

# Origins and rates of aneuploidy in human blastomeres

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**Objective:** To characterize chromosomal error types and parental origin of aneuploidy in cleavage-stage embryos using an informatics-based technique that enables the elucidation of aneuploidy-causing mechanisms.

**Design:** Analysis of blastomeres biopsied from cleavage-stage embryos for preimplantation genetic screening during IVF.

**Setting:** Laboratory.

**Patient(s):** Couples undergoing IVF treatment.

**Intervention(s):** Two hundred seventy-four blastomeres were subjected to array-based genotyping and informatics-based techniques to characterize chromosomal error types and parental origin of aneuploidy across all 24 chromosomes.

**Main Outcome Measure(s):** Chromosomal error types (monosomy vs. trisomy; mitotic vs. meiotic) and parental origin (maternal vs. paternal).

**Result(s):** The rate of maternal meiotic trisomy rose significantly with age, whereas other types of trisomy showed no correlation with age. Trisomies were mostly maternal in origin, whereas paternal and maternal monosomies were roughly equal in frequency. No examples of paternal meiotic trisomy were observed. Segmental error rates were found to be independent of maternal age.

**Conclusion(s):** All types of aneuploidy that rose with increasing maternal age can be attributed to disjunction errors during meiosis of the oocyte. Chromosome gains were predominantly maternal in origin and occurred during meiosis, whereas chromosome losses were not biased in terms of parental origin of the chromosome. The ability to determine the parental origin for each chromosome, as well as being able to detect whether multiple homologs from a single parent were present, allowed greater insights into the origin of aneuploidy. (Fertil Steril® 2012;97:395–401. ©2012 by American Society for Reproductive Medicine.)

**Key Words:** Aneuploidy, preimplantation genetic diagnosis, microarray, molecular karyotyping, segmental errors

In IVF, approximately 35% of fresh nondonor embryo transfers result in a live birth (1). Early-stage human embryos have been shown to frequently suffer from chromosomal abnormalities (2–9), and more than half of human embryos generated during IVF contain aneuploid cells (2–4). Chromosomal abnormalities lead to universally negative outcomes, such as failure to implant, spontaneous abortion, and the birth of a child with a trisomic condition (10–18). Understanding the mechanistic causes of aneuploidy along with their parental origin and the profile of various types of

aneuploidy within one blastomere holds the potential to significantly increase the success rates of IVF.

Most of our knowledge of chromosomal ploidy in the human cleavage-stage embryo is derived from karyotyping and fluorescent in situ hybridization (FISH) (19–23). Both of these methods can determine whole chromosome imbalances by providing a count of the number of each chromosome tested. However, FISH typically identifies ploidy states of only five to eight chromosomes and consequently misses a large portion of the aneuploidies (24, 25); moreover, FISH is associated with

high error rates, though these can be mitigated if reanalysis with additional probes is included (19, 26).

Newer preimplantation genetic screening (PGS) techniques, such as comparative genomic hybridization (CGH) arrays or single nucleotide polymorphism (SNP) arrays, allow investigation of ploidy across all 24 chromosomes (2, 4, 5, 24, 27–29). However, both of these techniques are subject to the variable levels of allele dropout and variable amplification across the chromosomes during single cell lysis and DNA amplification (30), which can lead to less-accurate results. Another major drawback of both methods is their inability to differentiate between ploidy types that contain a similar chromosomal count (e.g., euploidy and uniparental disomy) or to determine the parental origin of chromosomes.

These technological shortcomings can potentially be mitigated with the use of a sophisticated informatics tool that we previously described (6). The

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technique uses the relatively noisy SNP microarray intensity measurements from a single biopsied blastomere, combined with parental SNP data and crossover frequencies, to determine which haplotypes from which parent were inherited by each embryo. This allows the inference of the correct embryonic genotype and therefore significantly increases the statistical power with which the ploidy state of the blastomere can be determined. The technology is able to determine not only the chromosomal count in a cell but also parental origin of each chromosome, as well as major insertions, deletions, and translocations. Moreover, the method is able to detect when both haplotypes from one parent are present over a given segment of a chromosome, which is indicative of a meiotic error. In this study, we have used this technology to characterize the error types and origin of chromosomal abnormalities in 274 single blastomeres from 32 couples.

The detailed ploidy data presented in this article will allow greater insight into the origins of aneuploidy. Moreover, the data may have a direct clinical impact on the field of IVF. Recent studies have shown a high rate of mosaicism in human embryos (2, 5, 24, 31, 32), and other studies have shown that there is a significant degree of self-correction among mosaic embryos (33, 34). A detailed understanding of the different types of aneuploidy in a set of embryos in which a biopsied blastomere has tested aneuploid, combined with empirical data concerning the profile of ploidy states in mosaic embryos and their tendency to self-correct, may allow the selection of embryos with the greater likelihoods of developing into a healthy child (35). However, it is important to note that mosaicism prevalence and profiles are currently understood poorly, therefore the data presented here is not meant to be representative of the ploidy state of whole embryos; rather, it is meant to give a broad picture of the state of individual blastomeres in cleavage-stage embryos.

## MATERIALS AND METHODS

### Single Cell Isolation and DNA Amplification

Two hundred seventy-four blastomeres were obtained from 32 commercial IVF patients; 71 embryos were from women who had had more than two prior spontaneous abortions, and 59 embryos were from healthy egg donors. One blastomere was biopsied from each cleavage-stage day-3 embryos at IVF centers and shipped on dry ice the same day to our laboratory. Upon thawing, single cells were washed sequentially in three drops of wash buffer (5.6 mg/mL KCl, 6 mg/mL bovine serum albumin). Two lysis/amplification protocols were used in the analysis: [1] Rubicon whole genome amplification with Sigma proteinase K buffer (PKB), and [2] a modified multiple displacement amplification with PKB. For protocol 2, cells were placed in PKB (Arcturus PicoPure Lysis Buffer, 50 mM dithiothreitol), incubated at 56°C for 1 hour, and then heat inactivated at 95°C for 10 minutes. Multiple displacement amplification reactions were incubated at 30°C for 2.5 hours and then 95°C for 5 minutes. Genomic DNA from bulk tissue (Epicentre MasterAmp Buccal Swabs) was prepared using the DNeasy Blood and Tissue Kit (Qiagen). No-template controls (buffer blanks) were used for all amplification methods and all sample types and in all

cases produced intensities commensurate to the noise floor of the data.

### SNP Genotyping

Both amplified single cells and bulk parental tissue were genotyped using Illumina Infinium II genome-wide genotyping microarrays (HapMap CNV370Quad or CytoSNP-12 chips). For the bulk tissue, the standard Infinium II protocol ([www.illumina.com](http://www.illumina.com)) was used, whereas all single cells were genotyped using a modified Infinium II genotyping protocol, such that the entire protocol, from single cell lysis through array scanning, was completed in fewer than 24 hours, as previously described (6).

### Ploidy and Segmental Error Determination

Ploidy results are based on a novel statistical algorithm that makes use of high-throughput SNP measurements of parental and child samples to determine chromosome copy number of the child (6, 36, 37); details are given in the Supplemental Material (available online). The method is relevant for analyzing very small quantities of DNA for which direct measurements are inherently error prone; it does not rely solely on intensity measures and performs well even in the face of significant allele dropout rates (38). The Parental Support method has been validated on cells with known karyotype (6).

Throughout the document, *P* values are calculated using a two-tailed Welch's *t* test. The 95% confidence intervals are computed using exact binomial distributions where normal distributions are an inaccurate approximation.

## RESULTS

The method used for copy number determination has been previously described (6). The informatics method was applied to a dataset consisting of 274 blastomeres biopsied during PGS to provide statistics regarding the rates of various types of ploidy errors. The average maternal age for these biopsied embryos was 38.0 years (range 25.9–47.1 years). The data are segregated into lower and upper maternal age groups:  $\leq 36$  years (average 32.8 years) and  $>36$  years (average 40.9 years). With 102 blastomeres in the lower age group and 172 in the upper age group, there is sufficient power to observe the effects of maternal age on ploidy. Analysis of single blastomeres from a large number of embryos enables statistical assertions to be made on relatively rare aneuploidy events such as uniparental disomy (UPD) and segmental errors, including deletions, duplications, and unbalanced translocations, which are difficult to observe in smaller data sets. The euploid chromosome calls exclude any chromosomes with segmental errors or UPD; for all aneuploidy calls, the ploidy call is determined by the ploidy state present over the majority of the length of the chromosome.

A key feature of the informatics technology is its use of parental data, which allows us to identify the parental origin of every chromosome in the biopsied blastomere. Because the method takes into account the crossovers that occur during meiosis, it is able to determine which parental haplotypes

were involved in the formation of the embryo. Trisomies and uniparental disomies that contain at least one segment with both homologs from a single parent are referred to as both parental homolog (BPH) aneuploidies. Both parental homolog aneuploidies must have arisen either from a meiosis I error, or a meiosis II error on a recombined chromosome. In contrast, if only a single parental homolog (SPH) is detected across the full length of the chromosome, that is, the two chromosomes originating from the one parent are identical, then it either arose from a mitotic disjunction error, or from a meiosis II error on a chromosome that failed to recombine. (see Supplemental Material for an explanatory graphic). It is important to note that in cases in which the region of recombination is too small to be detected, BPH trisomies may appear as SPH trisomies. For the algorithm to detect a BPH aneuploidy, the minimum size of the region of recombination is between 5 and 15 Mb, depending on the number of SNPs on the segment.

### Aneuploidy Analyzed by Blastomere

Table 1 describes the chromosomal abnormalities for 274 blastomeres biopsied from cleavage-stage embryos during 32 IVF cycles with PGS. The results show that 27.7% (range 22.3%–33.1%) of these blastomeres were euploid, with women aged  $\leq 36$  years achieving marginally significantly higher euploidy rates compared with women aged  $>36$  years (34.3% vs. 23.8%) ( $P=.067$ ). Segmental errors (deletions and/or duplications), defined as at least 15% of the length of the chromosome being affected (therefore the cutoff is shorter on longer chromosomes), were found in a significant number (15.3%) of blastomeres. There was no significant difference in the rate of segmental errors observed between the different maternal age groups. Interestingly, the majority of segmental errors were present in blastomeres with other aneuploidies, and only seven blastomeres (2.6%) harbored only segmental errors.

Of all blastomeres, 31.4% were aneuploid owing to maternal errors only, and this fraction significantly increased

with maternal age, from 21.6% for women aged  $\leq 36$  years to 37.2% for women aged  $>36$  years ( $P=.0045$ ). In contrast, 6.2% of blastomeres had only paternal errors; 63.5% of blastomeres contained at least one maternal error, whereas 38.3% of blastomeres had at least one paternal error.

Both parental homolog trisomies, indicative of a meiotic error, were detected in 20.1% of blastomeres, whereas SPH trisomies (due to either meiotic errors on nonrecombinant chromosomes or mitotic errors) were detected in 25.3% of blastomeres. All observed BPH trisomies resulted from maternal errors, and their rate increased dramatically with maternal age (10.8% vs. 25.6%,  $P=.001$ ). In contrast, the rate of SPH trisomies did not change appreciably between the two maternal age groups (25.9% vs. 25.0%). To the extent that SPH trisomies are due to mitotic errors, these findings are consistent with previous findings that the rate of meiotic but not mitotic errors increases with maternal age (39).

The number of blastomeres diagnosed with a monosomy was numerically higher than the number with trisomy (44.5% vs. 39.8%;  $P=.26$ ), indicating that chromosome loss is a significant mechanism in aneuploidy formation. The majority of aneuploid blastomeres involve more than a single chromosome: 19.7% of tested blastomeres had a single chromosomal error, compared with 52.5% that involved errors on multiple chromosomes.

### Aneuploidy Analyzed by Chromosome

Table 2 describes the rates of different types of aneuploidy by chromosome. We specifically focused on the types of aneuploidy that cannot be determined using FISH or CGH, namely the different types of trisomies and uniparental disomies broken down by parental origin of chromosomes and by the number of parental haplotypes observed (i.e., BPH or SPH events). A more extensive breakdown of the data on a per-chromosome basis is shown in the Supplemental Material.

The rate of SPH trisomy per chromosome is 2.9% (range 2.5%–3.3%), and the rate of BPH trisomy is 4.3% (range 3.8%–4.8%). All BPH trisomies are maternal in origin, and

**TABLE 1**

#### Aneuploidy rates by blastomeres.

Parameter	Total count	Rate (%)	Maternal age < 36 y	Rate (%)	$\sigma$	Maternal age $\geq 36$ y	Rate (%)	$\sigma$
Total no. blastomeres	274		102			172		
Euploid	76	27.7	35	34.3	4.7	41	23.8	3.2
Any trisomy	109	39.8	33	32.3	4.6	79	45.3	3.8
Any monosomy	122	44.5	42	41.2	4.9	80	46.5	3.8
Any UPD	10	3.6	3	2.9	1.7	7	4.1	1.5
All nullsomy	12	4.4	6	5.9	2.3	6	3.5	1.4
Segmental error(s)	42	15.3	15	14.7	3.5	27	15.7	2.8
Only segmental error(s)	7	2.6	4	3.9	1.9	3	1.7	1.0
Maternal aneuploidy error(s) only	86	31.4	22	21.6	4.1	64	37.2	3.7
Paternal aneuploidy error(s) only	17	6.2	6	5.9	2.3	11	6.4	1.9
Both parent aneuploidy errors	88	32.1	35	34.3	4.7	53	30.8	3.5
BPH trisomy	55	20.1	11	10.8	3.1	44	25.6	3.3
SPH trisomy	69	25.3	26	25.9	4.3	43	25.0	3.3
1 chromosome with error	54	19.7	23	22.5	4.1	31	18.0	2.9
>1 chromosome with error	144	52.5	44	43.1	4.9	100	58.1	3.7

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TABLE 2

Aneuploidy rates by chromosome.										
Parameter	n	All			Maternal age < 36			Maternal age ≥ 36		
		Rate (%)	$\sigma$	n	Rate (%)	$\sigma$	n	Rate (%)	$\sigma$	
Total no. chromosomes	6,302			2,346			3,956			
Euploidy	4,811	76.34	0.54	1,808	77.07	0.87	3,003	75.91	0.68	
Trisomy	451	7.16	0.32	138	5.88	0.49	313	7.91	0.43	
Maternal BPH trisomy	270	4.28	0.26	63	2.69	0.33	207	5.23	0.35	
Paternal BPH trisomy	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	
Maternal SPH trisomy	135	2.14	0.18	57	2.43	0.32	78	1.97	0.22	
Paternal SPH trisomy	46	0.73	0.11	18	0.77	0.18	28	0.71	0.13	
Monosomy	540	8.57	0.35	201	8.57	0.58	339	8.57	0.44	
Maternal monosomy	275	4.36	0.26	79	3.37	0.37	196	4.95	0.34	
Paternal monosomy	265	4.21	0.25	122	5.20	0.46	143	3.61	0.30	
Uniparental disomy	10	0.16	0.05	3	0.13	0.07	7	0.18	0.07	
BPH UPD (heterodisomy)	9	0.14	0.05	2	0.09	0.06	7	0.18	0.07	
SPH UPD (isodisomy)	1	0.02	0.02	1	0.04	0.04	0	0.00	0.00	
UPD maternal present	10	0.16	0.05	3	0.13	0.07	7	0.18	0.07	
UPD paternal present	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	

*Rabinowitz. Origins of aneuploidy in humans. Fertil Steril 2012.*

the rate of maternal BPH trisomy increases with maternal age, as expected (2.7% vs. 5.2%,  $P < 10^{-5}$ ). Rates of SPH trisomies of both maternal and paternal origin do not increase with age.

The most striking result of this analysis is the absence of any BPH trisomies of paternal origin in the sample of 6,302 tested chromosomes. This finding is consistent with previous findings that the vast majority of meiotic errors are maternal in origin and that aneuploidy rates found in sperm are extremely low (40).

The rate of SPH trisomies of maternal origin is approximately three times higher than the rate of SPH trisomies of paternal origin, 2.14% vs. 0.73% ( $P < 10^{-5}$ ). Because SPH trisomies include both mitotic trisomies and also meiosis II errors with nonrecombinant chromosomes, there are two possible explanations for this finding.

The first possible explanation is that the maternal SPH trisomies are largely mitotic in origin and that nondisjunction during mitosis is three times more likely for maternal chromosomes.

The second possible explanation rests on the assumption that nondisjunction in mitosis occurs without respect to the parental origin of the chromosome. The absence of BPH paternal trisomies, noted above, implies that nearly all paternal trisomies are due to mitotic nondisjunction errors, and thus rates of paternal trisomy can be used as a proxy for rates of mitotic trisomy in general. If this is true, then approximately one-third of the maternal SPH trisomies are due to mitotic errors, and the remaining two-thirds are due to meiosis II nondisjunction combined with a failure to recombine in meiosis I.

Nonrecombination is infrequent or even absent in live births of euploid children, except for some of the smaller chromosomes (41). However, the studies of trisomies in trisomic children (especially trisomy 21) and products of conception showed that recombination is severely altered in the context of nondisjunction (42–45). In a substantial proportion of cases the trisomic homologs are nonrecombinant (44). This affirms the notion that recombination is important for

proper chromosomal segregation in humans. Our results are consistent with the notion (though do not show) that nonrecombination is common among aneuploid 3-day embryos.

Both explanations would entail surprising conclusions. The first would imply that maternal and paternal chromosomes behave differently in mitosis, with chromosomes of the maternal origin showing more lability. We have no evidence to support this possibility, but this is not beyond the realm of possibilities given the existence of epigenetic marks that distinguish chromosomes by parental origin. The fact that the rate of neither the maternal nor the paternal SPH trisomies increases with age supports this first explanation, given that we might expect that the incidence of the meiotic maternal SPH errors do increase with age. The second would suggest that a substantial proportion of meioses result in some chromosomes undergoing no recombination (at least 1.41% per chromosome). This rate is substantial: if we assume that nonrecombination occurs in meiosis for each chromosome independently, this rate would imply that approximately 28% of oocytes have at least one nonrecombinant chromosome. It would also suggest that the rate of meiotic SPH errors does not increase with age in contrast to the meiosis I, BPH errors.

Of maternal chromosome gains (most of which lead to triploidy but some of which lead to uniparental disomy), 32.5% (135 of 414) are SPH errors, and 67.5% (279 of 414) are meiotic BPH errors. However, this pattern varies substantially depending on the overall number of chromosomal losses and gains in the blastomere. In 95 blastomeres with a small number of chromosomal gains (one to nine), the majority of the maternal trisomies are SPH trisomies (56.2%; 86 of 153), with 43.8% (67 of 153) being BPH trisomies. In contrast, in highly aneuploid blastomeres with 10 or more chromosomal gains, maternal chromosomal gains are predominantly BPH (81.2%; 212 of 261). Note that of the 15 blastomeres in this category, only one is fully triploid,

indicating that these findings are not due to accidental polar body inclusion in the blastomere biopsy.

Figure 1 shows the overall rate of overall trisomy per chromosome, as well as the different types of trisomy that can be distinguished with this technology. Figure 2 shows the overall rate of monosomy, further broken down into maternal monosomy and paternal monosomy (defined as the loss of the maternal or paternal chromosome, respectively). The data indicate that the aneuploidy in cleavage-stage embryos occurs with roughly equal rates on the different chromosomes.

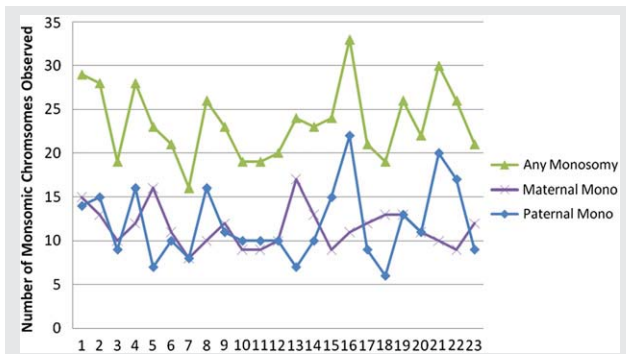
The overall monosomy rate does not change with maternal age. In addition, the rates of maternal and paternal monosomy are not noticeably different from each other (275 vs. 265). Given that the rate of paternal meiotic error is assumed to be low and given that we have found no evidence in our data of any paternal meiotic trisomies, these results are consistent with most monosomies being the result of chromosomal loss in mitosis.

This interpretation is also consistent with the observation that in our data UPDs are rare (0.16% by chromosome) and that they are all exclusively of maternal origin and found in the blastomeres with a large number of affected chromosomes: all blastomeres with a UPD chromosome had at least eight other aneuploid chromosomes. Uniparental disomy is likely to be mostly due to maternal meiotic chromosomal gain followed by mitotic chromosomal loss. Note that mitotic chromosomal loss on a chromosome affected by meiotic maternal gain would restore euploidy one-third of the time. Such cases should be rare and are expected to be found predominantly in blastomeres with a large number of maternal trisomies. Indeed, of the nine blastomeres with one or more UPDs, eight have at least one trisomy, with an average of 8.4 trisomies. Of the 76 trisomies in these blastomeres, all of them are maternal in origin, and 95% (72 of 76) are BPH trisomies.

## DISCUSSION

Presented here are results from an innovative technology that was developed with the goal to provide single cell chromosome copy number analysis with high accuracy as well as detail regarding the type of aneuploidy (6). The key feature of

FIGURE 2



Types of monosomies by chromosome number for 274 blastomeres. Rabinowitz. *Origins of aneuploidy in humans. Fertil Steril* 2012.

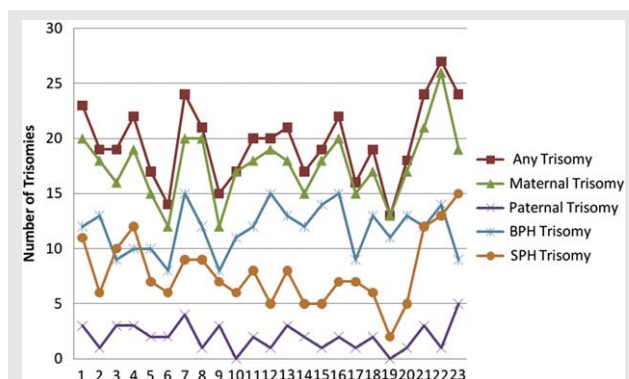
this technology is its statistical approach that utilizes parental genotype information to infer the correct genotypic information of the embryo across all 24 chromosomes. Because the technology allows the precise determination of the parental origin of each segment of each chromosome and determination of parental haplotypes—an ability not shared by either FISH or CGH—it has the ability to elucidate the aneuploidy-causing mechanisms.

The overall rate of trisomy rose with advancing maternal age, as expected. By differentiating the types of trisomy, it was possible to show that this increase was due exclusively to BPH maternal trisomy. In contrast, little dependence on maternal age was observed for monosomy, paternal trisomy, and most surprisingly, for maternal SPH trisomies. This suggests that maternal age does not significantly affect the occurrence of aneuploidy by mitotic errors during embryo development. Segmental errors are also roughly independent of maternal or paternal origin and of maternal age. In fact, all categories of aneuploidy analyzed here that show an increase in prevalence with increasing maternal age can be attributed to maternal disjunction errors during meiosis. Because segmental errors usually involve fewer than half of the chromosome, they will often not be detected by methods that use only a single probe per chromosome, such as FISH. This is particularly troublesome because, presumably, segmental errors are less likely than a full aneuploidy to result in a spontaneous abortion but can still result in the birth of a severely disabled child.

Overall, chromosome gains were predominantly maternal in origin and mostly occurred during meiosis; no chromosomal gains were observed that were definitively both paternal in origin and occurred during meiosis, whereas chromosome losses were not biased in terms of parental identity of the chromosome lost. The discrepancy between maternal and paternal SPH trisomies leads to two possible explanations, both surprising: either more than 1% of chromosomes fail to undergo recombination during meiosis, or else mitotic nondisjunction occurs disproportionately on maternal chromosomes.

When we focus on trisomies found on blastomeres with fewer than nine aneuploid chromosomes, we see that they

FIGURE 1



Types of trisomies by chromosome number for 274 blastomeres. Rabinowitz. *Origins of aneuploidy in humans. Fertil Steril* 2012.

are predominantly SPH trisomies, whereas trisomies found in blastomeres with more than 10 aneuploid chromosomes are overwhelmingly BPH trisomies. It seems that most of the highly aneuploid embryos are due to nondisjunction of multiple chromosomes during maternal meiosis. In contrast, mitotic errors seem to affect individual chromosomes separately, with global failures being less common.

Altogether, our results indicate that an informatics-based approach to determining the ploidy state of an embryo that leverages parental information provides a significant enhancement to PGS. It not only improves the accuracy of ploidy calls, but it also allows the determination of the number, type, and parental origin of each chromosome in the biopsied blastomere. These additional data provide information regarding whether the detected trisomy is meiotic vs. mitotic, whether a disomy is euploidy vs. uniparental disomy, and whether an aneuploidy is maternal vs. paternal in origin, thereby providing significant insights into the mechanistic causes of aneuploidy.

Mosaicism is a significant complicating factor: some studies have indicated that a majority of day-3 embryos are mosaic, calling into question the predictivity of blastomere analysis. Whereas our testing has corroborated some of these findings, significant differences in the measured prevalence of segmental errors in blastomeres by the two methods (2) further highlights the need for additional validation studies on both methods.

Nevertheless, our method may be able to partly overcome the problem of mosaicism. The knowledge of the type and parental origin of an aneuploidy may allow one to predict which embryos are more likely to be mosaic and thus have the potential to self-correct. For example, mechanisms of gamete formation imply that embryos with meiotic errors are more likely to be completely aneuploid, whereas embryos with mitotic errors are more likely to be mosaic. Thus we hypothesize that embryos associated with blastomeres with mitotic errors are more likely to self-correct than embryos associated with blastomeres with meiotic errors. More study into mosaicism profiles of day-3 embryos and their tendency for self-correction is needed to put on a firm footing clinical applications of these predictions.

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