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Let the data do the talking: the need to consider mosaicism during embryo selection

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Abstract

Chromosomal mosaicism, or the co-existence of cells with different chromosomal content, is a phenomenon that has been documented in human embryos for three decades. Early versions of preimplantation genetic testing (PGT-A) did not measure mosaicism, either because typically only a single cell was assessed, or because the technique could not accurately identify it. While this led to a straightforward diagnosis (an embryo was considered either normal or abnormal), it simply avoided the matter and in hindsight may have led to numerous misdiagnoses with negative clinical consequences. Modern PGT-A evaluates a multicellular biopsy with technologies capable of recognizing intermediate copy number signals for chromosomes or sub-chromosomal regions. We are therefore inevitably confronted with the issue of mosaicism and the challenge of managing embryos producing such results in the clinic. Here we discuss recent data showing that not only

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mosaicism in general, but specific features of mosaicism detected with PGT-A are associated with variable clinical outcomes. The conclusion is evident: mosaicism should be considered for more informed and improved embryo selection in the clinic.

Capsule:

Features of mosaicism revealed during preimplantation genetic testing for aneuploidy are associated with variable probabilities of achieving ongoing pregnancy and should be considered when prioritizing embryos for transfer.

Keywords

IVF; Preimplantation Genetic Testing; Next-Generation Sequencing; Embryo; Mosaicism

In the early 1990s, scientists using chromosome-labeling fluorescence in situ hybridization (FISH) probes on whole human preimplantation embryos made the interesting observation that, occasionally, single embryos contained a mixture of cells with different chromosomal counts (1). Mosaicism had been previously documented in several other contexts of animal and human physiology (2), but for the first time, visual evidence was captured demonstrating that human embryos could contain a combination of chromosomally normal and abnormal cells.

Over the past three decades, many reports have corroborated those observations. These included numerous FISH studies on whole embryos (3–10); comparisons of serial biopsies from individual embryos, regardless of the DNA-quantitation technology used (FISH, qPCR, SNP array, aCGH, or NGS) (11, 12); and analyses of single-cell RNA-seq data revealing chromosome-wide alterations in gene expression (13, 14). The post-zygotic chromosome-segregation errors of mitosis that give rise to mosaicism have also been observed live, just as they occurred, in murine and bovine embryos using fluorescent reporters (15, 16). The existence of mosaicism as a biological phenomenon in embryos is therefore backed by overwhelming evidence.

It should therefore come as no surprise that current PGT-A practices, which evaluate the collective chromosomal content of a multicellular biopsy of the trophectoderm (TE), should occasionally capture instances of mosaicism. State-of-the-art platforms for PGT-A offer high resolution and broad dynamic range, facilitating the detection of mosaicism as manifested by intermediate copy numbers (ICN) of chromosomes or sub-chromosomal regions. Such results are consistent with mosaicism, and the sampled blastocyst is said to be ‘mosaic’, with full understanding that the biopsy is only a sample of the embryo (a 5-10 cell ‘window’ on the chromosomal status of a ~200 cell blastocyst). The question is: How should such ‘mosaic’ embryos be managed in the clinic?

TRANSFERRING EMBRYOS OF THE MOSAIC CATEGORY

The first account of embryo transfers with prior knowledge of their mosaic diagnosis with modern PGT-A appeared in 2015 (17). Greco and colleagues discussed the transfer of

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mosaic embryos for 18 patients that had no euploid embryos available, resulting in the birth of six apparently healthy babies.

Since then, several centers and clinics have published their experiences with the transfer of mosaic embryos (18–25). All studies agreed that mosaic embryos could result in healthy pregnancies, but had lower clinical success rates compared to euploid embryos (Table 1). Specifically, mosaic embryos exhibited lower rates of implantation, ongoing pregnancy, and birth, along with increased rates of spontaneous abortions, as summarized by a meta-analysis of those reports (26).

The studies, however, disagreed on whether specific features of mosaicism detected with PGT-A were associated with variable clinical outcomes. That is a key point, as such associations could potentially inform guidelines for embryo prioritization in the clinic. Two features of mosaicism can be gleaned from PGT-A results. One is the mosaic ‘level’, or the inferred percentage of aneuploid cells in the sample. In case of disomy, PGT-A produces a result indicating chromosomal copy number of 2; for trisomy, copy number of 3, etc. If, for example, the results indicate a chromosomal copy number 2.4, it suggests that 40% of the cells are trisomic and 60% are disomic, and the sample is said to contain a ‘mosaic trisomy at the 40% level’. Conversely, if the results indicate a chromosomal copy number of 1.3, it suggests that 70% of cells are monosomic and 30% are disomic, and the sample is said to contain a ‘mosaic monosomy at the 70% level’. Often the results are not reported in such quantitative detail, and mosaic levels are grouped into ‘high’ and ‘low’ categories using a cutoff, such as 50%. From a biological standpoint, such mosaic levels are influenced by the timing and mechanism of chromosome mis-segregation. Specifically, mitotic errors occurring during early postzygotic divisions will propagate to a larger percentage of daughter cells, though we note that subsequent processes of natural selection could alter this ratio (27).

The other feature of mosaicism that can be distinguished from PGT-A results is the ‘type’. This refers to the form of aneuploidy involved: segmental, one chromosome (monosomy or trisomy), two chromosomes, or complex aneuploidies affecting three or more chromosomes. While errors such as mitotic non-disjunction and anaphase lag may typically affect one or two chromosomes, more catastrophic mechanisms such as multipolar mitosis disproportionately contribute to complex and ‘chaotic’ forms of mosaicism (28, 29).

While some studies have demonstrated an association between levels of mosaicism and outcome of mosaic embryo transfers, others reached conflicting conclusions (Table 1). There was also no consensus on whether any type of mosaicism was favorable over another (Table 1). Such inconsistency could be attributed to the use of different PGT-A platforms, varying definitions of mosaic results, or because of small samples sizes across the individual studies.

THE 1000 MOSAIC EMBRYO STUDY

In an effort to address outstanding questions surrounding mosaicism, a group of clinics and PGT-A centers embarked on a joint study with the following goals/principles: 1) Achieve a large sample size to increase the power of analysis, 2) Use a standardized PGT-A platform

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based on NGS, the state-of-the-art for assessing mosaicism (30–32), 3) 3) Use a uniform definition of mosaic results based on previously proposed threshold levels (20%-80%) (20, 33), 4) Validate the detection of mosaicism within each laboratory (see details below), and 5) Control for factors that could confound the retrospective comparison of the mosaic and euploid groups. The results of the study, which analyzed the transfers of one thousand mosaic embryos, were recently published (34).

Compared to clinical outcomes of a control group composed of over 5000 euploid embryos, the mosaic group had significantly lower rates of implantation, decreased rates of ongoing pregnancy (at the time of analysis) or birth (OP/B), as well as increased rates of spontaneous abortions (Table 2). All differences were more pronounced when only considering embryos in which the mosaicism affected whole chromosomes, i.e. excluding segmental mosaics (Table 2). These aspects of the study thus agreed with previous reports, highlighting the poorer outcomes of embryos classified as mosaic. In most of the existing studies, there was knowledge of embryos' PGT-A results prior to transfer, influencing decisions about whether to transfer mosaic embryos versus other embryos that may have been available, and thereby introducing a potential selection bias (e.g., if mosaic embryos are disproportionately transferred as a last resort). To mitigate any such bias, a sub-analysis in the thousand mosaic embryo study focused solely on mosaic embryos used at first transfer, which still exhibited significantly lower success rates compared to the euploid group (Table 2). For an additional 164 of the thousand mosaic embryos, there was no knowledge of the mosaic result at the time of transfer. In what can be considered a non-selection study/analysis, the clinical outcomes of that group were also significantly poorer than those of euploid controls (Table 2).

The combined mosaic group data was then further stratified according to mosaic features, considering the various permutations of mosaic level and type. The breakdown revealed that mosaic sub-groups exhibited different clinical outcomes. When sorted by clinical success rates, there emerged a ranking of mosaic sub-groups from having most to least favorable outcomes (Table 2). 'Segmental mosaics' were associated with the best success rates, but they were still significantly worse than the euploid control group. Low level mosaics (<50% aneuploid) were associated with better outcomes than high level mosaics (≥50% aneuploid), and within those groups, the type of mosaicism sorted thus from most to least favorable: one chromosome > two chromosomes > complex (Table 2). The sub-group with the poorest outcomes was the high level complex mosaics, but even those occasionally resulted in pregnancies and births. Together, those findings can serve as a template for an embryo prioritization scheme in the clinic.

Because embryo selection also involves the consideration of morphology, the outcome data was further refined through integration of PGT-A sub-category and embryo stage and grade. Again, further sub-trends became apparent within the various groups. The resulting matrix of values can serve prospectively in the clinic to rank embryos (34). A freely-accessible online tool allows the user to input the characteristics of two or more embryos and determine their relative potential for clinical success, based on the experience gathered from the thousand mosaic embryo study (https://embryo-score.web.app/).

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PREGNANCIES AND BIRTHS RESULTING FROM MOSAIC EMBRYO TRANSFERS

The hesitation to transfer embryos in which mosaicism is detected is obvious and justified. After all, chromosomal mosaicism is one underlying cause of human disorders and confined placental mosaicism (CPM), which can lead to placental dysfunction (2).

Of the 247 babies born in the thousand mosaic embryo study, none had notable birth defects (34). A more complete set of data was collected for 162 newborns, each with a matching baby born from a euploid embryo transfer. The average birth weight and length of gestation was equal between the two groups, and no overt symptoms associated with chromosomal abnormalities were reported in the babies from mosaic embryos (35). After mosaic embryo transfers, in over 100 amniocentesis results and over 200 prenatal testing results across all platforms (amniocentesis, CVS, and NIPT), there were five instances of abnormalities (34, 35). All five were amniocentesis cases, which identified segmental imbalances smaller than the resolution of contemporary PGT-A NGS platforms and were unrelated to the mosaicism detected with PGT-A. Therefore, in this sample group, the mosaicism observed at the blastocyst stage never persisted through gestation.

How could blastocyst-stage mosaicism ‘disappear’? Data from mosaic embryo transfers represents indirect clinical evidence for ‘self-correction’ via a mechanism of ‘clonal depletion’ (36), whereby aneuploid cells of mosaic embryos are outcompeted by euploid cells through differential proliferation and/or directed apoptosis. Indeed, there is a well-documented, universal link between aneuploidy and attenuated cell proliferation in humans and other organisms (37) (with the notable exception of cancer, where aneuploidy is common, but increased proliferation is primarily a consequence of mutations in oncogenes and tumor suppressor genes). Thus, mosaic embryos could develop into healthy babies if aneuploid cells are sufficiently diluted out during pregnancy, such that by the time of delivery (or much earlier, judging by prenatal testing results) there is no observable trace. In further support of this point, a study profiling the genomic landscape of fetal and placental tissues postpartum from both IVF and naturally conceived children showed that mosaicism was not preserved at later stages of prenatal development and that *de novo* numerical aberrations or large structural DNA imbalances occur at similar rates in IVF and naturally conceived neonates (38).

There is mounting experimental evidence for self-correction of embryonic mosaicism. Single-cell RNA-seq in mosaic embryos indicates that aneuploid cells downregulate proliferation genes, and the average incidence of aneuploid cells steadily decreases between the cleavage stage and the late blastocyst stage of development (13, 14). Extended *in vitro* culture assays show that mosaic blastocysts tend to become fully euploid in what is equivalent to the early stages post-implantation (39). Human gastruloids (models of gastrulation-stage embryos derived from embryonic stem cells), in which mosaicism was chemically induced, are prone to losing the aneuploid compartment over time due to directed apoptosis (14). Mouse chimeric blastocysts composed of euploid and aneuploid cells usually become fully euploid when the initial aneuploid-to-euploid ratio is equal or low, but tend to perish if the initial proportion of aneuploid cells is high (40). This mouse model of

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mosaicism shows attenuated proliferation and preferential apoptosis of aneuploid cells, which is compensated by increased proliferation of euploid cells (27). In point of fact, experiments using immunofluorescence show significantly different patterns of mitosis and cell death between human embryos classified as euploid and mosaic (22).

It is therefore logical that a disconnect should exist between mosaicism at the blastocyst stage and the (typically normal) karyotype later in pregnancy. There has only been one report to date of an amniocentesis reflecting the mosaicism detected with PGT-A (41). The pregnancy resulted in a live birth, and the phenotypically healthy baby showed evidence of mosaicism in a blood sample (but not in a buccal swab). The measured level of mosaicism declined over time from 35% with PGT-A to 2% with amniocentesis and 2% in one tissue at birth. This case argues for continued heightened surveillance of pregnancies from mosaic embryo transfers by careful monitoring of fetal growth and prenatal testing. Time and additional data will tell whether that single case is an outlier, or if persistence of mosaicism throughout gestation is more common than the bulk of the data currently indicates.

REDIFINING AND EXPANDING EMBRYO CATEGORIES IN PGT-A

In light of the data, the original binary classification system of embryos into ‘normal’ and ‘abnormal’ groups seems obsolete. If this system is retained, embryos that should be classified as ‘mosaic’ would be included in either the ‘normal’ or the ‘abnormal group’, over- or under-valuing their developmental potential, respectively. Failure to differentiate between ‘euploids’ and ‘mosaics’ may impact clinical success rates, considering the poorer outcomes of the latter. Conversely, grouping the mosaic category with the aneuploid category would mean discarding viable embryos, and if no euploids are available, denying patients a potential pregnancy. The mosaic category should be further stratified into sub-groups according to mosaicism level and type, such that, when given the choice, the embryo with the best chances of clinical success can be prioritized.

It is also important to note the distinction between self-correction in a mosaic setting (described above), and cell-intrinsic forms of self-correction (so called aneuploidy-rescue). In the latter, aneuploidies would be corrected within cells, and would allow a fully aneuploid embryo (arising from a meiotic error) to amend itself, at least in part. This could lead to the idea of transferring embryos with a PGT-A result indicating a uniform aneuploidy, with the hope that they would self-correct. However, the evidence for this mechanism in human IVF embryos remains scarce. Intracellular correction by endoreplication (for a monosomy) or trisomy rescue (42) would often result in uniparental disomy (UPD), an extremely rare occurrence in IVF-generated blastocysts (43). If by that stage the aneuploidy has not been corrected, the embryo is unlikely to result in a healthy pregnancy. In fact, transfers of embryos classified as uniformly aneuploid (non-mosaic) with PGT-A have virtually no chance of resulting in a healthy pregnancy, let alone a normal baby (44, 45). Therefore, under no circumstances should embryos with a mosaic result be conflated with those of an aneuploid result into one single ‘abnormal’ group.

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DIFFERENT PGT-A TECHNOLOGIES - SAME RESULTS?

It is crucial to assess the accuracy of each individual PGT-A platform for calling mosaicism. Specifically, platform validation should be performed in such a way to ensure that particular features of mosaicism (level and type) can be accurately assessed.

This can be accomplished with experiments in which cells or extracted DNA of euploid and aneuploid control samples are mixed in known proportions, subjected to the complete PGT-A protocol, and results compared with expectations. The reaction should contain 5-10 cells or amounts of DNA equivalent to that cell range, thereby mimicking a clinical TE biopsy. Using cell lines with different aneuploidies (both whole chromosome and segmental abnormalities) and preparing various mixture ratios (1:9, 2:8. 3:7, etc.) allows for thorough assessment of detection accuracy.

Several groups have successfully validated NGS-based PGT-A platforms for accurate ICN identification with such mixing experiments (18, 20–22, 31, 46–48). One study compared two widely adopted commercial platforms, confirming high resolution between mosaic intervals (20% for Veriseq, Illumina/Vitrolife and 30% for Reproseq, ThermoFisher) (49). This demonstrated degree of accuracy contradicts the notion that the intermediate outcomes for ‘mosaic’ embryos observed in clinical studies may be attributed to the fact that they are actually a combination of misdiagnosed uniform euploid and aneuploid embryos (a concept that also neglects the vast data documenting the common existence of mosaicism in embryos). It is equally important for each PGT-A platform and center to define appropriate quality control cutoffs with the noise-quantifying metrics of the analysis software to preclude ‘messy’ karyotype profiles (consequence of technical noise) from being classified as ‘mosaic’ simply because values fall in the ICN range. In such cases, the source blastocysts should be managed as undiagnosed embryos or be considered for re-biopsy.

It follows that given appropriate validation, a ‘high complex’ mosaic sample, for example, should produce the same results across laboratories, and so on for all mosaic sub-categories. Any PGT-A technology that correctly identifies mosaicism should ultimately produce similar clinical outcome associations to those observed in the thousand mosaic embryo study, and the ranking system should therefore be transferrable across clinics.

THE BIOPSY - AN IMPERFECT, ALBEIT USEFUL, PROXY OF THE EMBRYO

Regarding the predictive value of the embryo biopsy, PGT-A is very different from its cousin, diagnostic preimplantation genetic testing for monogenic disorders (PGT-M). In PGT-M, the biopsy serves as an ideal genetic representation of the remaining blastocyst, as well as the future fetus. In the case of chromosomal analysis with PGT-A, the phenomenon of mosaicism complicates the role of the biopsy as ‘representative’.

Aneuploidies derived from meiotic errors are present in the oocyte (or occasionally, the sperm), affect the resulting zygote, and thus uniformly impact all cells of the blastocyst (except in the unlikely event of aneuploidy rescue). Indeed, fully ‘euploid’ or ‘aneuploid’ PGT-A results from a TE biopsy tend to be perfectly concordant with the remaining blastocyst (12, 50, 51), though noting that meiotic and mitotic errors are not mutually

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exclusive and may co-occur. In contrast, a result indicating mosaicism in the TE biopsy is a poor predictor of the embryo's global chromosomal make-up (12), due to randomness in the sampling of cells. Furthermore, considering mosaic self-correction discussed above, a mosaic PGT-A result at the blastocyst stage will rarely (if ever) be predictive of the karyotype of the placenta or fetus later in the pregnancy. Hence, it might seem highly counterintuitive that mosaicism detected in the biopsy should be predictive in any capacity.

Nevertheless, the results of the thousand mosaic embryo transfer study are unequivocal; mosaic profiles, and what is more, levels and types of mosaicism detected with PGT-A strongly associate with specific outcomes (34). Therefore, while fully acknowledging the limitations of a biopsy, the data suggest it would be unwise to ignore the information regarding mosaicism provided by the biopsy to guide decisions of embryo prioritization according to their probabilities of clinical success.

How common is embryonic mosaicism? Can we determine the incidence of mosaicism from data gathered from biopsies? Not without contrived mathematical extrapolations. The biopsy can only directly inform us about the percentage of embryos classified as mosaic (as opposed to euploid or aneuploid). If, for example, 15% of embryos tested by PGT-A are classified in the mosaic category, it does not say much about the true incidence of mosaicism in all blastocysts from IVF—and yet the two values are often taken to be one and the same. To know the actual incidence, we would need to dissociate individual blastocysts and assess ploidy at the single-cell level in all their cells. Such an experiment has simply not yet been performed systematically in a large blastocyst sample size with modern DNA copy number quantitation methods, due to obvious limitations related to technology, cost, and sample availability. The data generated from aforementioned whole embryo FISH studies and analyses of single-cell RNA-seq information suggest that low level mosaicism (even as low as a single aneuploid cell being present amidst euploid cells) is quite frequent in early embryos, and might even be present in the majority of human embryos. However, such latent (ultra-) low level mosaicism is not likely detected by PGT-A due to sample randomness, and is likely inconsequential to embryo viability. This differs from cases with evidence of 'high' level mosaicism in the TE biopsy, which implies an early mitotic error event with clonal expansion.

Should we then use the terminology 'mosaic' embryos at all? Again, it must be understood that the categories in which we place embryos with PGT-A are solely based on the biopsy, not as a global assessment of the whole embryo. It follows then, that when we call an embryo 'mosaic', we are really saying that the embryo produced a PGT-A result that is consistent with mosaicism. In the same vein, a 'euploid' embryo is one with a biopsy producing results consistent with euploidy, and an 'aneuploid' embryo one with aneuploidy. It follows that those terms are used as short-hand to classify and manage embryos in the clinic.

CONLUSIONS

Advances in modern PGT-A have ignited the debate around mosaicism in embryos: should it be diagnosed, and how should such embryos be managed in the clinic? Many opinions,

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preferences, and beliefs have been expressed regarding this topic. The current data suggest that 1) Embryos with a mosaic diagnosis have poorer clinical outcomes than those with a euploid result, 2) Features of mosaicism detected with PGT-A are associated with specific clinical outcomes, 3) Babies born from embryo transfers following a mosaic diagnosis by PGT-A are largely indistinguishable from babies born following a euploid diagnosis.

More data is urgently needed to solidify, refine, or refute these current findings, and to dig deeper into the next set of questions, such as: Is mosaicism in different chromosomes associated with variable clinical success rates? Does the size or genomic content of mosaic segmental imbalances matter? Do rates or characteristics of mosaicism vary across cell types and tissues? Do products of conception from miscarried mosaic embryo transfers display chromosomal mosaicism? What other follow-up work should be done on neonates?

An international registry of mosaic embryo transfers that records outcome and, if available, prenatal testing and neonate information, would be a tremendously useful resource for the field. As new data emerges, the current statements will hold - or they will be modified accordingly. Such is the case with all of science, and embryo mosaicism should be no different. Here is a call to ‘let the data do the talking’.

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Table 1.

Studies analyzing the clinical outcomes of mosaic embryos.

Study	Year	'Mosaic' Embryos Transferred (n)	Transfers (n)	Knowledge of 'Mosaicism' at Transfer	PGT-A Method	PGT-A System	ICN Thresholds For Detection of Mosaicism	More Favorable Clinical Outcomes in 'Euploid' vs 'Mosaic' Embryos	More Favorable Clinical Outcomes in 'Low vs 'High' Levels of Mosaicism	Different Clinical Outcomes Between Mosaic Types (Segmental vs Whole Chromosome vs Complex)
Greco et al.	2015	18	18	Yes	aCGH	24sure Illumina	<3 x SD and/or ±0.3 loq2 ratio	Not assessed	Not assessed	Not assessed
Lledó et al.	2017	52	52	No	aCGH	Picoplex + Agilent	log2 ratio 1.7-0.3	Yes	Not assessed	Not assessed
Fragouli et al.	2017	44	39	No	NGS	Veriseq Illumina/ Vitrolife	20-80%	Yes	No	Yes Segmental Preferable over Whole Chromosome
Munné et al.	2017	143 (99+44)	138 (99+39)	Some	NGS	Veriseq Illumina/ Vitrolife	20-80%	Yes	Yes (Trend)	Yes Complex Mosaicism Less Favorable, All other Types Similar
Spinella et al.	2018	78	77	Yes	aCGH/ NGS	24sure / Veriseq Illumina/ Vitrolife	20-80%	Yes	Yes	Not assessed
Zhang et al.	2019	102	97	No	aCGH	24sure Illumina	SD of log2 ratio >0.1 deviation	Yes	Not assessed	Yes Segmental Preferable over Whole Chromosome
Victor et al.	2019	100	83	Yes	NGS	Veriseq Illumina/ Vitrolife	20-80%	Yes	No	Yes Segmental Preferable over Whole Chromosome, All other Types Similar
Munné et al.	2020	253	253	Some	NGS	Veriseq Illumina/ Vitrolife	20-80%	Yes	Yes	Yes Complex Mosaicism Less Favorable
Lin et al.	2020	108	108	Yes	NGS	Veriseq Illumina/ Vitrolife	20-80%	Not assessed	Yes	Not assessed
Lee et al.	2020	83	83	Yes	NGS	Veriseq Illumina/ Vitrolife	20-80%	Yes	Yes	No
Viotti et al.	2021	1000	957	Some Yes 84% No 16%	NGS	Veriseq Illumina/ Vitrolife	20-80%	Yes	Yes	Yes Segmental> One Whole Chromosome> Two Whole Chromosomes> Complex

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Table 2.
Summary of results of the '1000 mosaic embryo transfer' study.

Columns indicate the measured clinical outcomes, and rows indicate the (sub-) category of result obtained with PGT-A. Low <50%; High ≥50%; Complex = more than 2 aberrant chromosomes.

	Implantation per Embryo Transfer	Ongoing Pregnancy/ Birth per Embryo Transfer	Spontaneous Abortion per Implanted Embryo	*P* value
Euploid (n=5561)	57.2%	52.3%	8.6%	
Mosaic All (n=1000)	46.5%	37.0%	20.4%	<0.0001
Mosaic Whole Chromosome (n=517)	41.8%	31.3%	25.0%	<0.0001
Mosaic No Selection (n=467)	44.1%	35.3%	20.4%	<0.0001
Mosaic No Knowledge (n=164)	55.5%	37.2%	33.0%	<0.0001
Mosaic Low Segmental (n=385)	50.9%	43.9%	13.8%	<0.0001
Mosaic High Segmental (n=94)	52.1%	41.5%	20.3%	
Mosaic Low One Chromosome (n=190)	48.9%	39.5%	19.2%	
Mosaic Low Two Chromosome (n=93)	45.2%	39.8%	11.9%	
Mosaic Low Complex (n=88)	34.1%	25.0%	26.7%	
Mosaic High One Chromosome (n=67)	32.8%	22.4%	31.7%	
Mosaic High Two Chromosome (n=30)	33.3%	20.0%	40.0%	
Mosaic High Complex (n=38)	23.7%	13.2%	44.0%	

Compared to the 'Euploid' group, the following mosaic groups had significantly lower likelihood of achieving ongoing pregnancy/birth per embryo transfer: 'Mosaic All', 'Mosaic Whole Chromosome', 'Mosaic No Selection', and 'Mosaic No Knowledge' (Chisquare, $P<0.0001$).

The indicated order of mosaic sub-groups is statistically significant (Cochran-Armitage test for trend, $P<0.0001$).

For each clinical outcome, the colored shading indicates the relative success rates of each (sub-) category, from best (white) to worst (red).