse pregnancy outcomes. However, mosaic embryos display higher chances to implanting comparing to full aneuploid embryos. Thus, transfer of mosaic embryos may represent an option for those women that do not obtain euploid embryos after IVF.

The detection of chromosomal mosaicism in preimplantation embryos by PGT-A is technically challenging, and the accuracy of mosaicism predictions requires the use of a high-resolution next-generation sequencing (NGS) protocols validated for detection of mosaicism.[9, 13, 14]

As described in the following sections, the origin and characteristics of mosaic aneuploidies are different from those found in aneuploid embryos and the composition of the aneuploidies in mosaic embryos may influence the embryo's developmental capabilities. For these reasons, PGT-A guidelines recommend to offer appropriate genetic counselling explaining the different transfer options to the patient when faced with a mosaic embryo.[14-16]

Evidence acquisition

Chromosomal abnormalities and human reproduction

Chromosomal aneuploidy affects an exceptionally high number of human embryos and is recognized as one of the principal contributing factors in implantation failure and spontaneous miscarriage, providing a likely explanation for the relatively low success rate observed during IVF treatments. It has been demonstrated that the degree to which chromosomal abnormality affects human reproduction follows an inverse U-curve during maternal reproductive years.[17] Evidences in oocytes show that the curve is shaped principally by meiotic errors during oocyte formation that limits fertility in young and in women with advanced maternal age (AMA). Meiotic errors increase in frequency from 10-20% to >60% with increasing maternal age reaching 90% at 45 years old women. Even at the peak of a woman's fertility, at age 20-32, the incidence of chromosomal abnormality is about 20% in oocytes.[17]

Aneuploidy may involve deviation in copy number or structural rearrangement of chromosomes. Single or numerous chromosomes in a cell can be affected by aneuploidy. Aneuploidy errors in preimplantation embryos arise via whole-chromosome non-disjunction (where homologous chromosomes or sister chromatids fail to separate), or through unbalanced predivision (where homologous chromosomes or sister chromatids separate prematurely) via a mechanism that implicates breakdown of cohesin proteins in contributing to maternal age-related meiotic error.[18] Aneuploidy may also derive from meiotic

events in the father, but with a lower frequency ranging between 1-10%.[19] In addition to meiotic errors, mitotic errors are extremely common during the initial postzygotic cell divisions and produce mosaic embryos containing multiple distinct karyotypes. Unlike meiotic errors, the frequency of mitotic errors is constant with advancing maternal age.[17]

As with meiotic errors, post-zygotic errors of mitosis originate from non-disjunction or anaphase lagging, where sister chromatids fail to segregate correctly between two daughter cells.

An elegant study of McCoy suggests that embryos purged in early development often experienced catastrophic mitotic errors, while meiotic errors resulting in minor aneuploidy or simple polyploidy are comparatively viable through blastocyst formation. Consistent with this interpretation, McCoy showed that patients referred for PGT-A due to repeat IVF failure had higher rates of mitotic error than patients with other clinical indications, suggesting that some of these patients suffer systematically higher rates of preclinical pregnancy loss due to mitotic aberrations. Indeed, a high rate of mitotic spindle and cell division abnormalities have been documented in early embryos by independent methods. Due to the presence of aberrant spindles and abnormal cell division, affected embryos rarely survive to blastocyst formation and this explains the lower incidence of mosaic blastocyst compared to cleavage-stage mosaic embryos.[18]

Chromosomal mosaicism

The development of genomic technologies has revolutionized our capability to detect various kinds of genetic abnormalities in embryos.[20, 21] In 1993 chromosomal mosaicism, the coexistence of cells with a different karyotype, was described in human preimplantation embryos for the first time.[22] Since then many studies have been published on this topic, with mosaicism rates varying from 15%[23] to more than 90%.[24] One of the reasons for these varying rates of mosaicism in the literature are the different methods to diagnose mosaicism that have been used. Early versions of PGT-A did not measure it either because typically a single blastomere was used for analysis or because the technique could not accurately identify it.

In the current clinical practice, PGT-A usually involves sampling of 5–10 trophectoderm (TE) cells at blastocysts stage of embryo development providing a greater chance of highlighting mosaicism when present in the embryo. In addition, with the introduction of NGS protocols, which has now almost replaced traditional methods such as array - comparative genomic hybridization (aCGH),[14] the detection of mosaic embryos has become increasingly accurate. NGS technology is characterized by high throughput and is able to recognize intermediate copy number signals for chromosome or sub-chromosome regions, theoretically allowing even the presence of a single aneuploid cell in the biopsy to be detected.[8, 25, 26]

It is important to emphasize that this capability depends on the type of NGS platform used and on its appropriate use. If the NGS platform used for PGT-A is not accurate or adequately validated or the results are not properly interpreted, some mosaicisms could be not identified and the embryos are diagnosed as euploid or, on the contrary, some mosaic embryos are classified as aneuploid reducing the diagnostic accuracy and effectiveness of PGT-A.[14, 27]

This also explains the high range in incidence of mosaicism in PGT reports between fertility clinics and embryology laboratories, which in TE biopsies ranges from 4 to 22%.[15, 28]

The use of NGS for the analysis of few cells or entire embryo allows us to characterize chromosomal mosaicism and define the different form of mosaicism at cleavage and blastocyst stage.

In fact it has been demonstrated that mosaicism can affect different chromosome in the different stages of the embryo. At cleavage stage chromosomal mosaicism affects prevalently larger chromosomes and often involves many chromosomes simultaneously, originating a condition known as complex mosaicism.[18, 29, 30] On the contrary this is not found on Day 5. Between Day 3 and Day 5, chromosomes specific rates of mitotic error are positively correlated between these developmental stages.[30] At blastocyst stage, a small excess of post-zygotic chromosome gain has been reported compared to cleavage stage.[31] Mosaic embryos can either have a single chromosome loss or gain, a complex (three or more affected

chromosomes), or a structural aneuploidy, with varying ratios of normal to abnormal cells.[32] Apart from numerical abnormalities, structural abnormalities may also occur in both cleavage and blastocyst stage human embryos, leading to partial mosaicism of certain chromosomal segments.[33] NGS-based PGT-A, showed that 7.34% of mosaic embryos were with mosaic segmental abnormalities.[29, 33, 34]

Whether a mitotic error occurs early in development or later influences the distribution and the number of aneuploidy cells in the conceptus that contain the abnormality. For example, if mitotic errors occur at the time of the first or second cleavage, mosaic embryos will be composed of a greater proportion of abnormal cells than errors occurring during the third cleavage.[35, 36] Studies examining different sections of the same blastocyst or assessing the chromosomes of single-cell biopsies showed that there is no evidence for a preferential allocation of aneuploid cells to the trophectoderm of mosaic blastocyst stage human embryos. However, there remains a possibility that random distribution of abnormal cells could, by chance, leave the inner cell mass (ICM) unaffected and be present in trophectoderm (TE) only, or vice-versa.[37, 38] Recent studies analyzing four multifocal TE samples and ICM, provided evidence that when mosaicism impacted fewer than 50% of cells in the TE biopsy (low-medium mosaicism), only 1% of aneuploidies affected other portions of the embryo. In contrast, the outcome of high-grade mosaic embryos is clearly a different matter given that 65% of high-grade mosaic embryos were extensively affected, including in the inner cell mass.[39-41] Forms of mosaicism can be broadly classified into two groups. The first group, aneuploid mosaicism, is composed with two or more different abnormal cell lines (*e.g.*, mosaic-aneuploid) and no diploid cells are present in the embryo. Mosaic-aneuploid embryo originates when a mitotic error arises in an embryo already affected with a meiotic error. The second group, more common than mosaic-aneuploid, is represented by diploid–aneuploid mosaicism, which has been estimated to affect more than half of cleavage- stage embryos. Diploid–aneuploid mosaicism comprises a mixture of normal and aneuploidy cells and generally arises due to mitotic error in a cell descended from a diploid zygote.[42]

Despite their common origins in mitosis, the fitness consequences of each class of mosaicism are distinct; the mosaic aneuploid embryos have few chances to develop and high risk to result in aneuploidy fetus, while mosaic-diploid embryos have the potential to implant and results in healthy babies.[8] The clinical aspect of diploid-aneuploid mosaic embryos are discussed in more detail here.

Viability of mosaic embryos and clinical outcome

The first prospective study providing information on the developmental potential of mosaic diploid–aneuploid blastocysts was carried out by Greco *et al.*[11]

Up until that point, mosaic embryos were categorized aneuploid and not considered for transfer. After the Greco's study, other reports from other group using NGS-based PGT-A have reported transfer of mosaic embryos.[11, 13, 38, 39, 43, 44] All studies demonstrated that mosaic embryos had lower rates of implantation and higher likelihood of miscarriage than euploid embryos, but led to births with no overt medical conditions.[13, 29, 45] Thus, thanks to this studies the transfer of mosaic embryos is now considered as an option for women who undergo IVF resulting in mosaic embryos but no euploid ones after PGT-A.[46, 47]

The exact factors that affect implantation and miscarriage and govern which mosaic embryos will lead to normal outcomes are yet to be determined.

While there is a consensus among scientific societies that mosaic embryos are less favorable for producing good outcomes than euploids, there is still no agreement among published studies on the specific mosaic features (level, type, chromosome involvement) that affect implantation and miscarriage. Conflicting data exist on the clinical outcome data related to high- *versus* low-level mosaicism,[13, 44] or whether the type of mosaic (involving segmental, *versus* whole chromosome, *versus* complex abnormalities) has an effect on developmental potential of mosaic embryo.[38, 39, 44]

In a prospective study performed with NGS it

has been observed that mosaic blastocysts with mosaicism level >50% performed significantly better after transfer, compared to mosaic embryos with <50% abnormal cells, suggesting that the extent of mosaicism affects IVF success rate.[13] Confirming these results, data obtained from one thousand mosaic embryo transfers reported that whole chromosome mosaic embryos with level (percent aneuploid cells) <50% had significantly more favorable outcomes than the >50% group. Other studies reported that there was a tendency for mosaics with 20-40% mosaicism to have a better outcome over those with >40%.[38, 39] Zhang and colleagues found that pregnancy outcomes were globally decreased in the <50% abnormal cell embryo group compared with euploid embryos.[46] In contrast to these studies, one non-selection clinical trial found no evidence of inferior performance of low- and medium-grade mosaic embryos about pregnancy outcomes, including live birth rate, pregnancy loss, or chromosomal abnormalities in the pregnancy and in children.[48]

One report of amassed data from different centers to increase power of analysis evaluating 1000 mosaic embryo transfers.[29] The study collected data from several laboratories that all used the same parameters to diagnose mosaics, such as standardized PGT-A platform based on NGS, validated analysis system for the detection of mosaicism and uniform definition of mosaic result based on a 20-80% threshold.[29] The study revealed that different mosaic subgroup had different clinical outcome. When the subgroups were sorted by clinical success rate, a ranking of subgroup emerged from those having the most to those having the least favorable outcome. Segmental mosaics were associated with the best success rates, but they still had significantly worse success rates than euploid control group. Low levels of mosaicism were associated with better outcomes that high-level mosaic. Within these groups, the type of mosaicism was sorted providing the results showed in Table I.[49-51] Because embryo selection also involves consideration of morphology, the outcome data were further refined according to mosaic embryo level, type, and Gardner stage/grade assessment. Taking the average of the three indicated values for stage, ICM grade, and TE grade produces a ranking score for any given embryo. The resulting matrix of value is used prospectively in the clinic to rank embryos.[29] A freely accessible online tool allows the user to input the characteristics of two or more embryos and determine their potential for clinical success (https://embryo-score.web.app/). Ranking of mosaic embryo subgroups, sorted by favorable clinical outcomes is showed in Figure 1, 2.

Clinical cases of embryonic mosaicism identified with PGT-A persisting to pregnancy as true fetal mosaicism

Although there is certainly a need for comprehensive analyses of neonatal outcome data of transferred mosaic embryos from several studies reported that newborns have been invariably healthy based on routine neonatal examination for developmental defects and gross abnormalities. Mosaicism observed in TE biopsies by PGT-A has not been observed in matching CVS or NIPT samples (which test placental DNA) in existing publications.[29, 44, 48, 51] Together, these models provide a framework of how blastocysts with intrabiopsy mosaicism can result in healthy babies.

TABLE I.—*Cases of persistence of embryonic mosaicism during pregnancy.*

Author	PGT-A	Prenatal testing	POC	Ultrasound anomalies	Postnatal testing
Kahraman *et al.*,[49] 2020	Mos -2 (low level)	Mos +2 (low level)	NA	No	Mos -2 (blood cells)
Greco *et al.*,[50] 2023	Mos +1q; -7; -8; +9; -19; -20; +21 (low level)	Mos +21 (CVS+Amnio)	NA	Yes	NA
	Mos -1p36.33_p31.1 (low level)	Mos -1p36.33_p31.1 (Amnio)	Mos -1p36.33_p31.1	No	NA
Viotti,[51] ESHRE 2022	Mos +21 (low level)	Mos +21 (CVS+NIPT)	Mos +21	Yes	NA
	Mos +15 (high level)	Mos +15 (NIPT)	Mos +15 (placenta)	Yes	NA
	Mos +17 (high level)	NA	Mos +17	Yes	NA

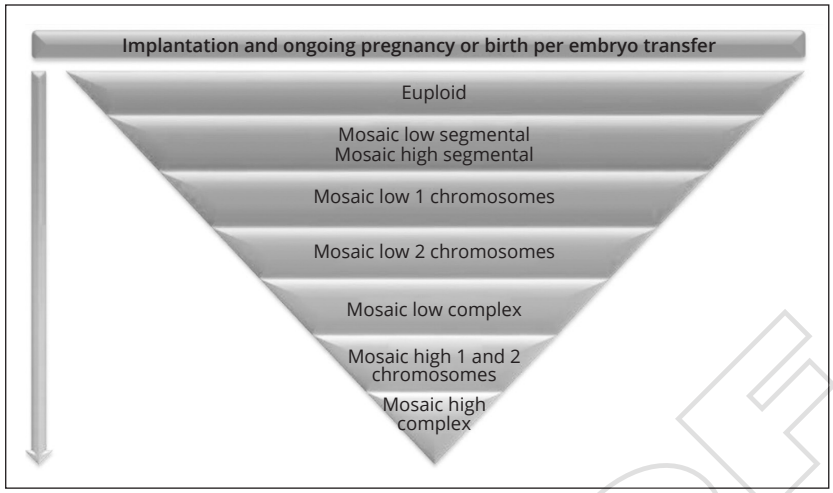

Figure 1.—Relationships between level of mosaicism and transfer outcome of mosaic embryo.

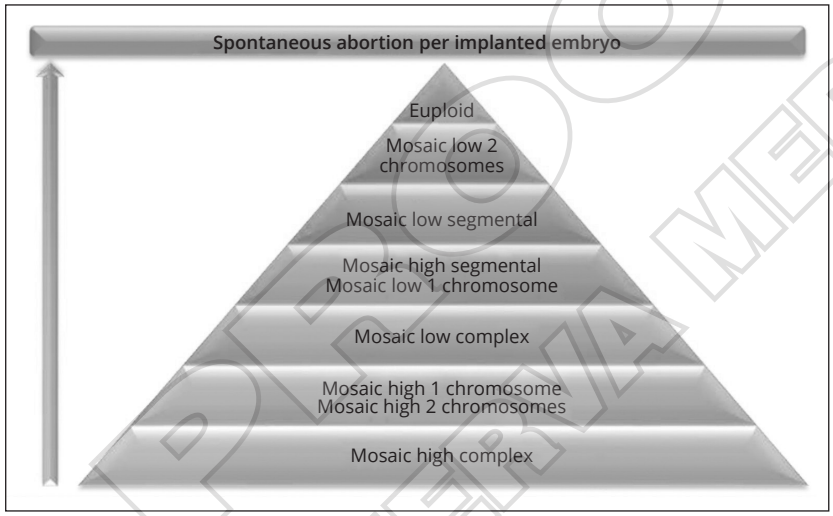

Figure 2.—Relationship between level of mosaicism and spontaneous abortions.

Another putative alternative to the fate of embryonic mosaicism is its persistence in fetal tissues through gestation, resulting in true fetal mosaicism (TFM). Some of the > 1000 mosaic embryo transfers reported to date had matching amniocentesis information (which tests fetal DNA), from patients that opted to share results.[29, 44] In the overwhelming majority of cases amniocentesis results were normal, and if an abnormality was detected it was independent of the mosaicism observed during PGT-A.

Considering the two studies with the highest number of cases,[49, 51] over 2155 pregnancies have been reported after transfer of mosaic embryos, of which 440 resulted in pregnancy and obtained a healthy baby. However recent finding reported cases in which mosaicism persisted in fetus. Up today, 6 case have been recently reported.[49, 51]

In the first case, reported by Kahraman *et al.*, in 2020,[49] the embryo showed 35% mosaic loss for chromosome 2 and amniocentesis detected a reciprocal chromosome 2 mosaic gain in 2% of cells. No pathological signs were revealed with ultrasonography analysis in the fetus and the pregnancy resulted in a healthy baby. Postnatal examination revealed a mosaic loss of chromosome 2 in 2% of peripheral blood cell draw and no aneuploidy cells in epithelial cells from a buccal smear sample. Recently, additional five cases were described[51] of which 2 cases were

reported in a recent publication by Greco.[50] In one of those, the 1p36 segmental deletion (40% mosaicism) detected in the transferred embryos was also revealed with the use of FISH probes in 15% of amniocytes demonstrating that the segmental deletion had persisted during gestation in a mosaic conformation. After termination of the pregnancy, fetal and placental samples (villous tissue) were analysed. No cells with a chromosome 1 deletion were revealed in tissue samples from the villi and heart. However, 1.5% of brain cells contained the 1p36 segmental deletion. In a second case, NGS-based PGT revealed the presence of a complex mosaic ((+1q;-7; -8; +9; -19; -20; +21) with 40% of aneuploid cells in the TE biopsy. Prenatal analysis, CVS and amniocentisis, detected only mosaic gain for chromosome 21. Ultrasound examination of the fetus and microscopic examination of abortion material confirmed a female fetus and the presence of abnormalities compatible with the trisomy 21, marked laceration at the level of the brain and carrying hypoplasia of the cerebellar vermis.

Presumably, in this case, the mosaicism for the other chromosome resolved itself during pregnancy, but somehow mosaic trisomy 21 evaded the self-correction. This provided strong evidence that embryos that contain even a low fraction of abnormal cells (<50%) can occasionally result in fetuses with mosaicism. The last three cases involved single trisomy for chromosome 21, 15 and 17 with >50% mosaicism. In all the cases, the aneuploidy was detected in the product of conception.[51] We should also mention a case in which the transfer of euploid embryo which after reanalysis was found to be high-level mosaic resulted in a child with a diagnosis of 15q duplication syndrome.[52]

These confirmed cases of embryonic mosaicism persisting through gestation to birth emphasizes the need of invasive prenatal testing, particularly amniocentesis, in pregnancies from mosaic embryo transfers.

Indication for mosaic embryos priorization

Recent attempts from scientific society have been made to assist with embryo selection decisions in situations whereby multiple embryos with mosaic results are under consideration for transfer.

The Preimplantation Genetic Diagnosis International Society (PGDIS)[15] suggests that if there is a choice between the transfer of two embryos with similar levels of mosaicism, preference may be considered based on embryo morphology, giving higher morphological grades to be tend to give better outcomes or on the nature of the variation (segmental mosaic embryo transfers are reported to give outcomes more similar to euploid embryos than are whole chromosome mosaic embryo transfers).

After these initial recommendations were issued, CoGEN released a position statement with analogous recommendations:[16] to prioritize for transfer embryos with lower levels (20%–40%) of mosaic aneuploidy,[16, 53-55] while discouraging[16] the transfer of mosaics involving chromosomes compatible with live birth in pure aneuploid form (*i.e.*, trisomies for chromosomes X, Y, 13, 18, 21, and 45,XO), ii. chromosome 16 (the most common cause of pregnancy loss due to a chromosomal issue), iii. or segmental aneuploidies; iv. Or in general trisomies that are associated with potential uniparental disomy (UPD; chromosomes 7, 14, 15).

It is important to note that the incidence of UPD is very low but one case after the transfer of high-level mosaic embryo was recently described.[56]

One of the main problems in formulating guidelines for selecting mosaic embryos for transfer is that data are collected from different studies based on different analytical methodologies and classification systems.

The transfer of low level mosaic embryos has variable reports with some groups suggesting outcomes similar to euploid embryos,[48] whereas other groups report higher pregnancy failure rates.[57]

As regards the value of reporting low-range mosaicism, a recent survey from ESHRE leaves the choice of whether to diagnose these types of mosaics or not to the experience of the clinics. Unfortunately, today, despite having the technologies to detect mosaics, we still do not know all the clinical implications associated with this phenomenon. This creates a lack of homogeneity in dealing with mosaicism. As reported by ESHRE the criteria for designating mosaicism, reporting

and transfer vary significantly across the ART/preimplantation genetic testing (PGT) centers replying to the survey. For these reasons, ESHRE recommends PGT centers to monitor emerging data on the topic and adapt or refine their policy whenever new insights are available from evidence. Due to several unknowns in chromosomal mosaicism and limited experimental data on parameters of mosaicism for transfer, patients considering transfer of mosaic embryos must receive thorough genetic counselling about potential pregnancy risks and outcomes. Counselling related to the genetic analysis should include the discussion of chromosomal mosaicism, the challenges associated with interpretation of the results, and, when appropriate, how this may affect diagnosis, embryo transfer and cycle outcomes. They should be made aware that these embryos may be associated with a higher miscarriage rate, fetal anomalies, termination of pregnancy and that an invasive prenatal diagnosis is strongly recommended. Genetic counselling should also inform the patient that mosaicism found in a TE biopsy may go on to produce a euploid placenta or a pregnancy with confined placental mosaicism (CPM). CPM of certain chromosomes (particularly 2, 7, 16, and possibly 22) may increase the risk of intrauterine growth retardation (IUGR) and other pregnancy complications, including fetal demise. Genetic counselling should include the technical and biological limitations associated with the detection of mosaicism, and the center's policy on transfer and cryopreservation of mosaic embryos. Further data are required to establish the percentage and the kind of mosaic aneuploidy which have more chance to implant and result in a pregnancy. Guidelines from ESHRE suggest for center reporting mosaicism to consider and report the level of mosaicism (as 'low-range' or 'high-range', using a cut-off of 50% to discriminate between low-range and high-range) in embryos to perhaps aid in decision making about the fate of the mosaic embryo. These could help decision making of patients.[10]

In the event that a patient proceeds with transfer of an embryo diagnosed as mosaic, counseling about the benefits, risks, and limitations of prenatal screening and diagnosis should be provided. While CVS offers the earliest prenatal diagnosis of aneuploidy, the cells obtained are placental in origin, whereas those obtained through amniocentesis are more representative of fetal tissues. However, amniocytes are derived only from the embryonic ectoderm and amnion, so even a normal amniocentesis does not exclude low-level mosaicism or aneuploid cells in other tissue types.

Factors contributing to the diagnosis of mosaicism

There is documented variability between IVF clinics in the percentage of mosaic embryos that are generated,[39, 58] also when the same PGT methodology is used, suggesting that protocols of oocyte retrieval, intracytoplasmic sperm injection (ICSI), and culture conditions might also contribute to the incidence of mitotic errors. For example, ovarian response to stimulation was positively related to the occurrence of segmental aneuploidy, while oocyte vitrification and ovarian response showed no effect on the mosaicism rate.[59] Because these abnormalities have a postmeiotic origin, it is conceivable that inadequacies of embryo culture play a role in the genesis of this problem, increasing the risk of chromosome malsegregation during mitosis. This remains highly speculative at this time, but there is some evidence that blastocyst mosaicism rates may vary between IVF clinics, hinting at procedure-related effects. The differences in platform specificity and sensitivity, the protocols for DNA amplification, and the threshold settings established for interpretation can lead to differences in the proportion of mosaicism and the number of euploid embryos to transfer.[60] Other factors associated with the biopsy technique, including the conditions surrounding cell loading and the number of cells biopsied, can affect the results.[61-63] The method of fertilization and laboratory conditions, such as oxygen concentration, pH and osmolality in the embryo culture medium, and temperature are related to an increased rate of mosaicism.[26]

Chromosomal mosaicism may not be associated with maternal age.[24] Some authors suggested a slight increase in mosaicism in younger patients compared to women over 37 years of age.[59] Indeed, as the increasing likelihood of meiotic

(oocyte-derived) aneuploidy increases with increasing maternal age means that the proportion of embryos that are euploid/aneuploid mosaics decreases with age, from 26.6% in women <35 years old to 10.5% in >42 year old. In particularly, low-degree of mosaicism and segmental aneuploidies, are the type of aneuploidies more associated with maternal age.[34, 59]

There is a higher proportion of mosaic embryos in PGT-A cycles with male infertility compared to patients with normal sperm parameters. The highest mosaicism rates is also related to the severity of male infertility. A high proportion of mosaic embryos was found in couples with low sperm concentrations.[64] In oligozoospermic and azoospermic men the prevalence of mosaic and chaotic aneuploidy in blastomeres ranges from 35% to 68%.[64, 65]

In addition, patients with repeated IVF failure had higher rates of mitotic error than patients with other clinical indications.[18]

Further data will be needed to define time points, mechanisms, and potential susceptibility factors associated with mitotic errors leading to mosaic segmental aneuploidies in blastocyst-stage human embryos.

Potential mechanism for self-correction of mosaic embryos

The favorable outcome obtained after the transfer of mosaic embryos[29, 48, 66, 67] supports the notion that self-corrective mechanisms are at play during development. According to that model, abnormal cells become diluted out of the mosaic mix between the embryonic stages and later pregnancy, to the point of clinical irrelevance. Although it remains unclear whether mosaic embryos may self-correct it has been hypothesized that aneuploid cells might arrest, become senescent or apoptotic, or become precursors of placental mosaicism,[26] and this is more likely when the mosaic presents small number of aneuploid cells. In some instances, residual aneuploid cells might become diluted to the point of being medically negligible.

In cases where aneuploid cells are confined to the placenta, resulting in CPM most cases result in healthy babies,[29] but occasionally and depending on the aneuploidy involved and percent abnormal cells, the condition can increase the risk of first trimester pregnancy loss.[5]

The concept that a mosaic blastocyst with a high percentage of aneuploid cells is less likely to succeed than one with lower percentage of aneuploid cells was extensively demonstrated in a mouse model. Experimental data suggests that aneuploid cells, when present at low levels (<50%), could be progressively depleted from the blastocyst stage onward, leading to the development of normal embryos.[68] In this regard, using an extended in vitro embryo culture protocol, Popovic *et al.* investigated the effects of chromosomal aberrations and blastocyst mosaicism on early preimplantation, up to 12 days post-fertilization (DPF). They found that human mosaic blastocysts diagnosed with a high percentage of abnormal cells were more likely to be non-viable at 12 DPF. These findings further support the presence of a mechanism for the depletion of abnormal cells in the embryo outgrowths.[69]

Several mechanisms have been proposed to explain the "self-correction" process of mosaic embryos. One of these implicates cell death or reduced proliferation of aneuploid cells compared to euploid cells.[70] Direct evidence revealed by studies in mice showed that depletion of aneuploid blastomeres first becomes apparent during blastocyst maturation, when abnormal ICM cells have increased apoptosis and abnormal TE cells exhibit limited proliferation, prior to implantation and later in the early developing embryo.[71] In Bolton's mouse model, it was shown that the fate of aneuploid cells in early embryos depends on lineage: aneuploid cells in the fetal lineage (*i.e.*, ICM) are eliminated by apoptosis, whereas those in the placental lineage (*i.e.*, TE) showed severe proliferative defects.[68] A recent article confirmed that in the human embryo, the dynamics of cell proliferation and death are different, on average, among euploid, mosaic, and aneuploid blastocysts. This could correspond to the proposed self-correction mechanism, as aneuploid cells might proliferate more slowly or undergo apoptosis, and euploid cells compensate by elevating their rates of proliferation.[69] Evidence that could support or refute this notion in human embryos, however, is currently lacking. To date, it is unknown whether minimum thresh-

old proportions of euploid cells are required to support normal development.

The self-correction hypothesis is also supported by the notion that the mosaicism rate is less than 1-2% in viable pregnancies,[72] which suggests that the phenomenon may also occur during intrauterine development to remove abnormal cells from mammalian embryos in the postimplantation period. The mosaic model used by Bolton *et al.*[68] was generated with a drug introducing massive chromosome abnormalities for multiple chromosome (complex mosaic) and it remains to be determined if mosaicism for one or few chromosomes results in similar effects on cell survival.

Discussion

At present, there is increasing evidence suggesting that mosaic embryos have real reproductive potential and can result in healthy live births after transfer.[11, 29, 39, 58, 66, 73]

It is important to consider that currently not all IVF centers report mosaic embryos. A recent survey based on data collected in 2020 showed that about 40% of the centers classifying embryos as euploid or aneuploid.[10] Undetected mosaicism may result in the transfer of embryos with a lower developmental potential compared to full euploid embryos. In contrast, labeling mosaic embryos as full aneuploid may result in discarding a embryo with potential to implant. The adoption of validated technologies such as NGS is mandatory for accurate identification of mosaic embryos, thus substantially reducing the risk of discarding potentially viable embryos.[74] This represents an important achievement, especially for patients with poor ovarian reserve, producing a limited number of embryos, or for those in which only chromosomally abnormal embryos have been detected, comprising a large percentage of IVF patients. Although further studies are needed to strengthen the preliminary results obtained, the transfer of mosaic embryos may give patients a chance to achieve a viable pregnancy. However, this practice should be used with caution and after a proper genetic counseling session.

Neonatal outcome and long-term follow-up of children born after assisted reproductive techniques (ART) are needed to provide more conclusive evidence on outcomes and implications, including neurodevelopment.[75, 76] But considering the meticulously conducted short- and long-term outcome studies completed so far, we can say that, overall, and after controlling for multiple gestations and preterm delivery, the results suggest that ART is a safe procedure, offering hope to many parent(s) wishing for a healthy child.[77] Ongoing issues on PGT field include the true frequency of chromosomal mosaicism, whether embryonic aneuploidies self-correct, and how practitioners manage embryos designated as mosaic.[78]

Conclusions

Ongoing studies will permit further refined ranking of embryos within the mosaic category improving embryo selection. In this context, the possibility to combine multiple parameters, including embryo morphology, chromosomal constitution and female age, that is the strongest predictor of embryo chromosomal abnormalities,[79] through artificial intelligence could represent a toll in the future to improve Treatment of Infertility and Assisted Reproduction Outcomes.[80]

The next set of studies should focus on maternal and fetal/new-born health from mosaic embryo transfers to obtain a more thorough understanding of their chromosomal and physiological health outcome.

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Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions
Francesca Spinella wrote the review and together with Ermanno Greco participated in the literature study, conception, design, and drafting of the manuscript; Manuel Viotti and Anil Biricik participated in the critical revision of the manuscript and final approval; Noemi Meschino prepared the graphic presentation; Pier F. Greco, Ilaria Listorti, Carlo Ronsini and Francesco Cucinelli participated in the critical appraisal of the literature. All authors read and approved the final version of the manuscript.

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