

# Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates

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**Objective:** To evaluate whether differences in results between studies that involve preimplantation genetic diagnosis for chromosome testing are affected by technology, such as the number of cells to be biopsied or by differences in study design.

**Design:** Evaluation of studies of aneuploidy testing according to the use of probes, fixation technology, error determination, and number of cells per embryo analyzed.

**Setting:** Preimplantation genetic diagnosis laboratories.

**Patient(s):** Patients in published studies who underwent preimplantation genetic diagnosis for infertility or repeated pregnancy loss.

**Intervention(s):** As determined by each evaluated study, the number of biopsied cells and its effect on further development was evaluated by a comparison of models of embryo freezing and partial cell loss.

**Main Outcome Measure(s):** Use of probes, fixation strategy, number of biopsied cells, and error rate determination of different published studies.

**Result(s):** Differences in results between studies can be explained by the technology used and are not affected necessarily by differences in design and patient allocation.

**Conclusion(s):** Studies that contradict the finding that aneuploidy screening improves implantation and lowers miscarriage rates all have  $\geq 1$  of the following aspects in common: (I) an excess of cells having been removed; (II) inadequate choice of probes; and (III) suboptimal fixation technology. (Fertil Steril® 2007;87:496–503. © 2007 by American Society for Reproductive Medicine.)

**Key Words:** Aneuploidy, mosaicism, single cell biopsy, efficacy of PGD

Until recently, there has been broad consensus about 2 closely related phenomena in human fertility: fecundity decreases with advancing maternal age, and reduced fecundity is associated markedly with chromosomal anomalies in the miscarried fetus. Although these notions remain fundamental, new evidence from a wide array of studies that involved chromosomal analysis of oocytes and early human embryos that were derived from IVF suggest that aneuploidy in oocytes occurs at a much higher frequency than previously assumed and that the proportion of affected embryos is much greater than expected (1–11).

Currently, it is accepted that at least 50% of human embryos are affected by aneuploidy and other chromosome abnormalities. However, the mounting evidence that has been collected by comparative genomic hybridization and fluorescent in situ hybridization (FISH) suggest that the true

incidence is higher still. These data indicate that as many as 70% of embryos that were derived from the most common age group of women with IVF who are treated in North America (35–37 years old) have chromosomal abnormalities (2, 4, 12). The proportion of affected embryos increases to 82% for women  $\geq 40$  years old (Table 1). Although this may suggest that chromosomal anomalies are related mostly to the infertile population, a recent study of young egg donors revealed that  $>50\%$  of embryos from these fertile patients were also affected by aneuploidy. This is particularly surprising given that only 9 chromosomes were tested (11) and suggests that the high frequency of such anomalies is not just confined to the infertile population. The question that is central to this issue therefore becomes whether the high incidences of anomalies are present in natural fertilization or whether they are related to follicular stimulation.

Follicular stimulation is 1 of the factors that may increase aneuploidy artificially. Its true impact and the mechanism by which the spindle or other factors that are involved in maintaining accurate chromosome segregation are affected largely remain unknown. Follicular stimulation may lead to the recruitment of smaller follicles that were unlikely to be recruited in the natural cycle and were predisposed to aneuploidy already. Some evidence from work in rabbits and mice show that gonadotropin dose can affect a matured

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**TABLE 1****Estimated frequency of chromosome abnormalities in assisted reproductive technology-produced embryos.**

Abnormality	Age (y)		
	20–34 (%)	35–39 (%)	40–47 (%)
Aneuploidy (9 chromosomes) <sup>a</sup>	24	27	39
Other aneuploidy (by comparative genomic hybridization) <sup>b</sup>	5	6	8
Postmeiotic abnormalities <sup>a,c</sup>	35	36	35
Total abnormalities	64	69	82

<sup>a</sup> Munne et al., 1995 (2); Marquez et al., 2000 (4).

<sup>b</sup> Gutierrez-Mateo et al., 2004 (12).

<sup>c</sup> Mosaic, polyploid, and haploid embryos.

*Cohen. Embryo biopsy and aneuploidy testing. Fertil Steril 2007.*

oocyte in vivo and result in chromosomal aberrations (13, 14). When follicles are cultured in vitro, the effect of FSH on the metaphase and prometaphase spindle appears to be dose-dependent (15). It is likely that ovulation induction in humans is a contributing factor, and it may increase embryonic aneuploidy, but the evidence is contradictory so far (16, 17).

Although the complex origins of aneuploidy remain ambiguous, the fact remains that aneuploidy frequency is extraordinarily high in human IVF embryos. In light of the evidence, it is not surprising that reproductive specialists increasingly are considering aneuploidy testing through preimplantation genetic diagnosis (PGD) for infertility or PGD for aneuploidy screening. A number of studies are now providing evidence that such procedures improve success rates by significantly lowering miscarriages and improving implantation in certain groups of patients (18–21).

Despite large studies that indicate the advantages of aneuploidy screening, the notion that PGD for infertility is beneficial is not shared by all reproductive geneticists and endocrinologists. One study that was performed by the Free University of Brussels argues that aneuploidy screening does not improve implantation rates. A second study from the same team shows that removing aneuploid embryos from their cohorts does not affect miscarriage rates (22, 23). Although these studies do help confirm previous investigations that were based on analyses of small groups of chromosomes (18), it is our argument that they were inadequately designed or harmed the embryos by extracting not 1 cell but 2. The discussion here focuses on the detrimental effects of removing 2 cells for aneuploidy testing. The argument that

PGD for infertility should be based on only randomized studies as has been suggested in recent reviews (24, 25) may be attractive, but not at the cost of ignoring fundamental embryologic concerns that are associated with supplementary cell loss in early embryos.

**BIOPSY OF THE CLEAVING EMBRYO AND SUBSEQUENT DEVELOPMENT**

More than 1,000 scientific publications about PGD have been published since the original report by Handyside et al. (26) in which pregnancies were obtained after Y-chromosome-specific amplification of single biopsied cells from 3-day old embryos. Most of the work that has followed emphasizes new technological breakthroughs in the detection of mutated genes and chromosomal anomalies. Only a few studies have underscored the importance of the biopsy procedure and general conditions during and after micromanipulation (27–29).

Much of the current understanding is based on an experiment that was conducted by Hardy et al. (27), which showed that cells that were removed initially by biopsy demonstrated no adverse effects on preimplantation development to the blastocyst stage other than a proportional loss of biomass. The embryos on which this experiment was performed were good in quality and cell number and were not necessarily representative of the diversity that was represented by embryos that were seen from infertile and fertile patients. Their fundamental experiment shows that the preimplantation phase of development is affected by blastomere biopsy, even though the authors' immediate interpretation suggested otherwise.

Later studies revealed that the biopsy of one-fourth of an embryo may reduce the ratio of inner cell mass to trophectoderm cells (28, 29). This would likely diminish viability beyond just a proportional decrease in biomass. However, the interpretation of these experiments is not consistent, and biomass reduction is discussed often in the context of a superfluous cell number and totipotency. Controlled experiments that compared the effects of biopsy on all stages of development, not just the fastest embryos, have not been conducted with ongoing pregnancy as an end point. The assumption that biopsy has little effect on the embryo simply remains unproven.

We postulate that embryo biopsy affects the embryo's development before transfer and may have lasting effects after replacement. This is based on the assumption that cell loss from biopsy can be compared with cell loss after thaw of frozen cleaved embryos. After cryopreservation, individual cells frequently are lost, although remaining cells have the potential to develop into a viable blastocyst depending on the quality of the embryo and the proportion of cells damaged at thaw. The implantation potential of thawed embryos that are fully intact and have no degenerate cells may resemble that of embryos that are transferred without cryopreservation (30, 31). With this analogy, implantation potential

TABLE 2

Outcome of cleaved embryo thaw procedures<sup>a</sup> according to the proportion of cells survived.

Cells survived after thaw (%)	Procedures with unique transfers (n)	Implantation rate (%)		Pregnancy rate: fetal heart beat (%)
		Sac	Fetal heart beat	
≤50	9	7.7	7.7	11.11
51–75	32	15.3	13.7	18.75
76–99	32	27.6	27.6	37.50
100	103	33.6	30.0	49.51

<sup>a</sup> Only thaws and transfers with uniform embryo cohorts are included.

Cohen. *Embryo biopsy and aneuploidy testing*. *Fertil Steril* 2007.

becomes a function of cell loss, the quality of the original embryo, and the number of cells that are present at biopsy.

An additional factor that should be considered is 1 that forms the basis of a common inaccuracy in human embryology. The days and steps of cleavage in equal increments of cell number that are often presented as fact (4 cells on day 2 and 8 cells on day 3) are actually an abstract simplification. Analyses of our EggCyte database have shown that the average cell number on day 3 is not 8, but 6.7 cells (unpublished data). This number is based on individual analyses of >75,000 embryos. The proportional effect of cell loss on implantation potential is therefore higher than might otherwise be expected.

Based on the work by Edgar et al. (30) and continuing with the cryopreservation analogy, the removal of a single cell from an 8-cell embryo is expected to reduce implantation potential by 12.5%. If the expected implantation potential in a hypothetical 8-cell embryo (for the purpose of this discussion) is set at 20%, a single cell biopsy is predicted to lower its implantation probability to 17.5%. Consequently, for preimplantation diagnosis to succeed in increasing implantation rates, it must more than compensate for this initial setback.

The challenge for PGD of infertility becomes considerably more difficult to overcome when 2 cells are removed. Following the model of Edgar et al. (30, 31), a loss of 2 cells from the same hypothetical embryo described earlier would reduce its implantation potential by 25%, giving it a 15% chance of implanting versus its initial probability of 20%. In this case, improved embryo selection that is achieved with PGD must bridge a larger deficit if it is to provide any advantage in terms of embryo implantation. Because the average cell number at the time of biopsy is only 6.7, rather than 8, the decline in implantation potential is expected to be even more pronounced. Given a theoretic implantation potential of 20% without biopsy, the implantation potential after a single- or 2-cell cell biopsy would diminish to 17.0% and 14.0%, respectively. It is clear from this analysis that a 2-cell biopsy significantly impedes embryo development and

is inadvisable in cases in which PGD is being used for the purposes of increasing IVF success rates.

In our work with cleaved embryos that were frozen on day 3, the model of Edgar et al. (30, 31) is confirmed, that is that the success rate of fully intact embryos after thaw is high (Table 2), whereas partially intact embryos may implant, but at an increasingly lower rate. Thirty percent of fully intact embryos formed a fetal heart beat after implantation. We analyzed only procedures in which thawed and replaced embryos had comparable cell loss. The analyses were carried out after internal review board approval by Saint Barnabas Medical center Institutional Review Board protocol 06–48. Embryo cohorts with mixed cell loss were excluded from this evaluation. Cell loss was expressed as a survived cell percentage.

Embryos between the 5- and 10-cell stage in which 1 cell was degenerated survived at a rate of >75%, and the rate of fetal heart beat was 27.6%. The second group of cell loss, those embryos whose blastomeres survived at a rate between 51% and 75%, included 5- to 10-cell thawed embryos with 2 lost blastomeres. However, some of those also represented 7- to 10-cell embryos in which 3 blastomeres were lost. The implantation rate (13.7%) of this second group of embryos did not diminish proportionally compared with those who only lost 1 blastomere, but loss was considerably more severe. This either indicates that, in our work, cell loss after freezing is not a proportional phenomenon like that seen in the model of Edgar et al. (30, 31), but that the loss of 2 blastomeres is relatively more severe than the loss of 1. On the other hand, this may mean that this group has been biased by fast embryos that lost 3 blastomeres and also had survival rates of >50%.

If a PGD study fails to detect any difference in implantation rate when comparing routine IVF cycles with cycles that use 2-cell biopsy and PGD, this does not mean that chromosome screening has had no effect. On the contrary, what such a study actually shows is that PGD has succeeded in compensating for the significant reduction in implantation that was caused by 2-cell biopsy. This is a vital distinction that

has not been acknowledged by the authors of the Brussels study (22) and has led to serious misinterpretations in subsequent reviews by others (24, 25).

## THE PREIMPLANTATION GENETIC DIAGNOSIS TRIAL

### Design and Implications

When planning PGD for infertility, the number of cells to be biopsied must be considered carefully. The decision to remove 1 or 2 cells should be based not only on reducing error rates and increasing the efficiency of the assay but also on the premise that embryo viability is diminished at least proportionally with each cell that is removed. It should be remembered that most patients who request PGD do so to improve their probability of a successful IVF cycle; their primary concern usually is not the avoidance of aneuploid syndromes such as Downs (although this is perceived as an added benefit of the procedure). Unlike patients who seek PGD for a single gene disorder, many PGD for infertility patients are not opposed to the termination of an abnormal pregnancy and do not consider preimplantation diagnosis as a replacement for prenatal diagnosis.

During the design of PGD studies, it is also of vital importance to choose the correct chromosome probe set for analysis, the most effective method of fixation, the best mode of visualization, the day of embryo transfer, and appropriate culture media.

We consider PGD for infertility to be a new system that the embryologist can use to be more specific about which embryo to choose for transfer. It is not the first and will not be the last method for the assessment of embryos. It is a component of the embryo selection system and not a substitute for prenatal diagnosis (the word *genetic* can be redundant or confusing in this context). There is no single, universally practiced protocol for PGD for infertility screening. It is an art much like IVF.

### Comparative Investigations

There have been 3 basic types of comparative trial investigations regarding PGD:

1. trials that used prospective datasets that compared patients who allowed PGD with patients who declined it (18–20),
2. trials that used prospective data in a randomized fashion (22) and
3. trials that used retrospective analyses (all other studies).

Here, we only consider the first 2 types of investigations.

The existing literature regarding PGD for infertility draws 2 starkly different conclusions: 1 group of investigators supports the hypothesis that PGD improves implantation and reduces miscarriage rates (18–20); the second group is not able to demonstrate any significant differences between control and PGD patients (22). What are the essential differences between these sets of observations, and are they able to

explain the diametrically opposed conclusions? The 4 main differences are the form of randomization, the number and type of chromosome probes applied, the type of cell fixation used, and the number of cells biopsied (Table 3).

Those studies that did not demonstrate a significant improvement in implantation rates or a reduction in miscarriage rates were relatively modest in size and were considered to be randomized. No study was randomized blindly prospectively because the embryologists were always aware of which patients had embryos that had been biopsied. These studies always used a restrictive probe set that was limited to only 6 chromosome pairs (X, Y, 13, 16, 18, 21, 22) and a type of fixation that produces the most errors of the 3 types that were used (33). The 2-cell biopsy technique was used for determining the best developing embryos (22, 23), but the studies failed to demonstrate a significant improvement in implantation or a reduction of the proportion of patients who miscarried.

Interestingly, in both studies, positive albeit nonsignificant trends were noted by the authors because of the small sample sizes. Indeed, there was a 5.6% increase in implantation rate and a 6.1% increase in ongoing implantation, when comparing PGD and control patients (22). Although this indicates an improvement of nearly 50%, the studies failed to reach statistical significance.

Other studies that demonstrated FISH was acceptably efficient at detecting aneuploidy and improving implantation rates or decreasing miscarriages were not randomized blindly prospectively. However, at least 2 studies used a prospective blinded allocation protocol that searched for a given control patient to a comparable PGD patient before the first pregnancy test (18, 20). The control patients were matched to each PGD patient according to age, number of oocytes retrieved, follicular response, attempt number, and batch of culture media. Although these and other studies of PGD for infertility may not have been ideal, we wonder why authors who review the literature regarding questions of PGD for infertility efficacy (25) neglect to include any reference to data from the largest published series in their review (18–20).

It has been known for a number of years that implantation rates do not show a marked improvement if the number of chromosomes that are assessed is too few (18), so it is not surprising that studies that have used a restricted set of chromosomes have failed to see improved implantation rates (22). However, published data clearly shows that, if the chromosomes screened are chosen carefully, the incidence of miscarriage can be diminished and the take-home baby rates can be increased, even when as few as 5 chromosomes are assessed and single cells are biopsied (18, 21).

Two published studies have used a single-cell biopsy technique and 9-chromosome probes. These studies demonstrated the efficiency of FISH for the detection of aneuploidy and resulted in improved implantation rates (19, 20). Their sample sizes were not appropriate to determine pregnancy rate improvement.

**TABLE 3**

**Summary of studies comparing PGD and control assisted reproductive technology outcome.**

Variable	Study					
	Munne et al., 1999 (18)	Gianaroli et al., 1999 (19)	Munne et al., 2003 (20)	Werlin et al., 2003 (32)	Staessen et al., 2005 (22)	Munné et al., 2006 (21)
Cells biopsied (n)	1	1	1	1	2	1
Chromosomes analyzed (n)	4–8 <sup>a</sup>	8	8	8	6	8
Type of study	Comp	Comp	Comp	Rand	Rand	Comp
Cycles (n)						
Control	117	127	138	28	141	8706 <sup>b</sup>
PGD	117	135	138	29	148	562 <sup>b</sup>
Average embryos replaced (n)						
Control	N/A	3.0 <sup>c</sup>	3.7 <sup>c</sup>	N/A	2.8 <sup>c</sup>	N/A
PGD	N/A	1.8 <sup>c</sup>	2.0 <sup>c</sup>	N/A	2.0 <sup>c</sup>	N/A
Implantation rate (%)						
Control	13.7	12.4 <sup>c</sup>	10.6 <sup>d</sup>	N/A	11.5	N/A
PGD	17.6	24.2 <sup>c</sup>	17.6 <sup>d</sup>	N/A	17.1	N/A
Pregnancy loss rate (%)						
Control	33.8 <sup>d</sup>	20.6	N/A	N/A	25.6	21.5 <sup>c</sup>
PGD	15.0 <sup>d</sup>	5.4	N/A	N/A	25.0	16.7 <sup>c</sup>
Ongoing implantation rate (%) <sup>e</sup>						
Control	10.6 <sup>d</sup>	10.2 <sup>c</sup>	N/A	N/A	10.4 <sup>f</sup>	N/A
PGD	15.9 <sup>d</sup>	22.5 <sup>c</sup>	N/A	N/A	16.5 <sup>f</sup>	N/A
Pregnancy rate (%)						
Control	29.9	25.1	N/A	20.7	27.7	N/A
PGD	35.9	29.1	N/A	43.0	19.6	N/A

Note: Comp = Prospective nonrandomized comparative study; Rand = prospective randomized study.

<sup>a</sup> Thirty-six cycles with 4 probes, 50 cycles with 5 probes, and 31 cycles with 8 probes.

<sup>b</sup> Pregnant cycles.

<sup>c</sup>  $P < .001$ .

<sup>d</sup>  $P < .05$ .

<sup>e</sup> Fetus ongoing  $\geq 12$  weeks/embryos replaced.

<sup>f</sup>  $P = .06$ .

Cohen. Embryo biopsy and aneuploidy testing. Fertil Steril 2007.

What is the quantitative difference if one uses 6 instead of 9 chromosome probes for FISH? This can be determined from data presented in 3 recent studies that used comparative genomic hybridization to analyze donated eggs and their polar bodies simultaneously (12, 34, 35). It appears that the standard 5-probe FISH panel X, Y, 13, 18, and 21 would detect only 37% of abnormalities (Table 4). Adding probes for 16 and 22, a 6 chromosome (7 probe) panel would detect 48% of all abnormalities, the

9-probe panel (X, Y, 13, 15, 16, 18, 21, and 22) would detect 57%, and adding 3 more probes (8, 14, and 20) for a total of 12 would detect 67% of chromosome abnormalities. The difference in detection between 6 and 9 probes is therefore at least 10%.

Another important difference between these studies and those studies that have failed to see an improvement in embryo implantation rates is the choice of blastomere fixa-

**TABLE 4**

**Egg and polar body complexes analyzed by comparative genomic hybridization and the predictive value of the probe number.**

Study	Egg/polar body analyzed (n)		Egg/polar body that would be abnormal by FISH (n)			
	Total	Abnormal	5 Chromosomes: XY,13,15,18	7 Chromosomes: 5 chromosomes + 16, 22	9 Chromosomes: 6 chromosomes + 15, 17	12 Chromosomes: 8 chromosomes + 14, 20, 8
Gutierrez-Mateo et al. (12)	25	11	4	4	6	6
Gutierrez-Mateo et al. (34)	21	21	3	9	11	12
Fragouli et al. (35)	100	22	13	13	14	18
<b>Total</b>	<b>146</b>	<b>54</b>	<b>20 (37%)</b>	<b>26 (48%)</b>	<b>31 (57%)</b>	<b>36 (67%)</b>

*Cohen. Embryo biopsy and aneuploidy testing. Fertil Steril 2007.*

tion technique. Successful PGD studies have used fixation methods that produce higher nuclear diameters than those without an improvement in outcome (22). Larger diameters of fixed nuclei reduce the likelihood that signals will fully or partially overlap, thus decreasing the incidence of false monosomy and FISH inaccuracy (33). Three methods of fixation were compared in the study by Velilla et al. (33). The first method was the modified acetic acid method (36); the second method was the Tween 20 HCl (37), and the third method was a combined technique (38). The error rates in these 3 methods were 13.5%, 57.7%, and 39.1% ( $P < .001$ ) respectively. Technologic quandaries with the potential to increase PGD inaccuracy, such as those in the Brussels study, may compromise the IVF outcome (22).

Essentially, the differences in results between the studies are explained only by the technology used and not by differences in design and patient allocation. Both reviews of the literature fail to evaluate the differences of the technology used by the PGD groups (24, 25). The 2-cell removal technique must have reduced the implantation potential by 20%–30% (22, 23). Yet these authors detected no significant differences between the implantation rates of control embryos and embryos that had 2-cell biopsy and FISH. This indicates that embryo selection based on PGD can make up for the initially large loss of developmental potential. We postulate that the study by Staessen et al. (22) would have been successful in demonstrating increased implantation if only a single cell had been removed from each embryo, and we hope these authors will undertake such an investigation in the future.

Given that the comparisons between these studies demonstrate that 2-cell biopsy is likely to be detrimental, this approach should be reserved for cases in which diagnostic accuracy is considered paramount, more important than embryo implantation (e.g., the most difficult cases of single-gene assessment in which an affected embryo is more likely to implant and the couple is presumably fertile).

**Errors of Fluorescent In Situ Hybridization and Mosaicism**

Preimplantation genetic diagnosis as a treatment modality for IVF patients has also been criticized on the notion that determining the chromosome complement is unpredictable because of high levels of mosaicism in the early human embryo. Preimplantation genetic diagnosis emphasizes this limitation because only 1 or, at most, a few cells are considered for each embryo, and mosaicism may go undetected as a result. Whereas such reservations are sometimes valid, they are not always based on biologic phenomena, and technology may play a role in artificially amplifying the true rates of mosaicism.

Disparate readings between cells of the same embryo are not necessarily true reflections of mosaicism but often are the results of technologic deficiencies. For example, it is known that certain types of fixation will increase signal

overlaps and error rates (33). A good example of this is the recent study by Li et al. (39) in which the rates of discordance were explained by mosaicism rather than by suboptimal technology. The true rate of mosaicism can be validated only by controlled studies that examine each and every nucleated cell of embryos that are donated to research (4, 8, 12, 35).

The error rate (defined as the incorrect diagnosis of a normal embryo as abnormal) is <6% for the approximate 30% of embryos that display mosaicism. The reason for this is that most cells in such mosaic embryos are found to be abnormal, although they may contain different abnormal cell lines. Error rates have been determined by reanalyzing every cell from embryos that were considered abnormal; presumably normal ones were either frozen or transferred (40). It is likely that this introduces a bias in the determination of error rates. The proportion of embryos that is allocated to reanalysis is likely to vary from program to program, based on differences in selection and allocation criteria. This may also explain partially the differences in reanalysis error rates. Mosaicism has an indirect technologic and biologic basis as well. Some patients seem to have embryos that are prone to mosaicism, whereas others do not. Changing intrafollicular and laboratory conditions may alter the rate of mosaicism (17). The error rate including all “no result” events, false-negative results, and positive results is 4.7% after reanalysis by additional probes of inconclusive cells with the use of a third panel (Colls, Munné, and Cohen, unpublished data). It is essential that these findings are considered when determining the rate of mosaicism and its associated error rate.

## CONCLUSION

There is agreement that aneuploidy rates among human IVF embryos are very high and that the presence of chromosome abnormalities usually is associated with a reduced probability of a successful pregnancy. This has led to the hypothesis that IVF success rates can be improved if cohorts of embryos are screened for aneuploidy and if those embryos that are found to be chromosomally normal are transferred preferentially. However, the mode of biopsy and technologic nature of PGD for infertility often varies between studies, which causes significant differences in the results. We suggest that the studies that contradict the finding that aneuploidy screening improves implantation rates and lowers miscarriage rates all have  $\geq 1$  of the following aspects in common:

1. an excess of cells having been removed,
2. poor choice of probes, and
3. poor fixation technology.

A fourth, as yet unproven, cause for this phenomenon may be the use of suboptimal embryologic techniques that increase mosaicism and reduce embryo viability. We suggest that, when reviewing the literature, users of PGD technology critically assess the IVF, biopsy, fixation, and assay method that was used to evaluate the patient groups.

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