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An evidence-based scoring system for prioritizing mosaic aneuploid embryos following preimplantation genetic screening

Francesca Romana Grati [a,*], ***Gloria Gallazzi*** [a], ***Lara Branca*** [a], ***Federico Maggi*** [a], ***Giuseppe Simoni*** [a], ***Yuval Yaron*** [b]

[a] *R and D, Cytogenetics and Medical Genetics Unit, TOMA Advanced Biomedical Assays S.p.A., Busto Arsizio, Varese, Italy*

[b] *Prenatal Genetic Diagnosis Unit, Genetic Institute, Tel Aviv Sourasky Medical Centre and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

Francesca Romana Grati graduated is R&D Director of Istituto TOMA Advanced Biomedical Assays and contract professor at the School of Medical Genetics in Milan. Her research interests are the epidemiology of fetal chromosome abnormalities, genetics and epigenetics of placenta and feto-placental mosaicisms.

KEY MESSAGE

Mosaic aneuploid embryos are occasionally encountered during PGS, and often these are the only embryos available for transfer. It is currently unclear whether mosaic embryos should be considered for transfer. The aim of this study was to devise an evidence-based scoring system for prioritizing mosaic aneuploid embryos for transfer.

A B S T R A C T

The aim of this study was to devise an evidence-based scoring system for prioritizing mosaic aneuploid embryos for transfer. A retrospective analysis was performed of all sequential cytogenetic and molecular results on chorionic villi samples (n = 72,472) and products of conception (n = 3806) analysed at a single centre. The likelihood that a mosaic aneuploidy detected in chorionic villi samples will involve the fetus, the incidence of clinically significant fetal uniparental disomy in the presence of a mosaic in chorionic villi and the chance of the mosaicism culminating in miscarriage were used to generate a scoring system for prioritizing mosaic aneuploid embryos detected by preimplantation genetic screening. A composite score was obtained for each individual mosaic aneuploidy after assignment of an individual risk score based on the incidence/likelihood of each adverse outcome. A final additional score was assigned to viable full or mosaic aneuploidies with a well-defined phenotype. The higher the composite score the lower the priority for embryo transfer. In conclusion, due to the paucity of prospective studies on the actual transfer of mosaic aneuploid embryos, we suggest using this evidence-based scoring system to provide a useful tool for clinicians, embryologists and patients.

* Corresponding author.
E-mail address: fgrati@tomalab.com (FR Grati).

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Introduction

Aneuploidy is the most common type of chromosome abnormality and is the leading cause of implantation failure, miscarriage and congenital abnormalities in humans (Hassold et al., 1996). This fact prompted the introduction of preimplantation genetic screening (PGS). The hypothesis was that if embryos obtained by IVF were screened for aneuploidy prior to transfer, implantation and pregnancy rates would improve and miscarriage rates decrease (Munné et al., 1993). This approach would be particularly useful in patients at an increased risk of having aneuploid embryos, such as patients of advanced age, those with recurrent implantation failure or cases with repeated miscarriage. Initially, PGS was performed by fluorescence in-situ hybridization (FISH) on fixed cells and day 3 biopsy. However, the effectiveness of this approach has been questioned by several randomized control trials (Mastenbroek et al., 2007, 2011; Twisk et al., 2005, 2006). One of the reasons why PGS with FISH may not have been successful is that only a limited number of chromosomes were analysed. Other reasons may include technical proficiency with biopsy and fixation of cells for FISH analysis (Cohen and Grifo, 2007; Munné et al., 2007a, 2007b; Simpson, 2008). The development of novel molecular approaches has ushered in the concept of PGS 2.0. In this approach, comprehensive chromosomal screening (CCS) of all 24 chromosomes is performed by array comparative genomic hybridization (aCGH), real-time quantitative PCR (qPCR) or, more recently, next-generation sequencing (NGS) (Forman et al., 2014; Rubio et al., 2017; Scott et al., 2013; Yang et al., 2012). The analysis is usually performed on several trophectoderm (TE) cells removed from a day 5–6 blastocyst. However, when such genome-wide approaches are employed, particularly when several cells are analysed, mosaic aneuploidy is occasionally detected, specifically in 4% of embryos by aCGH (Greco et al., 2015) and 21% of embryos by NGS (Munné and Wells, 2017). This usually implies that aneuploidy is present in only some of the cells whereas others are normal (euploid). Following PGS, preference is obviously given to euploid over mosaic embryos. In some cases, however, there are no euploid embryos, and only mosaic aneuploid embryos are available for transfer. The possibility that viable embryos may be discarded due to concerns over mosaicism represents one of the greatest challenges currently facing PGS, because there are several reports of healthy children being born following the transfer of such mosaic embryos (Fragouli et al., 2017; Greco et al., 2015; Munné and Wells, 2017). Nonetheless, the transfer of mosaic embryos is associated with significantly poorer outcomes than those of the control euploid embryos, having lower implantation and ongoing pregnancy rates and higher rates of miscarriage. It thus remains to be determined whether all mosaic embryos should be considered for transfer, and if so, what types of mosaic aneuploidy are more likely than others to be associated with adverse outcomes.

While it is not yet common practice to transfer mosaic embryos, it has been suggested that this may be considered under some circumstances, as proposed by some authors (Munné et al., 2016). A recent Preimplantation Genetic Diagnosis International Society (PGDIS) Position Statement on chromosome mosaicism in PGS has suggested a guideline to prioritize mosaic embryos for transfer, based on the level of mosaicism and the specific chromosome involved (PGDIS, 2016). Likewise, following the 2016 CoGEN meeting in Barcelona, an updated position statement was issued (CoGEN Statement). Subsequently, it has also been established that there are, in fact, no differences in pregnancy outcomes between monosomic and trisomic mosaics (Munné and Wells, 2017). While these recommendations provide some framework for clinical decision making, there are scant prospective follow-up studies on the outcome of pregnancies achieved following transfer of mosaic aneuploid embryos. Until such data become available, it is possible to extrapolate from cytogenetic analyses of chorionic villus samples (CVS) performed for prenatal diagnosis.

The gold standard for cytogenetic analysis of CVS is by investigating both the cytotrophoblast by direct preparation (DP) and the placental mesenchyme by long-term culture (LTC) (Grati et al., 2006; Ledbetter et al., 1992). Using this approach, placental mosaic aneuploidy can be detected in about 2% of cases (Hsu et al., 1997; Malvestiti et al., 2015). When mosaicism is detected on CVS, it is necessary to follow up with confirmatory amniocentesis to assess whether the mosaic state involves the fetus itself or is only confined to the placenta. The likelihood of aneuploidy also being present in the fetus depends on: (i) the chromosome involved; (ii) the type of aneuploidy; (iii) the percentage of abnormal cells; and (iv) the tissue distribution (cytotrophoblast, mesenchyme, or both).

Thus, both CVS and PGS attempt to predict the chromosomal status of the embryo by analysing the cells of the trophoblast. In fact, the TE cells removed for PGS at the blastocyst stage are the precursors of the placental cytotrophoblast. One may therefore view TE biopsy for PGS as a 'very early' direct preparation CVS.

In order to devise an evidence-based scoring system for prioritizing mosaic aneuploid embryos for transfer, the likelihood that a mosaic aneuploidy detected in the trophoblast by CVS is also present in the fetus was analysed. The impact of mosaicism on the occurrence of uniparental disomy (UPD) was also reviewed. This is because a clinically significant UPD has been reported in 2.1% of fetuses with a normal karyotype on amniocentesis following the detection of a mosaic aneuploidy on CVS (Malvestiti et al., 2015). To further assess the impact of mosaic aneuploidy on pregnancy outcome, its incidence in products of conception (POC) was also studied, as these would more likely be associated with non-viability. Finally, an additional risk score was assigned to those mosaic or full aneuploidies that can lead to viable affected births with a well-characterized phenotype.

Methods

The study included cytogenetic samples analysed at a single centre (TOMA Advanced Biomedical Assays S.p.A., Busto Arsizio, Italy). The study received a notification of exempt determination from the TOMA Laboratory Institutional Review Board (approval #0000015) in December 2014. In order to evaluate the likelihood that a mosaic aneuploidy detected in the trophoblast is also present in the fetus we reviewed chorionic villus sampling performed between May 2000 and December 2016, including data previously published (Grati, 2014; Grati et al., 2006; Malvestiti et al., 2015). Cytogenetic analyses were performed in agreement with Italian and European guidelines (Linee Guida per la Diagnosi Citogenetica Consensus, 2007 and 2013, www.sigu.net; Specific Constitutional Cytogenetic Guidelines ECA, July 2012, www.e-c-a.eu), which were progressively updated during the study period. Standard protocols were used to set up the cultures and chromosome preparations (Babu and Verma, 1995) and a Q-banding technique (QFQ) was used for the entire series. Karyotype results were formulated according to the International System for Human Cytogenetic Nomenclature ((ISCN, 1995, 2005, 2009, 2013, 2016). Methods used for karyotyping of chorionic villi (CV) and amniotic fluid (AF) and UPD

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