Relevance of Embryo Aneuploidy in Medically Assisted Reproduction

71

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71.1 Background

The prevalence of primary and secondary infertility has been estimated in 1.9% and 10.5%, respectively, in women of 20–44 years of age from 190 countries according published data in 2010 [1]. Aneuploidy is the main genetic factor that influences human reproductive success [2]. As it has been published, aneuploid embryos account for at least 10% of human pregnancies, and the incidence could exceed 50% for women over 35 years of age [3, 4]. Most aneuploidies compromise the implantation of the conceptuses that perish in utero, and those that implant may result in an early miscarriage or cause congenital birth defects.

Medically assisted reproduction (MAR) allows for the treatment of most infertile couples with the aim of securing a healthy birth. Therefore, in vitro fertilization (IVF) laboratories are challenged to reduce the risk associated with multiple pregnancy. For that, most of the IVF clinics have moved to the strategy of a single embryo transfer, diagnosed as chromosomally normal, since selecting just the morphologically normal ones to transfer is not enough to guarantee its success. Morphology of an embryo is weakly correlated with its viability and, hence, with its chromosome constitution. All type of uniform aneuploidies can survive to the blastocyst stage [3, 5–13]. Moreover, 40–50% blastocysts with optimal morphology can be chromosomally abnormal [14, 15], and euploid embryos do not always demonstrate better morphology than chaotic mosaics [16]. On the other hand, there is a correlation between aneuploidy and maternal age due to an increase of premature sister chromatids separation and meiotic nondisjunction of homologous chromosomes [17]. As an example, aneuploidy increases from 40% in fertile egg donors to 80% in patients of 41–42 years old [18]. However, Harton et al. in 2013 [19] demonstrated that if a chromosomally normal embryo is transferred to the uterus, the chance to implant is independent of maternal age. The transfer of abnormal embryos in an IVF cycle is related to higher rates of implantation failure and miscarriage. Although there is a direct correlation between embryo aneuploidy and maternal age, there is also positive correlation with other factors such as sperm chromosome abnormalities, altered male meiosis, or nongenetic male factor [20, 21].

Preimplantation genetic testing (PGT) has been used since the 1990s to diagnose genetically abnormal embryos for selecting, with some certainty, those genetically normal embryo(s) to be transferred to the uterus with the maximum guarantees to implant and to reach term. PGT has been incorporated into IVF laboratories to improve the efficiency of ART, increasing implantation rates while lowering pregnancy loss rate [22–29]. The success of PGT for aneuploidy screening (PGT-AS) is not limited to the technique itself but depends on different factors: (1) the optimization of the PGT-AS technique; (2) the proper selection of patients for PGT-AS; (3) the number of analyzed chromosomes (limited or comprehensive chromosome screening, CCS); and (4) the protocols of ovarian stimulation, in vitro embryo culture, and embryo(s) transfer. Focusing on PGT-AS technique, over the past years, different methodologies have been optimized to overcome many of the technical limitations intrinsic to the analysis of a single cell or a few number of them. Fluorescence in situ hybridization (FISH) on fixed nuclei from biopsied blastomeres was the technique of choice over the past two decades. However, the classic FISH technique analysis was limited to a restricted number of chromosomes [30] restricting the improvement of IVF outcomes with PGT-AS, as reported by several authors [31–38] and advised by the ESHRE PGD Consortium [39]. Therefore, the natural evolution of the PGT-AS has driven to the development, clinical validation, and application of the new emerging CCSs methodologies. Currently, the available CCS techniques developed and clinical validated for PGT-AS are array comparative genomic hybridization (aCGH) [5, 11, 15, 40], 24-chromosome FISH

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(FISH-24) [41], single nucleotide polymorphism (SNP) microarray [42], quantitative real-time polymerase chain reaction (qPCR)-based CCS [43], and more recently next-generation sequencing (NGS) [44–50]. The application of CCS techniques also produces a change in the protocol of biopsy, moving from day 3 to day 5 of embryo development in order to have more quantity and quality of DNA for amplification and to overcome the high rate of mosaicism detected on cell stage embryos that can lead to misdiagnosis. Among these technologies, NGS seems to detect with higher accuracy for segmental imbalances [51, 52] and chromosomal mosaicism [51, 53–55]. Recently after three randomized control trials (RCT) testing day 5 blastocyst biopsies in good prognosis patients, there appear to be significant improvements in ongoing pregnancy rates [26, 27, 56], encouraging physicians to recommend PGT-AS on trophectoderm samples.

71.2 Is There an Optimal PGT-AS and Embryo Transfer Program?

One of the more recent discussions about PGT-AS using the new CCS platforms is which is the most efficient operating way in terms of maximizing pregnancy rates. When PGT-AS by FISH was established, most centers did the biopsy on day 3, and euploid embryos were transferred in day 5 in a fresh cycle, but pregnancy rates were not as good as expected. In the last few years, there is published evidence showing that transferring cryopreserved embryos in a non-stimulated cycle increases clinical implantation rates [57–63] and decreases low birth weight and preterm delivery rate [64, 65]. Coates et al. in 2017 [66] published a RCT comparing both approaches: to perform day 5 biopsy and vitrify all embryos while waiting for the PGT-AS results and to carry out the euploid embryo transfer in a non-stimulated cycle versus biopsing embryos at day 5 and transferring the euploid embryos on day 6 in a fresh cycle. Embryos showing slow development were biopsied on day 6 and kept frozen for a future non-stimulated transfer, in case of failed outcomes. The study was performed in a US institute with a long standing experience in embryo vitrification, embryo culture, and biopsy procedures, and the results showed, in terms of ongoing pregnancy rates and live birth rates, a trend in favor of a freeze all strategy and transference of the euploid embryos in non-stimulated cycles. Another RCT published by Rubio et al. [67] compared the effectiveness of clinical outcome with and without PGT-AS in women with advanced maternal age (from 38 to 41 years old) after embryo analysis by aCGH. They published a higher delivery rate per transfer after the first transfer attempt (52.9% vs 24.2%) and higher delivery rate per patient (36.0% vs. 21.9%) in the group that performed PGD aneuploidy screening compared to the group that did not perform PGD.

The main issue when applying the freeze all strategy is that the laboratory must optimize its culture conditions to achieve the highest rates of blastocyst formation. Moreover, vitrification and thawing protocols must be optimized in order to achieve the highest post-warming survival and cleavage development rate. Unfortunately, not all IVF laboratories around the world have standardized protocols, and, even among those following the highest quality standards, results may drastically differ from one center to another. This suggests that although publications are in favor of a specific strategy, each center should analyze its own laboratory efficiency and which strategy is the best for them. For one laboratory that does not have a good established blastocyst vitrification protocol and presents a high incidence of lysed cells and low development rate post-warming, the best approach would be to perform day 5 biopsy and transfer in a fresh cycle and only keep vitrified the D6 biopsied blastocyst for a second transfer. Another scenario may be a laboratory that presents a poor embryo culture conditions. In that way, the best approach should be to biopsy on day 3 and transfer in day 3/4 in a fresh cycle to avoid losing embryo potential.

71.3 Mosaicism

Transferring high morphological quality euploid embryos has increased pregnancy rates, but we are still faced with the challenge that some euploid embryos with a good morphology fail to implant. In this scenario, many programs have started to utilize time-lapse PGD-AS studies to correlate morphokinetics parameters and the type of aneuploidy in an attempt to identify which embryos have better competence to implant, but that said, efficiency is still not 100%. This can mainly be due to two factors: mosaicism and technical limitations.

Embryo mosaicism is one of the main sources of error when performing PGT-AS [68–76]. To establish the rate of mosaicism in preimplantation embryos is a complex task since it varies according to the embryo stage, the technology used for the diagnosis, and the skills of the genetic laboratory for the interpretation. In cleavage-stage embryos, the estimated levels of mosaicism vary from 15 to 75% while in blastocyst have been estimated in 3–24%, according to a published review [77]. The great variability on reported data can also be influenced by different factors other than PGT-AS procedure itself, including the etiology of infertility, female's age, or even in vitro culture and environmental conditions. All these elements can also impact the abnormal chromosome segregation leading to embryonic mosaicism. However, it appears that there is a general agreement for the observation that a gradual decrease in aneuploidy takes place during embryo development most probably due to self-correction mechanisms and preferential development of euploid cells.

Mosaicism occurs during mitotic division of the embryo, giving rise to chromosomally different cell lines. When analyzing one cell from the embryo, it is assumed that the result is representative of the whole embryo. In order to avoid mosaicism misdiagnosis when performing PGT-AS, two different strategies have been proposed. The first is to perform polar body 1 and 2 analysis. Using this strategy, only chromosomal abnormalities of maternal meiotic origin are analyzed, while paternal meiotic abnormalities and abnormal chromosomal mitotic segregations are not evaluated. The second is to perform trophectoderm biopsy at blastocyst stage, analyzing more than one embryonic cell in in a developmental stage with a lower rate of mosaicism compared to day 3 embryos. This strategy can be used only if a good system for day 5 embryo culture is available and if a high number of embryos is achieved. However, although mosaicism rate is lower, it can be present so there is still a risk of misdiagnosis. At the blastocyst stage, different types of mosaics have been described [78]: mosaicism that affect both trophectoderm (TFE) and inner cell mass (ICM), when the abnormal cells are confined to the TFE or ICM exclusively or when the ICM is normal TFE is abnormal (or vice versa). Depending on the type of mosaicism we are facing and the TFE cells we are biopsing by chance will condition PGT-AS misdiagnosis rate on blastocyst stage. Some studies have tried to estimate this correlation between ICM and TFE cell lines by biopsing two or three different groups of cells of the same embryo. They observed a diagnosis correlation of 95–100%, and the discordance between ICM and TFE cell lines was estimated to be around 3–4% [79, 80].

Another strategy to avoid misdiagnosis due to mosaicism on the PGT-AS results has been to perform two cells biopsy on day 3. However, this strategy has been demonstrated to be detrimental for embryo developmental competence and has not been recommended any longer.

New CCS platforms for PGT-AS such as NGS can detect low levels of diploid/aneuploid mosaicism with high accuracy (lower than 20%). Mosaic or potentially mosaic embryos have become a new category to classify embryos [81]. According PGDIS recommendations [82], embryos with a mosaicism rate lower than 20% can be considered as euploid (and then transferable), while embryos with more than 80% of abnormal cells are classified as aneuploid. The remaining ones (20–80%) can be classified as mosaics. However, to establish the thresholds between which the embryos can be considered transferable or not is a controversial issue. Simon et al. recently suggested [81] that one consider above 50% of mosaicism embryo to be classified as aneuploid and non-transferable. According to a worldwide survey from 32 countries, <10% of the analyzed embryos are classified as mosaics [81]. These embryos have a theoretically decreased implantation rate and increased risk of miscarriage, pregnancy complications, and clinically affected life births [81, 82]. Transferring embryos categorized as mosaic, although can raise some ethical considerations, is generally accepted when the couple does not have any euploid embryos [82, 83]. Different factors should be taken into consideration such as the methodology used for testing, the involved chromosomes, or the reproductive medical history of the couple [81–83]. Regarding this matter, PGDIS consortium published a suggested guideline to prioritize mosaic embryos for transfer. Patients may consider transferring a mosaic embryo only after a proper genetic counselling about the risks of miscarriage and adverse outcomes they can face.

71.4 Conclusion

One of the most important challenges for the embryologist is to discern which is the most competent embryo to transfer. Many efforts to find the magical wand have been made in studying the cytoplasmic and nuclear competence, the morphology, and morphokinetics during embryo development or in developing the most paramount technique to detect all chromosome aneuploidies. Yet still, just when we thought that we had the most comprehensive technology that permits us to screen all chromosomes, some new question arises and makes us go back in time and question all we know. Is embryo mosaicism an indicator of euploidy? Do we have to discard mosaic embryos?

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