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

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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.

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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.

Materials and 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

Table 1 – Different types of chromosomal mosaicism based on the tissue involvement.

Type	Nature	Trophoblast (direct preparation)	Mesenchyme (long-term culture)	Amniocytes
I	CPM	Abnormal	Normal	Normal
II	CPM	Normal	Abnormal	Normal
III	CPM	Abnormal	Abnormal	Normal
IV	TFM	Abnormal	Normal	Abnormal
V	TFM	Normal	Abnormal	Abnormal
VI	TFM	Abnormal	Abnormal	Abnormal

CPM = confined placental mosaicism; TFM = true fetal mosaicism.

investigation were described in detail by Malvestiti et al. (2015). Briefly, CV karyotyping was performed routinely by combining DP with LTC and at least 10 metaphases were scored and analysed for each method. Karyotyping on confirmatory AF was performed by scoring approximately 50 metaphases derived from 20–25 colonies from in-situ cultures. Mosaic trisomy in CV was defined as the presence of at least two cells showing the same abnormality and mosaic monosomy was defined in the presence of at least three cells with the same abnormality. The different types of mosaicism, depending on the tissues involved, are presented in **Table 1**.

True fetal mosaicism (TFM) was defined as the presence of at least two colonies from two AF cultures presenting the same abnormality previously observed in CV. To evaluate the risk of mosaicism resulting in clinically significant uniparental disomy (UPD), all UPD molecular analyses performed on CV samples in the same sample population (May 2000 to December 2016) were reviewed, some of which were previously reported (Grati, 2014; Grati et al., 2006; Malvestiti et al., 2015). Briefly, UPD testing was performed by segregation analysis of microsatellite markers located on the chromosome of interest using DNA from the fetus and the parents. UPD analysis was performed only in cases where the mosaic cytogenetic abnormality in CV involved a proposed or documented imprinted chromosome (6, 7, 11, 14, 15, 16), regardless of the number of abnormal metaphases (Dawson et al., 2011; Kearney et al., 2011). To further assess the impact of mosaicism on pregnancy outcome, we also reviewed cytogenetic abnormalities detected in first trimester miscarriages (POC) by karyotyping, collected from 1995 to 2015, including some cytogenetic data and methods previously published (Grati et al., 2013).

Results

Likelihood that a mosaic aneuploidy detected in the trophoblast is also present in the fetus

Of the 72,472 CVS samples analysed during the study period, chromosomal mosaicism was detected in 1524 cases (2.1%). Of these, 1166 cases were subsequently investigated by amniocentesis as well. In 1011 (86.7%) of the AF samples, there were no signs of aneuploidy. However, in 155 cases (13.3%), aneuploidy was demonstrated in the fetus as well (TFM).

Mosaicism involving both placental layers (type III CVS mosaicism) has the highest likelihood of fetal involvement (37.7%) compared with mosaicism involving only one layer: 11.9% for mosaicism detected in the mesenchyme only (type II CVS mosaicism) and only 3.9% in mosaicism found in the cytotrophoblast only (type I CVS mosaicism).

Table 2 – The likelihood that a mosaicism (aneuploid/normal) detected in the cytotrophoblast is also found in the fetus.

Abnormal cell line in mosaicism	Total (*n* = 316)	CPM I and III (*n* = 280)	TFM IV and VI (*n* = 36)	Fetal involvement (%)
Trisomy 1	0	0	0	–
Trisomy 2	8	8	0	–
Trisomy 3	26	26	0	–
Trisomy 4	1	1	0	–
Trisomy 5	3	3	0	–
Trisomy 6	0	0	0	–
Trisomy 7	47	47	0	–
Trisomy 8	16	16	0	–
Trisomy 9	6	6	0	–
Trisomy 10	3	3	0	–
Trisomy 11	4	4	0	–
Trisomy 12	1	1	0	–
Trisomy 13	30	29	1	3.3
Trisomy 14	8	7	1	12.5
Trisomy 15	20	20	0	–
Trisomy 16	4	3	1	25.0
Trisomy 17	0	0	0	–
Trisomy 18	18	14	4	22.2
Trisomy 19	1	1	0	–
Trisomy 20	18	17	1	5.6
Trisomy 21	18	10	8	44.4
Trisomy 22	1	1	0	–
45,X	66	51	15	22.7
47,XXX	7	4	3	42.9
47,XXY	8	6	2	25.0
47,XYY	2	2	0	–

CPM = confined placental mosaicism; TFM = true fetal mosaicism.

To more closely approximate PGS of trophectoderm (TE) cells, we focused specifically on mosaic aneuploidies presenting in the cytotrophoblast (CVS mosaicism types I and III). These included 280 cases of confined placental mosaicism (CPM) and 36 cases of TFM (**Table 2**). In these samples, no cases of trisomy for chromosomes 1, 6 and 17 were detected. This may be due to negative selection when present in the placenta and/or the fetus.

Mosaic aneuploidies show different likelihoods of fetal involvement and may therefore be assigned one of the following arbitrary risk scores:

- 3. High risk (>15%): trisomy 16, 18, 21 and 45,X, 47,XXY, 47,XXX
- 2. Intermediate risk (5–15%): trisomy 14 and 20
- 1. Low risk (1–4%): trisomy 13
- 0. No risk (<1%): trisomies 1–12, 15, 17, 19, 22 and 47,XYY

Incidence of clinically significant fetal UPD in cases with a mosaic aneuploidy in CV

During the same study period, UPD investigations were performed on a subgroup of 169 cases due to increased risk of clinically significant UPD of chromosomes 6, 7, 11, 14, 15 and 16, according to the current guidelines (Dawson et al., 2011; Kearney et al., 2011). Results presented in **Table 3** demonstrate that UPD was detected in nine cases (5.3%): three cases (21.4%) with trisomy 14 CPM (two cases of CPM type I and one case of CPM type III); two cases (6.5%) of trisomy

Table 3 – The likelihood of clinically significant UPD as a result of mosaicism (aneuploid/normal) detected in the cytotrophoblast.

Abnormal cell line in mosaicism	Cases investigated	Cases with UPD	Type of mosaicism	UPD incidence (%)
Trisomy 6	3	0	–	–
Trisomy 7	90	0	–	–
Trisomy 11	6	0	–	–
Trisomy 14	14	3	2 (CPM I) + 1 (CPMII)	21.4
Trisomy 15	31	2	1 (CPMII) + 1 (CPMIII)	6.5
Trisomy 16	25	4	3 (CPM III) + 1 (TFM VI)	16.0
Total	169	9	8 CPM and 1 TFM	5.3

CPM = confined placental mosaicism; TFM = true fetal mosaicism; UPD = uniparental disomy.

15 CPM (one CPM type II and one CPM type III), and in four cases (16%) of trisomy 16 CPM (three cases of CPM type III and one case of TFM type VI). The following risk scores for clinically significant UPD resulting from mosaic aneuploidy were assigned:

- 3. High risk (>10%): trisomy 14 and 16
- 2. Intermediate risk (1–10%): trisomy 15
- 1. Low risk (<1%): trisomy 6, 7, 11
- 0. No risk – all others

Incidence of mosaic aneuploidies in POC

During the study period, 3806 samples from POC were cytogenetically analysed. Of these, 1242 (32.6%) did not provide a result because of culture failure, maternal cell contamination or poor chromosome morphology. Of the remaining 2564 reportable samples, an abnormal karyotype was noted in 1269 (49.5%) cases. Of these, 75 cases demonstrated mosaic chromosomal aberrations. In 11 cases, however, there was no normal cell line and in seven others, the abnormal cell line was complex, non-aneuploid (e.g. isochromosome, deletion, translocation, additional euchromatic material, inversion). The remaining 57 cases are presented in **Table 4** and included 14 cases (25%) of mosaic tetraploidy, probably representing a culture artefact due to cell endo-reduplication, eight cases (14%) of mosaic 45,X, five cases (9%) of mosaic trisomy 16, and three cases (5%) each of mosaic trisomy 2, 8 and 20. Mosaic aneuploidies likely to be associated with miscarriage may be assigned one of four risk scores based on the incidence of each individual mosaic aneuploidy in POC:

- 3. High risk (>10%): mosaic 45,X/46,XX
- 2. Intermediate risk (4–10%): trisomies 2, 6, 8, 16, 17, 20, 21, 22
- 1. Low risk (1–3%): trisomies 4, 5, 7, 9, 11, 13, 14, 15, 18, 47,XXY, and mosaic 45,X/46,XY
- 0. Very low risk (<1%): trisomies 1, 3, 10, 12, 19

Generating an evidence-based scoring system for prioritizing mosaic aneuploid embryos

Table 5 summarizes the composite score for each type of mosaic aneuploidy based on (i) the likelihood that the mosaicism detected in the trophoblast is also present in the fetus; (ii) the incidence of clinically significant fetal UPD resulting from the mosaic aneuploidy; and (iii) the incidence of the mosaic aneuploidy in POC. Additional risk scores were assigned to viable aneuploidies: a score of 4 for trisomy 13, 18, 21 and 45,X; a score of 3 for the well-described viable mosaic trisomy 16; a score of 2 for viable mosaic aneuploidies of chromosomes 8 and 9; and a score of 1 for other sex chromosome aneuploidy (47,XXX, 47,XXY and 47,XYY).

Discussion

Mosaic aneuploidy has no doubt always existed in preimplantation embryos. In fact, it has been suggested that most human preimplantation embryos are mosaics of euploid and aneuploid cells (van Echten-Arends et al., 2011). The survival of blastocyst mosaics to term depends, among other factors, on the abnormal cell load. Using a mouse model, Bolton et al. (2016) demonstrated that in chimeric embryos, containing aneuploid and euploid cells, the aneuploid cells in the fetal lineage are eliminated by apoptosis, whereas the aneuploid cells in the placental lineage show severe proliferative defects. They have shown that the aneuploid cells are progressively depleted from the blastocyst stage onwards. They concluded that mosaic embryos may have full developmental potential if they contain a sufficient proportion of euploid cells.

Table 4 – The incidence of mosaicism (aneuploid/normal) detected in products of conception (POC).

Abnormal cell line in mosaicism	Number	Incidence (%)
Trisomy 2	3	5
Trisomy 4	1	2
Trisomy 5	1	2
Trisomy 6	2	4
Trisomy 7	1	2
Trisomy 8	3	5
Trisomy 9	1	2
Trisomy 11	1	2
Trisomy 13	1	2
Trisomy 14	1	2
Trisomy 15	1	2
Trisomy 16	5	9
Trisomy 17	2	4
Trisomy 18	1	2
Trisomy 20	3	5
Trisomy 21	3	5
Trisomy 22	2	4
45,X/46,XX	8	14
45,X/46,XY	1	2
47,XXY/46,XY	1	2
Monosomy 21	1	2
Tetraploidy	14	25
Total	57	100

Table 5 – An evidence-based scoring system for prioritizing mosaic aneuploid/normal embryos for transfer following PGS.

Abnormal cell line in mosaicism	Risk of fetal involvement	Risk of UPD	Risk of miscarriage	Risk of viable aneuploidy	Composite score
Trisomy 1					0
Trisomy 2			2		2
Trisomy 3					0
Trisomy 4			1		1
Trisomy 5			1		1
Trisomy 6		1	2		3
Trisomy 7		1	1		2
Trisomy 8			2	2	4
Trisomy 9			1	2	3
Trisomy 10					0
Trisomy 11		1	1		2
Trisomy 12					0
Trisomy 13	1		1	4	6
Trisomy 14	2	3	1		6
Trisomy 15		2	1		3
Trisomy 16	3	3	2	3	11
Trisomy 17			2		2
Trisomy 18	3		1	4	8
Trisomy 19					0
Trisomy 20	2		2		4
Trisomy 21	3		2	4	9
Trisomy 22			2		2
45,X	3		3	4	10
47,XXX	3			1	4
47,XXY	3		1	1	5
47,XYY				1	1

However, the new high-resolution techniques used for CCS, particularly NGS, can now detect its presence. It is obvious that some mosaic aneuploid embryos, if transferred, may result in the birth of healthy children (Fragouli et al., 2017; Greco et al., 2015; Munné and Wells, 2017). In a prospective study, Greco et al. (2015) described the transfer of mosaic embryos in 18 consecutive women who had no euploid embryos. These resulted in eight pregnancies, six of which resulted in a euploid live birth as confirmed by CVS, and two were biochemical pregnancies. Using our scoring system, cases resulting in a euploid live birth had a lower mean composite score compared with those that had not (2.5 versus 5.7), suggesting that they would have had higher priority. Fragouli et al. (2017) used NGS to retrospectively analyse 44 archived trophectoderm biopsies from 39 patients. Compared with the control group, mosaic embryos were associated with significantly lower implantation (30.1% versus 55.8%) and ongoing pregnancy rates (15.4% versus 46.2%) and higher miscarriage rates (55.6% versus 17.2%). Of these, 12 were mosaics for one or more segmental aneuploidies, with a pregnancy rate of 66%; 20 had mosaic aneuploidy of more than one chromosome resulting in one ongoing pregnancy (5%) and two miscarriages (10%). Of the 12 embryos with a single whole-chromosome aneuploidy, three embryos with mosaic aneuploidy of chromosomes 14, 20 and 7 resulted in ongoing pregnancies (25%) and three embryos with mosaicism for chromosomes 11, 17 and 19 resulted in miscarriage (25%). Finally, in a retrospective study, Munné et al. (2017) described the transfer of 143 mosaic embryos as determined by NGS in four different centres (44 of which were previously reported by Fragouli et al., 2017). Compared with matched controls, the implantation rate (IR) was lower (53% versus 71%), the fetal loss rate was significantly higher (24% versus 10%; $P = 0.002$) and the ongoing IR significantly lower (41% versus 63%; $P < 0.006$). Complex abnormal mosaics, involving three or more chromosomes, had a significantly reduced ongoing IR (10%) compared with mosaic single aneuploid (50%), double mosaic (45%) and mosaic segmental (41%). There was no difference in ongoing IR between mosaic monosomy and mosaic trisomy. Likewise, no significant differences were noted in ongoing IR between single-chromosome mosaic embryos and double-chromosome mosaic embryos (46% versus 45%). Embryos with 40–80% abnormal cells had an ongoing pregnancy rate of 22% compared with 56% in those with <40% abnormal cells. Nonetheless, despite these initial studies it is currently unclear what type of mosaic aneuploidy is tolerable and what degree of mosaicism is acceptable.

A recent 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). Accordingly, euploid/monosomic mosaic embryos are preferred over euploid/trisomic ones because the former (with the exception of 45,X) are not viable. Types of mosaic aneuploidy to be avoided include: (i) mosaic aneuploidies that may lead to a viable affected birth (chromosomes 13, 18, 21); (ii) those implicated in intrauterine growth restriction (chromosomes 2, 7, 16); or (iii) those that may be associated with uniparental disomy (UPD) (chromosomes 14, 15). Mosaic aneuploid embryos to be considered for transfer include those trisomic for chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 17, 19, 20, 22, X, Y.

The subsequent CoGEN Position Statement on Chromosomal Mosaicism Detected in Preimplantation Blastocyst Biopsies (CoGEN Statement, 2017) includes the following recommendations for prioritizing mosaic embryos for transfer: (i) embryos mosaic for trisomies capable of live born viability (chromosomes 13, 18, 21, 22) are of lowest priority; (ii) embryos mosaic for trisomies associated with uniparental disomy (chromosomes 14, 15) are low priority; (iii) embryos mosaic for trisomies associated with intrauterine growth retardation (chromosomes 2, 7, 16) are low priority; (iv) mosaicism involving

chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 17, 19, 20, have not been associated with the aforementioned adverse outcomes; and (v) mosaic monosomies should be considered to have similar risk as their counterpart trisomies.

Such recommendations provide some guidance for clinical decision making until sufficient prospective data accumulate. However, in the presence of several mosaic aneuploid embryos, it may be difficult to assign the correct priority, as no clear scoring system is provided.

In this study we sought to develop a scoring system for prioritizing preimplantation mosaic aneuploid embryos, based on clinical evidence derived from actual cytogenetic analyses of first trimester CVS and from POC. This approach was chosen because the trophectoderm (TE) cells used for PGS correspond embryologically to the cytotrophoblast that is analysed in a CVS direct preparation (Bianchi et al., 1993). The likelihood that a mosaic aneuploidy detected in CVS will involve the fetus, the risk for clinically significant UPD and the chance of the mosaicism culminating in miscarriage was used to generate a scoring system for prioritizing mosaic aneuploid embryos detected by PGS. **Tables 2, 3 and 4** describe the incidence of each of these adverse outcomes, based on which an arbitrary score was assigned for each chromosome. A higher score implies a higher likelihood of an adverse outcome. Additional scores were assigned to viable full or mosaic aneuploidies. **Table 5** summarizes the composite scores for all these adverse outcomes. The lower the composite score the higher the priority for embryo transfer.

Based on this scoring system, we suggest that mosaic aneuploid embryos should have the following priority for transfer:

- Mosaic trisomies 1, 3, 10, 12 and 19 have a composite score of 0 and have the highest priority for transfer because of a very low risk of any of these adverse outcomes.
- Mosaic trisomies 4 and 5 and 47,XYY have a composite score of 1 and are the second group to be considered for transfer, albeit with a disclaimer of a slightly increased likelihood of miscarriage (**Tables 4 and 5**) or a viable aneuploidy (47,XYY).
- Mosaic trisomies 2, 7, 11, 17 and 22 have a composite score of 2 and are the third group to be considered for transfer, having a slightly higher risk of miscarriage or a relatively low risk for UPD (trisomies 7 and 11).
- Mosaic trisomies 6, 9 and 15 have a composite score of 3 due to increased risk of miscarriage, UPD or viable aneuploidy. The possibility for transfer should be considered with caution and only after detailed discussion with the prospective parents.
- Mosaic trisomies 8, 20, 47,XXX and 47,XXY have a composite score of 4–5, due to high risk of fetal involvement and a slightly increased risk for miscarriage and viable aneuploidy. The possibility for transfer could be considered after extensive discussion with prospective parents regarding the possible clinical manifestations thereof.

The remaining mosaic aneuploidies are best avoided: trisomies 13, 14, 16, 18, 21 and 45,X.

The aforesaid priority scores regarding autosomal trisomies should also apply to the respective mosaic autosomal monosomies. This is because these are most likely the result of post-zygotic non-disjunction which would give rise to two daughter cells: one trisomic and one monosomic, in addition to the euploid cell line already present in the conceptus. In such circumstances, the possibility of a complementary trisomic cell line in the embryo remains a possibility (Turchetti et al., 2011).

Retrospective studies on mosaic blastocysts and prenatal diagnosis data do not always match. As suggested by Munné et al. (2017), this could be due to different mechanisms, such as cell load of abnormal cells determining the chance of producing a viable pregnancy (Bolton et al., 2016; Munné et al., 2017) and the observation that later on the trophoblast acquires aneuploid cells that become invasive and implant (Weier et al., 2005).

Additional consideration should also be given to the detected degree of mosaicism. In a recent survey held by CoGEN on the IVF-Worldwide Website, the views and practices regarding mosaicism in PGS were assessed among 102 IVF centres from 32 countries, representing a total of 108,900 annual IVF cycles (Weissman et al., 2017). Of all respondents, 31% consider embryonic mosaic aneuploidy when detected in >20% of the cells, fewer consider mosaicism when detected in >30% (12.6–14.6%) and only a minority consider mosaicism when the percentage of aneuploid cells is >50%. The results of this survey represent the relevant opinions of PGS practitioners and specialists. However, the clinical utility of the detected degree of mosaicism is generally unknown. In fact, it might only reflect a technical bias due to the random sampling of a restricted trophoblastic area rather than the real degree of mosaicism in all of the embryos. Munné et al., (2017) reported that embryos with 40–80% abnormal cells had an ongoing pregnancy rate of 22% compared with 56% in those with <40% abnormal cells. However, the correlation between the chromosome-specific degree of mosaicism and the implantation outcome is limited and prospective studies are needed to explore these aspects. Therefore, until sufficient follow-up data are available, the degree of mosaicism should be used with caution as a criterion for prioritizing embryos. If such studies demonstrate a predictive role for this criterion, the suggested scheme could then assign an additional score for the degree of mosaicism to be included in the embryo-specific composite score. Future studies will probably discover further biological predictive criteria whose introduction will be beneficial for the refining of the composite score for the prioritization of mosaic embryos, with a progressively more complex algorithm.

In case of an ongoing pregnancy after the transfer of mosaic aneuploid embryos, prenatal diagnosis on amniocytes should be highly recommended due to the a priori increased risk of fetal aneuploidy. CVS is not recommended due to the higher likelihood of encountering the same mosaic aneuploidy detected at blastocyst stage by PGS. In case of a normal karyotype after a standard analysis, an extended analysis and/or cell count on amniocytes should be performed to exclude the presence of low-level mosaicism in the fetus. This is the same laboratory procedure that is recommended for the analysis of amniocytes after the detection of a mosaic condition in the CV (Grati et al., 2015; Munné and Wells, 2017). Likewise, non-invasive prenatal testing by cell-free DNA is not recommended as the cell-free DNA fragments are not derived from the fetus itself but from the apoptosis of the cytotrophoblastic cells.

In conclusion, although the transfer of mosaic embryos may be associated with poorer outcome, there is also a compelling concern that viable embryos may be unjustifiably discarded due to concerns over mosaicism. Thus, until further prospective follow-up studies on the actual transfer of mosaic aneuploid embryos are available, we suggest using this scoring system as a tool for clinicians and embryologists, both for prioritizing embryos for transfer and as a counselling aid for discussing possible outcomes following transfer of mosaic aneuploid embryos detected by PGS.

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