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Editorial

The Disputable Discourse on Accuracy and Effectiveness of PGT-A in Light of Advancements in Genetic Tools

In the past three decades, the main concern regarding infertility treatment was whether checking embryos for chromosome aneuploidy before transfer to uterus has positive effect on results of in vitro fertilization (IVF), and correspondingly implantation, pregnancy, and live birth rates. During this long period of developments in the diagnosis and treatment of infertility, researchers made their best efforts to improve embryo quality by selecting the best ones for transfer and subsequently increasing outcomes of infertility treatment; yet, the substantial revolutions in the methods of genetic evaluation and performing preimplantation genetic testing for aneuploidies (PGT-A) assisted them for such purpose. Despite these developments, researchers have not yet been able to experimentally or clinically validate the effectiveness of these techniques. On the contrary, there is growing evidence that the effectiveness of these procedures is being questioned due to biological characteristics of early human embryos at pre-implantation stage (1).

PGT-A, originally known as preimplantation genetic screening (PGS), was performed on a limited number of chromosomes using FISH on one blastomere of cleavage embryo. Today, the development of high throughput genomic methods such as next generation sequencing (NGS) and microarray-based comparative genomic hybridization (aCGH) and TE biopsy at blastocyst stage results in accurate diagnosis of aneuploidy; however, the case of mosaic embryos is a new challenge of IVF for which the application of PGT-A is matter of concern and debate (2).

Regarding mosaicism detection in embryos, three conditions can be reported for trophectoderm biopsy and PGT-A results at blastocyst stage: (1) the reported euploid embryos may be 100% euploid or contain aneuploid cells elsewhere in the trophectoderm or inner cell mass. The actual explanation is that this embryo is not completely aneuploid, may be fully euploid or mosaic; (2) the reported aneuploidy embryos may actually be 100% aneuploid or may be mosaic due to presence of euploid cells elsewhere in the trophectoderm and inner cell mass. In fact, this embryo is not completely euploid; (3) the reported mosaic embryo is certainly mosaic, although it is not possible to reliably determine the true percentage of euploidy and aneuploidy through few cells collected from trophectoderm (1).

Thus, the reported mosaicism is much lower than the actual rate, whereas the clinical significance of mosaicism in PGT-A has been overestimated. Still, many researchers falsely claim that mosaicism occurs in a small number of embryos at blastocyst stage, while *in vivo* and *in vitro* studies on mice and human embryonic stem cells have shown that the mosaicism at blastocyst stage is essentially a natural physiological phenomenon. On the other hand, mosaicism is one of the common findings of PGT-A following new generation of diagnostic tools. In addition, several studies have shown that ongoing pregnancies and live birth can be achieved even by transfer of mosaic embryos. For example, in one study, transfer of 102 mosaic embryos resulted in 46.6% live births, while the transfer of 268 euploid embryos resulted in 59.1% live births, respectively (3).

It has been shown that mosaic embryos have different characteristics compared to euploid embryos. Based on this finding, this process has been proposed as a mechanism for aneuploidy correction in which aneuploid cells are selectively removed from the embryo. In mice, aneuploid embryonic cells are removed by autophagy and apoptosis and a similar mechanism has recently been identified in human embryos. In addition, it has been shown that the elimination of aneuploid cells from inner cell mass (ICM; embryonic cells) is greater than trophectoderm cells (TE; extraembryonic cells) (4).

Application of spent culture media or blastocoelic fluid (BF) containing cell free DNA (cfDNA) is a new option for noninvasive PGT-A. The elimination of aneuploid cells through self-correction process of embryos leads to release of cfDNA in blastocoel and culture media. An interesting finding was that the cfDNA in BF of aneuploid embryos is more likely to be amplified, showing that more DNA is released form apoptosis of aneuploid cells. This could mean that the amount of cfDNA in BF could predict euploidy status of embryos. Therefore, the use cfDNA for PGT-A may lead to false positive results and subsequently elimination of euploid embryos from the total number of embryos of a couple (4, 5).

In order to use any clinical test for diagnostic purposes, adequate levels of sensitivity and specificity must be achieved. Both invasive trophectoderm biopsy and the noninvasive cfDNA techniques have high levels of sensitivity and specificity for the diagnosis of euploid embryos, while their diagnostic ability for aneuploidy is very disappointing; therefore, high frequency of false positive results leads to elimination of numerous

healthy euploid embryos. The evidence for this claim is based on various reports of the live birth of healthy neonates from the embryos which were tagged as aneuploid or mosaic through PGT-A (5).

According to recent findings, the human early embryos have self-correction ability to remove the aneuploid blastomeres. Therefore, with the development of the embryo from the cleavage to the blastocyst stage, the aneuploidy of embryos decreases; also, lower rate of aneuploidy among day 5 embryos compared to day 3 confirms such decline. Currently, the maximum time for culturing and maintaining early embryos outside the uterus is 6 days, after which the embryos should be frozen or transferred into the uterus. Therefore, if the advancement of science and technology lays the ground for longer *in vitro* culture of human embryos without interfering with implantation rate, it will be possible to select more euploid embryos for transfer into the uterus and considerably enhance the success rate of infertility treatment (1, 4).

Despite huge improvements in genetic testing and increasing the accuracy and efficiency of methods, the effectiveness of PGT-A in IVF has always been challenged. Simply put, based on the available evidence, the usefulness of these methods had not been proven in the last three decades, therefore, the rationale for recommending PGT-A routinely for all IVF cycles cannot be justified now since it may decrease the chance of a couple to have a child and waste their time, money, and energy for continuing treatment.

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Mohammad Reza Sadeghi
Editor-in-chief