Chromosomal mosaicism detected during preimplantation genetic screening: results of a worldwide Web-based survey

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Embryonic mosaicism, the presence of more than one distinct cell line within an embryo, has recently become the focus of growing attention and controversy in the context of preimplantation genetic screening (PGS). To evaluate the extent of mosaic aneuploidy in clinical practice and to gain insight on the practices and views regarding this issue, we conducted a survey using a prospective, 20-item Web-based questionnaire with questions related to practices and views regarding mosaicism in PGS. A total of 102 in vitro fertilization (IVF) units from 32 countries that performed 108,900 IVF cycles annually responded to the survey. More than half responded that embryonic mosaic aneuploidy is reported by the laboratory, but 31.9% stated that samples are reported as euploid or aneuploid only. If mosaic aneuploidy is reported, 46% stated that it was present in $\leq$10% of the embryos. More than two-thirds were of the opinion that next-generation sequencing is required to reliably detect mosaicism. Among centers performing PGS, 47.9% consider embryonic mosaicism when detected in >20% of the cells, and nearly two-thirds believe that mosaic aneuploid embryos should be stored for potential therapeutic use after extensive and appropriate counseling. In summary, mosaicism has always existed in preimplantation embryos, and new technologies can now detect its presence with higher resolution. More studies are needed before definite conclusions can be drawn. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy, chromosomal aberrations, in vitro fertilization, mosaicism, preimplantation genetic screening

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Chromosomal aberrations are the leading cause of implantation failure, miscarriage, and congenital anomalies in humans (1). It has been shown that a great proportion of human preimplantation embryos exhibit chromosomal aberrations, mostly aneuploidies (trisomies and monosomies). It has therefore been suggested that performing preimplantation genetic screening (PGS) for aneuploidy detection would improve outcomes of assisted reproductive technology (ART). The underlying hypothesis was that by transferring only euploid embryos, implantation and pregnancy rates would improve and miscarriage rates decrease (2). Proponents have suggested that PGS would be particularly useful for patients who are at higher risk of aneuploidy, such as women of advanced age, those with recurrent miscarriages, or those in couples who have experienced repeated implantation failure.

Initially, PGS has been performed using fluorescence in situ hybridization (FISH) for five to nine chromosomes in blastomeres biopsied from cleavage-stage embryos. Despite the initial enthusiasm, subsequent randomized controlled trials (RCTs) and a meta-analysis indicated that PGS using fluorescence in situ hybridization failed to show improved reproductive outcomes (3, 4). Subsequently, professional societies discouraged the use of PGS in this form, and its use declined. Current PGS practice, nicknamed PGS 2.0, employs comprehensive chromosomal screening of all 24 chromosomes using advanced platforms such as array comparative genomic hybridization

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(aCGH), single-nucleotide polymorphism (SNP) microarrays, quantitative or real-time polymerase chain reaction, and next-generation sequencing (NGS). In most cases genetic analysis is performed on multicellular biopsy samples obtained from the trophectoderm of day-5 to day-6 blastocysts, which, subsequently, may be vitrified. The effectiveness of this approach is still a matter of controversy (5–7). To complicate matters even further, these advanced techniques, particularly NGS, have unveiled the phenomenon of embryonic mosaic aneuploidy—the presence of cell lineages with different chromosomal constitution.

When PGS is performed and both euploid and mosaic aneuploid embryos are detected, preference is obviously given to the former. However, in some instances, only mosaic aneuploid embryos are present. The transfer of such mosaic aneuploid embryos has been reported to result in the occasional birth of healthy children (8), but there is a great deal of uncertainty about the transfer of such embryos and the circumstances and conditions for doing so. Recent position statements on chromosome mosaicism in PGS have proposed guidelines to prioritize mosaic embryos for transfer, depending on the chromosome(s) involved (9, 10).

Nonetheless, in lieu of sufficient data on the outcomes of pregnancies achieved after transfer of mosaic aneuploid embryos, it is a matter of speculation as how to best deal with this phenomenon. To further assist clinicians and researchers dealing with this matter, we conducted this survey to evaluate the extent of mosaicism in PGS clinical practice and to gain insight on the views and practices of the ART community regarding this issue.

MATERIALS AND METHODS

A 20-item survey entitled "Survey on Mosaicism in Preimplantation Genetic Screening (PGS): What Is Your Opinion?" was compiled and posted on the IVF-Worldwide Web site from December 1 through December 31, 2016. The survey questions can be accessed at: www.ivf-worldwide.com/survey/survey-on-mosaicism-in-preimplantation-genetic-screening-pgs.html. The survey questions focused on various aspects of PGS, including technical aspects and laboratory practices. The attitudes and opinions regarding mosaicism in PGS were assessed with particular distinction between centers that perform PGS and those that do not.

Quality Assurance

Minimization of duplicate reports from a clinical unit as well as possible false data was achieved via a computerized software program that assessed the consistency of three parameters from self-reported data of the unit surveyed with existing data of units registered on the IVF-Worldwide Web site, as previously described elsewhere (11). These parameters included the name of the unit, country, and e-mail address. At least two of the parameters from the survey had to match archived data on the Web site in order for the data reported by the clinical unit to be included in the study.

Statistical Analysis

The analysis was based on the number of IVF cycles reported by the unit, not on the number of units in the study. Thus, the relative proportion of answers reflects the total proportion of IVF cycles represented rather than the proportion of individual respondents to the survey questions. The survey was structured as a sequence of multiple-choice questions in which respondents could select a single answer for most questions, although in two questions multiple answers were possible. The results were calculated by using the following formulas as described in previously reported research from the IVF-Worldwide network (11):

$$\%\, a = \frac{\sum \text{Number of cycles in units that answered } a}{\sum \text{Number of cycles in all units}} \times 100$$

$$\%\, b = \frac{\sum \text{Number of cycles in units that answered } b}{\sum \text{Number of cycles in all units}} \times 100$$

$$\%\, c = \frac{\sum \text{Number of cycles in units that answered } c}{\sum \text{Number of cycles in all units}} \times 100$$

$$\%\, d = \frac{\sum \text{Number of cycles in units that answered } d}{\sum \text{Number of cycles in all units}} \times 100$$

This article does not contain any studies with human or animal subjects. Because the survey does not involve human subject research, formal institutional review body approval was not obtained. The survey was available as an open-access questionnaire to the members of the IVF-Worldwide.com who voluntarily answered the study questions. Data collected for this research were anonymous. The study did not involve the use of laboratory animals, and the authors declare that they have no conflicts of interest.

RESULTS AND DISCUSSION

The results represent responses from 102 IVF centers from 32 countries around the world, representing a total of 108,900 annual IVF cycles. Of these, 87 centers, representing 99,300 annual IVF cycles, routinely performed PGS, and 15 centers, representing 9,600 annual IVF cycles, did not perform PGS. The detailed responses to all the questions can be accessed through the IVF-Worldwide Web site at www.ivf-worldwide.com/survey/survey-on-mosaicism-in-preimplantation-genetic-screening-pgs/results.html. Although the survey respondents were heavily biased toward PGS, this would not have affected the overall conclusions because comparisons were made within each group separately (i.e., centers that perform PGS versus those that do not). The geographical distribution of IVF units participating in the survey is presented in Table 1.

In centers where PGS is routinely performed, 90% perform trophectoderm biopsies on day 5, or days 5 to 6, depending on blastocyst development, whereas only 10% performed blastomere biopsy of ≥ 1 cells from day-3 cleavage-stage embryos. None of the respondents perform PGS