Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments

Francesca Spinella, Ph.D., Francesco Fiorentino, Ph.D., Anil Biricik, Ph.D., Sara Bono, Ph.D., Alessandra Ruberti, B.Sc., Ettore Cotroneo, Ph.D., Marina Baldi, Ph.D., Elisabetta Cursio, B.Sc., Maria Giulia Minasi, B.Sc., and Ermanno Greco, M.D.

GENOMA Group, Molecular Genetics Laboratories; and Reproductive Medicine, European Hospital, Rome, Italy

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OBJECTIVE: To assess whether the extent of chromosomal mosaicism can influence the success rate of IVF treatments.

Design: Prospective study.

Setting: Private genetic and assisted reproduction centers.

Patient(s): The transfer of mosaic embryos was offered to 77 women for which IVF resulted in no euploid embryos available for transfer.

Intervention(s): All embryos were cultured to blastocyst stage; trophoderm biopsy was performed on day 5/6 of development. Comprehensive chromosome screening was performed using either next-generation sequencing or array-comparative genomic hybridization methodologies.

Main Outcome Measure(s): The clinical outcome obtained after transfer of mosaic embryos with low (<50%) and high (≥50%) aneuploidy percentage was compared with that resulting from a control group of 251 euploid blastocysts.

Result(s): A significantly higher implantation rate (48.9% vs. 24.2%), clinical pregnancy rate/ET (40.9% vs. 15.2%), and live-birth rate (42.2% vs. 15.2%) were observed comparing embryos with mosaic field <50% and ≥50%. Mosaic embryos with high aneuploidy percentage (>50%) showed a significantly lower clinical pregnancy rate/ET (15.2% vs. 46.4%), implantation rate (24.4% vs. 54.6%), and live-birth rate (15.2% vs. 46.5%) than euploid blastocysts. In contrast, embryos with lower aneuploidy percentage (<50%) have a clinical outcome similar to euploid embryos.

Conclusion(s): The results of this study further confirm that mosaic embryos can develop into healthy euploid newborns. We demonstrated that the extent of mosaicism influences the IVF success rate. Mosaic embryos with low aneuploidy percentage have higher chances of resulting in the birth of healthy babies compared with embryos with higher mosaicism levels. (Fertil Steril® 2017;___:

Key Words: Preimplantation genetic screening, mosaic embryos, array-comparative genomic hybridization, next-generation sequencing, embryo aneuploidy

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Chromosomal aneuploidy is a recognized significant contributing factor in implantation failure and spontaneous miscarriage, providing a likely explanation for the relatively low success rate observed during IVF treatments.

Preimplantation genetic screening (PGS), a technique enabling the assessment of the numerical chromosomal constitution of embryos, was introduced into the IVF field to test embryos for abnormal chromosome copy number (aneuploidy) and select for transfer chromosomally normal (euploid) embryos (1, 2). The rationale for PGS 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. However, while the premise behind PGS is widely accepted, its benefits with regard to cumulative live-birth rate per started cycle have not been demonstrated (3–5). Although a recent series of clinical trials (6–9) have reported a significant improvement in implantation and delivery rates, and reduction of miscarriage rates and time to pregnancy in different categories of patients, the clinical utility of PGS is controversial and still represents an open debate (10).
It is widely accepted that PGS cannot create a healthy embryo or improve the health of an embryo. In addition, if the technology used for PGS is not accurate or adequately validated or the results are not properly interpreted, embryo screening may lead to reduced diagnostic accuracy and effectiveness of PGS. As a consequence, this may result in discarding chromosomally normal embryos because of possible false positives, thus causing a decrease in the cumulative live-birth rate. However, improved PGS technology allowing a more accurate selection of embryos with the normal number of chromosomes for transfer has the potential to reduce the time in treatment to achieve a healthy live birth and reduce the risk of miscarriage or a profoundly disabled child due to an abnormal number of chromosomes.

In the current clinical practice, PGS usually involves sampling of 5–10 trophoderm (TE) cells at blastocysts stage of embryo development and aneuploidy detection for all 24 chromosomes, to provide a more accurate assessment of the reproductive potential of embryos. Among the different methodologies for comprehensive aneuploidy screening currently available for clinical use (11–16), array comparative genomic hybridization (array-CGH) was the first technology to be widely available (11). This approach has been extensively validated using cells of known karyotype (16, 17), and it is now used extensively around the world.

New technologies, such as next-generation sequencing (NGS) technology, are now emerging in the PGS field (18). Compared with other methods, NGS may offer more potential advantages including lower cost, reduced time for testing, and higher chromosomal analysis resolution (19–22).

The availability of robust and accurate methodologies allowing comprehensive aneuploidy screening has empowered a series of clinical studies reporting high implantation rates achieved after transfer of morphologically normal euploid blastocysts (6–8). However, a large percentage of such embryos still fail to progress to delivery. Chromosomal mosaicism could represent a likely explanation for some failures after the transfer of PGS-screened embryos (23, 24).

Embryonic mosaicism is a phenomenon characterized by the presence of two or more genetically distinct cell lineages, typically one with a chromosome abnormality and the other showing a normal chromosome constitution (25, 26). Although its impact on implantation and the developmental potential of embryos is not fully known, it is reasonable to assume that mosaicism is likely to influence the implantation rate. Such a phenomenon is relatively common in human preimplantation embryos, affecting 15%–90% of cleavage-stage embryos and 30%–40% of blastocyst-stage embryos (25–29). Mosaicism arises from mitotic segregation errors occurring after fertilization via a variety of mechanisms, including anaphase lag, mitotic nondisjunction, inadvertent chromosome demotion, and premature cell division before DNA duplication (26, 30, 31). The percentage of abnormal cells within a euploid/aneuploid mosaic embryo is influenced by the cleavage stage in which the chromosomal segregation error occurs. For example, errors occurring at the time of the second cleavage may result in a greater proportion of abnormal cells than errors occurring during the third cleavage (25, 26).

Recent studies reported that array-CGH and NGS methodologies are able to accurately distinguish uniform aneuploidies from mosaic diploid/aneuploid aneuploidies in TE samples biopsied from embryos at the blastocyst stage (29,32–34). This finding also demonstrated the feasibility of reliably determining the mosaicism percentage, after a proper calibration and validation of the methodologies [23, 33, 34].

The application of these techniques to TE biopsies and whole embryos has provided relevant insight into the incidence and nature of chromosomal mosaicism (35).

In a recent study, we have demonstrated that euploid/diploid mosaic embryos hold the potential to implant and result in the birth of healthy babies (32). These findings have implications for women who undergo IVF resulting in mosaic embryos but no euploid embryos. As a consequence, the transfer of these embryos is now offered as an option for these patients. We also hypothesized that the extent of mosaicism may affect the IVF success rate. However, our study was small and the data available were insufficient to test this hypothesis.

The aim of this study was to assess whether the extent of chromosomal mosaicism may influence the developmental potential of mosaic embryos. To test this hypothesis, we enlarged our previous study, offering the transfer of mosaic embryos, at different aneuploidy percentage, to 77 women for which the IVF/PGS cycle resulted in no euploid embryos available for transfer.

**MATERIALS AND METHODS**

**Experimental Design**

The study was organized into two steps. The first step assessed the ability of NGS to detect chromosomal mosaicism and defined its limit of detection (i.e., the minimum ratio of aneuploid to euploid cells that is needed to detect a copy number variation). This involved cell mixing experiments at different ratios of euploid and aneuploid cells to mimic chromosomal mosaicism, followed by analysis of mixed cells with both NGS and array-CGH methodologies. The second step involved a prospective evaluation of the clinical outcome obtained after transfer of mosaic embryos, diagnosed with either array-CGH or NGS-based PGS, performed between May 2013 and March 2016. Specifically, from May 2013 to September 2014, PGS was performed by array-CGH. In October 2014, because of a technology change, NGS replaced array-CGH in our routine PGS practice. From that period, blastocysts were analyzed with NGS.

The study was approved by the Institutional Review Board of both the European Hospital and the GENOMA laboratory. No specific funding was obtained for this study. None of the authors have any conflict of interests to declare. IVF and embryo biopsy procedure are described in the Supplemental Material.

**Reconstructed mosaicism experimental model.** To validate the NGS method for detection of chromosomal mosaicism on embryos, single cells from a euploid (46,XY) cell line were mixed with aneuploid cell lines (47,XX,+18 and 47,XX,+21) at different ratios. The cells were isolated using a flow sorter (FACS Aria II SE, BD Biosciences) and mixed...
in the following proportions: 10 aneuploid cells to 0 euploid cells; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9; and 0:10 (Supplemental Data, Supplemental Tables 1 and 2, and Supplemental Figure 1). Cells were lysed and processed for whole genome amplification and subsequent analysis with array-CGH or NGS methods (see Supplemental Data).

Case referrals and patient counseling. The demographic characteristics of the patients enrolled in the study are summarized in Supplemental Table 3. The couples were initially seen by a clinical geneticist. Genetic counseling consisted of a review of the couple’s clinical history, followed by an explanation of the PGS process. A calculation of the possible genetic outcomes, the likely success rates, the possibility of no euploid embryos available for transfer, and the risk of misdiagnosis were also discussed. Finally, the possible risks related to the transfer of mosaic embryos were specified and confirmatory prenatal diagnosis by for any ensuing pregnancy was recommended. Amniocentesis was suggested as the ideal follow-up in place of chorionic villus sampling, to overcome the confined placental mosaicism issue related to the latter invasive prenatal testing procedure.

After genetic counseling, patients were referred to the collaborating IVF clinic to arrange the clinical aspects of the treatment.

A written informed consent was obtained from each couple enrolled in the study, as approved by the Institutional Review Board of both GENOMA and the collaborating IVF clinic European Hospital.

Array-CGH and NGS analysis. Array-CGH and NGS analyses were undertaken according to our previously validated protocols using 24 sure microarrays (Illumina) (15) and VeriSeq PGS (Illumina) (21, 22), respectively, according to the manufacturer’s protocols and detailed in the Supplemental Data.

Classification of results. NGS and array-CGH results were classified as described earlier (15, 21, 22). Briefly, for array-CGH, the thresholds values for disomic results were established according to the log2 ratio obtained after the analysis of euploid samples. Autosomal profiles were assessed for chromosomally normal status using a 3 × standard deviation (SD) assessment greater than 0 ± 0.04 log2 ratio. A trisomy (partial or full) was automatically assigned by the software for chromosome ratios above 0.33 × SD, or +0.3 log2 ratio call (gain). A monosomy (partial or full) was scored for chromosome ratios below 0.33 × SD, or -0.3 log2 ratio call (loss). A chromosome was scored as mosaic when the log2 ratio was between 3 × SD 0.08 ± 0.04 and 0.033 ± 0.02.

For NGS results, the thresholds values for disomic results were established according to the copy number obtained after the analysis of euploid samples and defined as 1.97 ± 0.04; gains (partial or full) and losses (partial or full) were scored when the copy number value for each chromosome was above and below the copy number state of 3 or 1, respectively. A “mosaicism” result was manually assigned for each chromosome with a copy number between 2 and 3 or 2 and 1.

Clinical data and definitions. The number of fertilized (two pronuclei) oocytes and the number of biopsied embryos were calculated on the basis of the total number of mature injected oocytes. The absence of an identifiable pregnancy on ultrasound examination after a positive pregnancy test was termed “biochemical pregnancy loss” (36). Clinical pregnancy was defined as ultrasound demonstration of a gestational sac at 7 weeks after ET. Miscarriage was classified as “early” (<12 weeks post-ET) or “late” (>12 weeks post-ET). Implantation rate was defined as the number of gestational sacs per transferred embryo (expressed as a percentage), and ongoing pregnancy rate as the number of fetuses with fetal cardiac activity beyond 20 weeks of gestation per patient (expressed as a percentage).

Ethical approval. The material used in this study was obtained with patients’ informed consent and Institutional Review Board approval from both European Hospital center and GENOMA laboratory.

Statistical Analysis
Clinical outcome between different groups of mosaic embryos or between euploid and mosaic embryos was compared using a χ² test or Fisher’s exact test for significance. P < .05 was considered statistically significant using PRISM software (GraphPad Software).

RESULTS
The Impact of the Extent of Chromosomal Mosaicism on the Development Potential of Embryos

To determine the impact of mosaicism on embryo viability, the clinical outcome of patients who accepted the transfer of mosaic embryos was assessed. Seventy-seven patients (median age, 37.6 years; range, 29–47) decided to transfer at least one mosaic embryo and were enrolled in the study (Supplemental Table 3). The characteristics of mosaic embryos obtained after PGS are summarized in Supplemental Table 4. After the transfer of 78 embryos in 77 transfer cycles, 37 women achieved a positive beta-hCG (48.1% per ET), eight of which resulted in biochemical pregnancies only. A total of 30 embryos implanted and led to the presence of a gestational sac (38.5% implantation rate); six pregnancies miscarried within the ninth week of gestation (Table 1). Twenty-three pregnancies continued and were confirmed with at least one fetal sac and heartbeat (30.0% clinical pregnancy rate per ET). All ongoing clinical pregnancies went to term, resulting in the birth of 24 babies (Table 1), confirmed to have a normal karyotype through sampling of the chorionic villi and/or amniotic liquid.

The comparison of the clinical outcome obtained after transfer of mosaic embryos with low (<50%) and high (≥50%) aneuploidy percentage was performed to assess a statistically significant difference in the development potential between the two groups (Table 2).

A significantly higher implantation rate (48.9% vs. 24.2%, P = .039), clinical pregnancy rate/ET (40.9% vs. 15.2%, P = .021), and live-birth rate (42.2% vs. 15.2%, P = .021) were observed comparing embryos with mosaicism <50% and ≥50% (Table 2). The biochemical pregnancy
rate (11.4% vs. 9.1%, \(P = .95\)) and miscarriage rate (6.8% vs. 9.1%, \(P = .95\)) were not significantly different between the two groups.

In addition, we compared the clinical outcomes of embryos with a different number of chromosomes involved in mosaicism. We found that embryos with two or more mosaic diploid/aneuploidy chromosomes resulted in a significantly lower clinical pregnancy (5.3% vs. 35.3%, \(P = .0002\)) and live-birth rate (5.3% vs. 44.7%, \(P = .0002\)) compared with single or double trisomy and in a significantly lower implantation (15.8% vs. 50.0%, \(P = .02\)), clinical pregnancy (30.0% vs. 46.4%, \(P = .014\)), and live-birth rates (30.8% vs. 46.6%, \(P = .019\)) compared with the transfer of euploid embryos (Table 1).

Embryos with chromosomal mosaicism showed a significant reduction in the implantation (38.5% vs. 54.6%, \(P = .0002\)), clinical pregnancy (30.0% vs. 46.4%, \(P = .014\)), and live-birth rates (30.8% vs. 46.6%, \(P = .019\)) (Table 1). Mosaic embryos with high aneuploidy percentage (\(\geq 50\%\)) resulted in clinical pregnancy (15.2% vs. 46.4%; \(P = .0013\)), implantation (24.2% vs. 46.4%; \(P = .0019\)), and live-birth rates (15.2% vs. 46.6%; \(P = .0013\)) being reduced to approximatively one-third compared with that achieved after the transfer of euploid blastocysts. The number of biochemical pregnancies (11.4% vs. 9.6%, \(P = .8\)) and the miscarriage rate (6.8% vs. 8.0%; \(P = .79\)) were not significantly different between the two groups (Table 3).

In contrast, embryos with lower aneuploidy percentage (<50%) resulted in clinical outcomes similar to euploid embryos (clinical pregnancy/ET, 40.9% vs. 46.4%; \(P = .45\); implantation rate, 48.9% vs. 46.4%; \(P = .5\); miscarriage rate, 6.8% vs. 8.0%; \(P = .79\); live-birth rate, 42.2% vs. 46.6%; \(P = .75\); Table 3).

In addition, embryos with mosaicism involving more than two chromosomes resulted in clinical pregnancy (5.3% vs. 46.4%; \(P = .0002\)), implantation (15.8% vs. 46.4%; \(P = .001\)), and live-birth rates (5.3% vs. 46.6%; \(P = .0002\)) being significantly reduced compared with euploid blastocysts. In contrast, the clinical pregnancy, implantation, miscarriage, and live-birth rates obtained from mosaic embryos with one or two mosaic chromosome abnormalities were not statistically different compared with the control group (Supplemental Table 5).

**DISCUSSION**

Here we provide the first evidence on the impact of chromosomal mosaicism on IVF clinical outcomes. We found that mosaic embryos have poorer clinical outcomes compared with euploid embryos and that their implantation and developmental potential is influenced by the extent of mosaicism.

Embryonic mosaicism has recently become a controversial topic in the context of PGS, with debate over their potential viability (37).

Typically, mosaic embryos have not been considered useful for transfer in IVF treatment because, similar to euploid embryos, they were considered abnormal. However, in a recent study, we have demonstrated that mosaic embryos hold the potential to implant and result in the birth of healthy babies (32).

There are now multiple studies confirming our earlier finding, with no apparent evidence of mosaicism found in the babies who have been born after the transfer of mosaic embryos.
Embryos (24, 38, 39). As a consequence, the transfer of these embryos is now considered an option for women who undergo IVF resulting in mosaic embryos but no euploid embryos (37).

In our previous study, we also hypothesized that the extent of mosaicism may influence the IVF clinical outcomes (32). However, no definitive conclusion could be drawn because our study was small and the data available were insufficient to test this hypothesis.

In this study we reported the clinical outcome of an increased number of transfers of mosaic embryos as compared with our previous paper (32). For the purpose of the study, the mosaic embryos were classified in two categories: low (<50%) or high (≥50%) mosaicism percentage.

The results achieved confirmed that the delivery of healthy babies was possible after the transfer of mosaic embryos. However, mosaic embryos with a high percentage of chromosomally abnormal cells (≥50%) resulted in a statistically significant reduction in clinical pregnancy rate/ET (15.2% vs. 40.9%, *P* = .021), implantation rate (24.2% vs. 48.9%, *P* = .039), and live-birth rate (15.2% vs. 42.2%, *P* = .021) compared with mosaic embryos with a lower aneuploidy percentage (<50%).

In addition, our findings demonstrated that the clinical pregnancy (30.8% vs. 46.4%, *P* = .019), implantation (38.5% vs. 54.6%, *P* = .02), and live-birth (30.8% vs. 46.6%, *P* = .019) rates achieved after the transfer of mosaic embryos were significantly lower compared with euploid embryos. Interestingly, not all mosaic embryos had the same impact on embryo viability. Blastocysts with low-level mosaicism (<50%) were associated with outcomes similar to euploid embryos, while embryos with higher aneuploidy percentage (≥50%) resulted in statistically significant reduced clinical pregnancy, implantation, and live-birth rates.

A recent study on artificially produced mosaic embryos from mouse models (40) demonstrated that mosaic embryos with sufficient normal euploid cells (>50%) have full developmental potential. In contrast, when the proportion of normal blastomeres decreased to one third, there was a partial rescue of embryonic lethality. These results obtained in mice are confirmed by our findings and suggest that the reproductive potential of a mosaic euploid/aneuploid blastocyst is likely inversely correlated with the abnormal-to-normal cells ratio.

The reduced viability of mosaic embryos was recently described in a retrospective study involving reanalysis of archived TE biopsy specimens from transferred blastocysts (24). In this study, embryos with mosaicism in one or more whole chromosomes resulted in a 30.1% implantation rate and 15.4% ongoing pregnancy rate, significantly lower than a well-matched nonmosaic euploid control group (55.8% implantation rate, 46.2% ongoing pregnancy rate) (24). Concordant with our data, the investigators reported that blastocysts with 40%–80% aneuploid cells in the TE sample have a significantly reduced potential to achieve a pregnancy (22% ongoing pregnancy rate), compared with embryos with <40% abnormal cells (56% ongoing pregnancy rate) (24, 35).

It is important to emphasize that, although the clinical outcome obtained after transfer of euploid embryos versus embryos with <50% mosaicism was not significantly different, embryos with a different karyotype than those transferred in this study could have different outcomes. Indeed, the type of aneuploidy involved in mosaicism might also influence the clinical outcome as well as the extent of mosaicism. To date, no sufficient clinical data are available on the implantation potential of mosaic embryos with different types of aneuploidy and at different mosaicism percentages. For this reason, mosaic embryos should be considered as a distinct category in terms of development potential and risk for the fetus, lying between euploid and fully aneuploid embryos.

The results of this study suggest that priority for transfer should be given to euploid embryos and mosaic embryos with low-level mosaicism (<50%) compared with mosaic embryos with high mosaicism levels (≥50%), although our findings have shown that mosaic embryos with ≥50% of aneuploid cells can still result in healthy newborns.

However, the results of our study present several limitations to be considered. Due to the intrinsic nature of mosaicism, the chromosomal makeup achieved from a TE biopsy represents only a small part of the embryo and does not necessarily reflect the karyotype of the entire embryo. In this view,

### TABLE 3

Comparison of clinical outcomes between euploid embryos and mosaic embryo with different mosaicism level.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Euploid, n (%)</th>
<th>Mosaic, &lt; 50%, n (%)</th>
<th><em>P</em> valuea</th>
<th>Mosaic, ≥50%, n (%)</th>
<th><em>P</em> valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos transferred</td>
<td>251</td>
<td>45</td>
<td></td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Transfers</td>
<td>250</td>
<td>44</td>
<td></td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Positive beta-hCG</td>
<td>160 (64.0)</td>
<td>26 (59.1)</td>
<td>.36</td>
<td>11 (33.3)</td>
<td>.001</td>
</tr>
<tr>
<td>Biochemical pregnancies</td>
<td>24 (9.6)</td>
<td>5 (11.4)</td>
<td>.8</td>
<td>3 (9.1)</td>
<td>.8</td>
</tr>
<tr>
<td>Embryos implanted</td>
<td>137 (54.6)</td>
<td>22 (48.9)</td>
<td>.5</td>
<td>8 (24.2)</td>
<td>.0019</td>
</tr>
<tr>
<td>Early abortions</td>
<td>20 (8.0)</td>
<td>3 (6.8)</td>
<td>.79</td>
<td>3 (9.1)</td>
<td>.9</td>
</tr>
<tr>
<td>Ongoing clinical</td>
<td>116 (46.4)</td>
<td>18 (40.9)</td>
<td>.75</td>
<td>5 (15.2)</td>
<td>.0013</td>
</tr>
<tr>
<td>Pregnancy/ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>116 (46.4)</td>
<td>18 (40.9)</td>
<td>.45</td>
<td>5 (15.2)</td>
<td>.0013</td>
</tr>
<tr>
<td>Babies born</td>
<td>117 (46.6)</td>
<td>19 (42.2)</td>
<td>.75</td>
<td>5 (15.2)</td>
<td>.0013</td>
</tr>
</tbody>
</table>

a Euploid embryos versus mosaic embryos with <50%.

b Euploid embryos versus mosaic embryos with ≥50%.

the mosaicism level deduced from a single TE biopsy might not unequivocally represent the exact mosaicism percentage of the remaining the TE cells or the inner cell mass (ICM) constitution.

Whether data on mosaicism obtained from a small portion of the embryo may predict the chromosomal status of the remaining embryo still remains an important open question. Further studies are needed to demonstrate the correspondence between mosaicism results obtained from TE biopsies performed in different sides of the embryos and the ICM or the whole embryo.

A further open question is how mosaic embryos can “self-correct,” resulting in healthy babies with no evidence of mosaicism or abnormalities, even when >50% of aneuploidy cells are present in a mosaic embryo. We are unable to provide an explanation for such normalization. The reported potential mechanisms for embryos correction include preferential growth of the euploid cells or preferential allocation of the normal cells to the ICM [28, 41, 42]. Another hypothesis suggests that trisomic cell populations may self-correct by losing the extra chromosome via anaphase lag or nondisjunction [43]; however, this explanation is less likely, given the low rate of detection of uniparental disomy (UPD) among blastocysts [44]. Live-embryo imaging and single-cell tracking in mosaic embryos from mouse models suggest that aneuploid cells in the TE have a growth disadvantage, while those in the ICM are eliminated by processes such as apoptosis, leading to a decline in their numbers as development progresses, ultimately resulting in a normal placenta and fetus [40].

We have excluded the possibility of a self-correction mechanism by investigating the presence of UPD in our samples. In addition, although our data are still limited, the lack of confined placental mosaicism in chorionic villus sampling excludes the hypothesis of preferential allocation of aneuploidy cells.

Thus, we can hypothesize that, during embryo development, aneuploid cell lines may either be eliminated or proliferate slower compared with euploid cells, leaving only an irrelevant population of cells within the embryo.

Moreover, long-term follow-up data collected after the birth of children resulting from the transfer of mosaic embryos are still missing. As a consequence, additional clinical data must be obtained before this approach can be evaluated for routine integration into PGS programs in women undergoing IVF. Recently released guidelines recommend the preferential transfer of embryos showing mosaic monosomies over mosaic trisomies [45]. Autosomal monosomies are generally not viable, whereas certain trisomies can result in live births with associated physical and cognitive impairments. Thus, the transfer of mosaic embryos with purportedly “viable” aneuploidies should be considered with extreme caution.

Patients considering the transfer of mosaic embryos should undergo comprehensive genetic counseling, reviewing the possible risks and outcomes related with the transfer of such embryos [31]. Confirmation of the fetal karyotype by invasive prenatal diagnosis, preferably amniocentesis, should also be suggested for any ensuing pregnancy.

**Conclusion**

The results of this study further confirm that mosaic embryos can develop into healthy euploid newborns and demonstrate that the extent of mosaicism influences the IVF success rate. Mosaic embryos with low aneuploidy percentage (<50%) have higher chances of resulting in the birth of healthy babies compared with embryos with higher mosaicism levels (≥50%). The difference between the two delivery rates was statistically significant, suggesting that priority for transfer should be given to mosaic embryos with low levels of aneuploidy. These findings have implications for women who undergo IVF resulting in mosaic embryos but no euploid embryos. In these patients, the transfer of mosaic embryos may ultimately reduce the risk of discarding potentially viable embryos and increase the chances of establishing a pregnancy. However, as demonstrated by our results, euploid embryos have a higher implantation potential than embryos with high mosaicism levels (≥50%), thus the transfer of such mosaic embryos should be considered an option only for women who undergo IVF resulting in mosaic embryos but no euploid embryos.

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