ORIGINAL ARTICLE: ASSISTED REPRODUCTION

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

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

[a] GENOMA Group, Molecular Genetics Laboratories; and [b] Reproductive Medicine, European Hospital, Rome, Italy

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; trophectoderm 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 mosaicism <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. 46.6%) 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;■: ■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Preimplantation genetic screening, mosaic embryos, array-comparative genomic hybridization, next-generation sequencing, embryo aneuploidy

Discuss: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/20505-24473

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).

Received June 6, 2017; revised September 5, 2017; accepted September 21, 2017.
F.S. has nothing to disclose. F.F. has nothing to disclose. A.B. has nothing to disclose. S.B. has nothing to disclose. A.R. has nothing to disclose. E.C. has nothing to disclose. M.B. has nothing to disclose. E.C. has nothing to disclose. M.G.M. has nothing to disclose. E.G. has nothing to disclose.
Reprint requests: Francesco Fiorentino, Ph.D., GENOMA Group, Molecular Genetics Laboratories, Via di Castel Giubileo, Rome 11 00138, Italy (E-mail: fiorentino@laboratoriogenoma.it).

Fertility and Sterility® Vol. ■, No. ■, ■ 2017 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2017.09.025

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 trophectoderm (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 demolition, 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