Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing

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Objective: To determine the pregnancy outcome potential of mosaic embryos, detected by means of preimplantation genetic screening (PGS) with the use of next-generation sequencing (NGS).
Design: Retrospective study.
Setting: Genetics laboratories.
Patient(s): PGS cycles during which either mosaic or euploid embryos were replaced.
Intervention(s): Blastocysts were biopsied and processed with the use of NGS, followed by frozen embryo transfer. Trophectoderm (TE) biopsies were classified as mosaic if they had 20%–80% abnormal cells.
Main Outcome Measure(s): Implantation, miscarriage rates, and ongoing implantation rates (OIRs) were compared between euploid and types of mosaic blastocysts.
Result(s): Complex mosaic embryos had a significantly lower OIR (10%) than aneuploidy mosaic (50%), double aneuploidy mosaic (45%), and segmental mosaic (41%). There was a tendency for mosaics with 40%–80% abnormal cells to have a lower OIR than those with <40% (22% vs. 56%). However, few embryos (n = 34) with a mosaic error in 40%–80% of the TE sample were replaced. There was no difference between monosomic and trisomic mosaics or between entire chromosome mosaicism or segmental mosaicism. Implantation rates were significantly higher (70% vs. 53%), miscarriage rates lower (10% vs. 25%), and OIRs higher (63% vs. 40%) after euploid embryo transfer than after mosaic embryo transfer.
Conclusion(s): Forty-one percent of mosaic embryos produced an ongoing implantation. Complex mosaic blastocysts had a lower OIR than other mosaics. Mosaic monosomies performed as well as mosaic trisomies and mosaic segmental aneuploidies. The results suggest that embryos with >40% abnormal cells and those with multiple mosaic abnormalities (chaotic mosaics) are likely to have lower OIRs and should be given low transfer priority. (Fertil Steril® 2017;108:62–71. ©2017 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)
Key Words: Mosaicism, PGD/PGS, next-generation sequencing, pregnancy outcome

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Mosaicism has been described in human embryos since the beginning of preimplantation genetic screening (PGS) for aneuploidy (1–4). Although meiotically derived aneuploidy was found to increase with advancing maternal age, there was no clear relationship with other embryologic factors, such as dysmorphism (defined as presence of multinucleation, fragmentation, and unevenness) (5, 6). Conversely, other types of chromosome abnormalities, such as mosaicism occurring due to post-zygotic malsegregation, polyploidy, and haploidy, were found to increase with dysmorphism, but not with maternal age, with the exception of aneuploid embryos carrying a combination of meiotic and mitotic (mosaic) errors (5,7–12). Whole cleavage-stage embryo analysis, with strict criteria to classify an embryo as mosaic if at least two of its cells are abnormal, showed that ~30% of them were mosaic (12). On the other hand, lower rates of mosaicism have been detected in blastocysts (13–17).

With the advent of molecular techniques (array comparative genome hybridization [aCGH], single-nucleotide polymorphism array, quantitative polymerase chain reaction [qPCR], next-generation sequencing [NGS]), the DNA of all of the cells in the biopsy is analyzed as a single entity and compared to a control DNA sample. This has therefore precluded the analysis of mosaicism. In addition, some of these techniques do not have enough sensitivity to detect the presence of mosaicism in a biopsied embryo sample. Array CGH, the most widely used of these methods, can not detect mosaicism with a great degree of accuracy. The software used for aCGH analysis (Bluefuse; Illumina) has not been validated for mosaicism detection, and a noisy profile or a mosaic profile could look alike. With perfect profiles, we would be comfortable detecting 40%–60% mosaicism. Below 40% we would classify it as normal and above 60% as abnormal. Recently Greco et al. (18) reported a 4.8% rate of mosaicism (mosaicism range 35%–50%), compared with 15%–37% with the use of fluorescence in situ hybridization (FISH) (15–17,19).

In the past few years another technique, NGS, was validated and then implemented for PGS (20–23). NGS has a higher dynamic range compared with aCGH, and it can detect the presence of ~20%–80% abnormal cells in a blastocyst biopsy (21, 24, 25). There are several different NGS platforms, and not all of them can detect mosaicism to the same extent, because they have different resolutions. The platform we use is based on Illumina's Veriseq NGS strategy (high-resolution NGS [hr-NGS]) can detect mosaicism when aneuploidy is present in 20%–80% of TE cells biopsied from a blastocyst. Considering that a blastocyst biopsy has on average 5–10 cells, we are able to detect mosaicism being present in 1/5 (20%) to 4/5 (80%) of the TE cells. With the use of hr-NGS, we reported 21% of embryos to contain euploid/aneuploid cell lines and ~10% aneuploid/aneuploid (but with different aneuploid cell lines) (26). These findings are similar to the historical data obtained with the use of FISH when analyzing all cells individually. In addition, and similarly to the FISH data, we did not observe an increase of mosaicism rates with advancing maternal age (26). This preliminary work demonstrated that hr-NGS is a much more sensitive method, compared with aCGH and can accurately identify the presence of mosaicism (24, 27). Another NGS platform, CNV-Seq (23), can also detect 20%–80% mosaicism, but in that study only 13% of embryos were classified as mosaic. Lower-resolution NGS, such as Embryvu, has not reported the detection of mosaicism.

The ability of hr-NGS to accurately identify mosaicism in TE samples has led to significant disagreement and confusion regarding the biologic meaning of these findings. A recent investigation has even suggested that low-level mosaicism is indistinguishable from technical background noise (28). Another opinion paper (29) suggested that mosaicism detection is flawed, because if the same amount of trisomic and monosomic cells are present in a TE biopsy, then the average result will be of euploidy. However, a preliminary study (30) in which several biopsies from the same embryo were analyzed with the use of hr-NGS found that only two out of 28 embryos had monosomic and trisomic cell lines for the same chromosome abnormality, suggesting that this event is rare, because by the time the embryo reaches the blastocyst stage one of the abnormal cell lines has taken over, or compartmentalization is unlikely to produce that result.

There is also scant information on the clinical implications of replacing embryos classified as mosaic by means of hr-NGS. After replacing 44 mosaic blastocysts classified as such by means of hr-NGS but euploid by means of aCGH, 38% (17/44) implanted but 29% (5/17) of those miscarried, compared with 57% (29/51) implanting and 24% (5/29) miscarrying for a well matched control euploid embryo group (27). This is similar to 38% implantation rate after replacing mosaic embryos identified by means of aCGH (18). The difference between these two studies is that hr-NGS detects many more mosaic blastocysts than aCGH (29% vs. 5%).

In another study, reanalysis by means of hr-NGS of surplus DNA from embryos classified as euploid by means of aCGH that ended up in miscarriage showed that 46% were euploid and the rest mosaic or polyploid (31). Using the same approach, we also found significantly more mosaic embryos in lost pregnancies than in ongoing pregnancies ($P=.0062$) (24). The miscarriage rate after replacing embryos classified as euploid by means of NGS has been determined to be 6%, compared with 13% for aCGH (32) and 20% for qPCR (33).

Thus the evidence suggests that embryos classified as being mosaic by means of NGS miscarry more and implant less, but ~40% of them can still result in a viable pregnancy. To date there is little information about which of these NGS mosaics have the highest chance to make a viable pregnancy. Therefore the purpose of the present study was to retrospectively analyze the pregnancy outcomes of replaced mosaic embryos and compare them to the type of mosaicism seen according to several different criteria, such as percentage of abnormal cells, chromosome abnormality type (segmental, trisomy, monosomy, complex abnormal), and chromosomes involved.

METHODS

Patients and Embryos

This study included patients that received transfer of mosaic embryos only and for which the clinical outcome was known. Forty-four of these embryos (series 1) were included in a