PGT-A for Elderly maternal

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Contributor: Chenming Xu

Preimplantation genetic testing for aneuploidies (PGT-A) is widely used in women of advanced maternal age (AMA). However, the effectiveness remains controversial.

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comprehensive chromosomal screening
embryo biopsy

1. Introduction

Preimplantation genetic testing (PGT) was introduced over thirty years ago as an early form of prenatal genetic diagnosis performed with multiple assisted reproductive technologies, such as embryo biopsy, embryo vitrification, and embryo transfer [1]. This procedure is mostly performed to reduce the transmission of genetic disorders for patients with monogenic diseases (PGT-M), chromosomal segmental rearrangement (PGT-SR), or aneuploidies (PGT-A) [2]. It is well known that the karyotypically embryotic anomaly is one of the main causes of recurrent pregnancy losses [3]. The occurrence of embryotic aneuploidies increases with maternal age. As a result, women of advanced maternal age (AMA) have a higher risk of miscarriages, implantation failures after in vitro fertilization (IVF), fetal malformations, and fetuses born with chromosomal disorders [4][5]. However, the effectiveness of PGT-A in women of AMA is still controversial.

A meta-analysis reported in 2011 showed no evidence of a beneficial effect of PGT-A on live birth rate after IVF [6]. Possible reasons for the disappointing results might be associated with the PGT-A procedure per se, such as damages caused by embryo biopsy and misdiagnoses owing to embryotic mosaicism. As reported, blastomere biopsy might delay the compaction and blastulation of embryos, thus affecting the implantation [7][8]. Additionally, fluorescence in situ hybridization (FISH), the first and the most widely applied in PGT-A, can only assess a select number of chromosomes (5 to 12 chromosomes) simultaneously [9]. Furthermore, the accuracy of FISH was always affected by signal overlaps, signal splits, signal diffusion, and probe inefficiency [10][11].

In the past decade, PGT has developed rapidly with advanced methods of biopsy and strategies of genetic analysis. On the one hand, embryo biopsy has evolved into trophectoderm biopsy, which entails removing 5–10 trophectoderm cells at the blastocyst stage, which reported more accuracy than cleavage-stage biopsy and has less impact on embryo viability, leading to a higher implantation rate [12][13][14]. On the other hand, techniques of genetic testing have been developed from FISH to the comprehensive chromosome screening (CCS) technology, including real-time quantitative PCR (qPCR), array comparative genomic hybridization (aCGH), and next-

generation sequencing (NGS) [15][16][17][18]. Although the qPCR method is ineffective in the detection of segmental aneusomy, an improvement in implantation and delivery rates has been observed in qPCR-based PGT-A [19]. Better than the qPCR method, aCGH has been commonly used in detection of segmental abnormalities and unbalanced translocations in PGT procedures [20]. Moreover, NGS is introduced as a meaningful approach of aneuploidy screening characterized by its high throughput, low cost, and high sensitivity and specificity. NGS can also be used in the detection of copy number variations, unbalanced translocations, and point mutations [21].

2. Comprehensive Chromosome Screening

Compared with FISH, aCGH could detect copy number variations (CNV) and unbalanced translocations effectively by mixing the fluorochrome-labeled test DNA with a control sample and hybridizing them onto an array platform [22]. Fragouli et al. applied both aCGH and FISH to 12 embryos donated from 5 patients [23]. It was observed that the results of nine embryos (75%) were consistent, while two aneuploid embryos were not identified by FISH and were theoretically detected by the probes, and one embryo was recognized with a trisomy of chromosome 8, which was out of the scope of FISH [23]. Additionally, NGS was used in PGT-A as a reliable and high-throughput strategy, which enabled higher sensitivity in the diagnosis of mosaicism with greater resolution compared to aCGH. Various studies validating the accuracy of the NGS approach for CCS of embryos demonstrated a 100% diagnosis consistency with aCGH [21][24]. A comparison between NGS and aCGH applied to PGT-A has also been performed and evaluated [25]. The implementation of NGS for PGT-A revealed higher implantation rates and live birth rates compared to aCGH, which might be attributed to the advantages of NGS in detecting small chromosomal deletions and duplications and mosaicism. In addition, the NGS could be used for the diagnosis of single-gene disorders, translocations, and haplotype analysis in PGT.

3. Stage of Embryo Biopsy

Embryo biopsy, obtaining genetic materials from oocytes or embryos, is a significant step during the PGT procedure. It can be performed at different stages, including polar body biopsy (polar bodies), cleavage-stage biopsy (a single blastomere), and blastocyst biopsy (5 to 10 trophectoderm cells). The first and second polar bodies were produced during meiosis of oocytes and seemed to be not necessary for embryo development; therefore, polar body biopsy was considered less damaging than cleavage-stage biopsy and blastocyst biopsy. However, polar body biopsy cannot analyze the genetic information from paternity or the later development stage of embryos, which are important factors affecting its predictive power [26]. More frequently, embryo biopsy was performed on Day 3 by extracting 1–2 blastomeres. Nevertheless, the major drawback of cleavage-stage biopsy was misdiagnosis or missed diagnosis of mosaicism because of the limited materials [27]. With the technical innovation of embryo culture and vitrification, blastocyst biopsy emerged and was conducted by removing 5–10 trophectoderm cells on Day 5, which provided more testing samples than cleavage-stage biopsy for detecting mosaicism and reducing the risk of amplification failure. Moreover, it was reported that the aneuploidy rate was lower in blastocyst biopsy than in cleavage-stage embryos, as euploid cells showed a growth advantage in the embryo development [23]. However, undesirable effects of the embryo biopsy reducing the embryonic development

potential have been reported, such as cleavage arrest in polar body biopsy and blastulation delay in blastomere biopsy [8][14][28].

4. Embryo Mosaicism

Embryo mosaicism, a phenomenon of both euploid and aneuploid cells observed in the same embryo, was another significant factor accounting for the ineffectiveness of PGT-A. It was derived from mitotic errors at all post-zygotic stages of the embryo, which increased with maternal age in mitotic aneuploid mosaicism [29]. However, the criteria of mosaicism that the threshold percentage of abnormal cells had been still undefined. Two aspects playing vital roles in the diagnosis of mosaicism were the techniques of genetic analysis and materials obtained from embryo biopsy. Compared to aCGH, NGS was presented with a higher resolution, which could detect mosaicism as low as 20% in aneuploid cells [30]. It was estimated with an incidence ranging from 2% to 13% through the strategy of trophectoderm biopsy combined with NGS analysis [31]. However, their developmental potential remains to be determined. Several studies demonstrated that embryos diagnosed as mosaic were more likely to miscarriage than euploid embryos [31]. Nevertheless, a comparable rate of live birth and ongoing pregnancy was reported between euploid embryos and low-percent mosaicism (<50%) [32]. Accordingly, a standardized assessment is necessary to be finalized for the clinical decision and care of mosaicism in PGT-A.

5. Summary of Results

The results of our systematic review and meta-analysis could be summarized as two key findings. First, the utilization of CCS in the PGT-A procedure could improve the pregnancy outcomes as it accurately assessed the embryo euploidy status. Second, the blastocyst biopsy might be advantageous in the PGT-A procedure in women of AMA. Although the overall analysis showed that the pregnancy outcomes of the PGT-A group were not better than those of the control group, it was found that the live birth rate, the primary outcome, was higher after IVF with PGT-A than in the control group. Meanwhile, the ongoing pregnancy rate showed the same trend, which was higher in the PGT-A group with CCS compared with those with FISH. Regarding stages of embryo biopsy, seven of the included trials in this study were cleavage-stage biopsy, one trial was polar body biopsy, and one was blastocyst biopsy. In the group of blastocyst biopsy, IVF with PGT-A showed a higher rate of ongoing pregnancy and live birth than that in the control group of women of AMA, whereas comparable rates were observed in the polar body biopsy group and the cleavage-stage biopsy group.

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