Transfer of embryos with segmental mosaicism is associated with a significant reduction in live-birth rate

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Objective: To evaluate the impact of segmental mosaicism on pregnancy outcomes from the transfer of embryos previously designated as euploid.
Design: Retrospective cohort analysis.
Setting: Single, private, high-volume fertility center.
Patient(s): Three hundred and twenty-seven women who underwent 377 frozen single euploid embryo transfers.
Intervention(s): Trophectoderm biopsy of embryos cultured to the blastocyst stage, where all transferred embryos were designated euploid by high-density oligonucleotide array comparative genomic hybridization (aCGH); after ascertaining all outcomes, revaluation of aCGH results for evidence of segmental mosaicism (defined as mosaicism on a portion of a chromosome).
Main Outcome Measure(s): Live-birth rate and spontaneous abortion rate.
Result(s): Of the 377 embryos transferred, 357 were euploid with no mosaicism, and 20 embryos had segmental mosaicism. Segmental mosaics had a statistically significantly lower live-birth rate compared with euploid controls (30.0% vs. 53.8%). When controlling for age and day of Trophectoderm biopsy, the odds for live birth after transfer of segmental mosaics were reduced by 66% compared with euploid controls (0.34; 95% confidence interval, 0.13–0.92). The spontaneous abortion rate was statistically significantly higher after transfer of segmental mosaics compared with euploid controls (40.0% vs. 18.2%).
Conclusion(s): Blastocysts with segmental mosaicism have reduced reproductive potential but retain the ability to result in live birth. These results support reporting segmental mosaicism to optimize selection of a single embryo for transfer that will maximize the chance of life birth. (Fertil Steril® 2019;111:69–76. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Embryonic mosaicism, aCGH, segmental aneuploidy, PGT, trophectoderm biopsy

Use of preimplantation genetic testing for aneuploidy screening (PGT-A) has greatly increased since its introduction in the early 2000s (1). PGT-A is used to screen for numeric chromosomal abnormalities with the goal of improving the live-birth rate (LBR) per embryo transfer (2), and current technology allows for detection of chromosomal abnormalities on all 23 pairs of chromosomes (3). The most robust protocols involve trophectoderm (TE) biopsy (4, 5) and genetic analysis using single-nucleotide polymorphism (SNP) array, quantitative polymerase chain reaction (qPCR), array comparative genomic hybridization (aCGH), and, most recently, next-generation sequencing (NGS). The newer technologies (high-density aCGH and NGS) have improved sensitivity with the ability to detect both embryonic mosaicism and segmental imbalances (6). Three randomized controlled trials have demonstrated that PGT-A in good prognosis patients leads to improved ongoing pregnancy rates (7, 8) and live-birth rates per embryo transferred when compared with controls (9). However, despite use of PGT-A, there are clinical scenarios when euploid embryos will fail to implant. One potential explanation may be embryonic mosaicism (10).

Mosaicism is defined as two or more cell populations with different genotypes and is thought to arise from postfertilization mitotic errors,

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including nondisjunction and anaphase lag (11). Mosaicism is more commonly found in cleavage-stage embryos compared with blastocysts, with an occurrence rate of up to 70% in cleavage-stage embryos (12) versus 5% to 15% at the blastocyst stage (13, 14). This may be due to the limited ability of the early embryo to adequately respond to and correct damage secondary to environmental factors before the activation of the embryonic genome (15). It has been postulated that after activation of the embryonic genome the embryo has improved mechanisms for self-correction, including increased cell cycle arrest, apoptosis of aneuploid cells, auto self-correction, and preferential allocation of aneuploid cells to the placenta, which may explain the reduced rate of mosaicism at later stages of embryo development (16–19).

Segmental errors occur when small portions of DNA are duplicated or deleted and can occur by de novo generation by a meiotic error during gametogenesis, by a mitotic error during embryo development or inherited from a carrier of a balanced translocation (20). If the error occurs during mitotic division, the embryo will possess mosaicism of the segmental error whereby some of the cells have normal chromosome copy numbers and others may have segmental duplications or deletions of the chromosome (6). The frequency of segmental errors in the embryo at the blastocyst stage range from 4% to 19% (21, 22). Little is known about the clinical impact of segmental mosaicism in reproductive medicine.

Significant controversy surrounds the transfer of mosaic and segmental mosaic embryos, given the very limited retrospective data on clinical outcomes. Studies suggest reduced implantation rates (IR)/clinical pregnancy rate (CPR) and increased miscarriage rates in mosaic embryos compared to euploid controls (23, 24). The paucity of long-term neonatal and childhood clinical data after the transfer of mosaic embryos makes the fate of these embryos uncertain. Although various societies, including the Preimplantation Genetic Diagnosis International Society (PGDIS) and the American Society for Reproductive Medicine (ASRM), offer recommendations regarding the transfer of mosaic embryos in general (25, 26), there are no guidelines specifically addressing transfer of segmental mosaic embryos. Segmental mosaic embryos pose a unique challenge given the heterogeneity of abnormalities in addition to minimal data on reproductive potential and long-term outcomes. The purpose of this study was to evaluate the impact of retrospectively identified segmental mosaicism on pregnancy outcomes and live-birth rates in a cohort of embryos previously designated as euploid at the time of transfer.

MATERIALS AND METHODS

All patients who underwent in vitro fertilization (IVF) with PGT-A using high-density oligonucleotide aCGH and had a single euploid frozen embryo transfer between January 2015 and December 2015 were screened for inclusion in the study. The detailed aCGH results from all frozen euploid embryos were then retrospectively unmasked by the testing laboratory to determine the presence of unreported segmental mosaicism. After the PGT results were unmasked, the euploid or segmental mosaic embryos that were transferred were included. Embryos with whole chromosome mosaicism were excluded for the purposes of this study, as several prior studies have evaluated the clinical impact and potential controversies associated with whole chromosome mosaicism. Embryos derived from autologous and donor oocytes were included. Pregnancy outcomes as well as patient age and day of embryo biopsy were collected on all transfers before unmasking of the PGT results. The study was approved by the institutional board review at University of California, Los Angeles.

Ovarian Stimulation, Embryo Culture, and Biopsy

The protocol for ovarian stimulation, embryo culture and biopsy has previously been described elsewhere (27). Briefly, controlled ovarian hyperstimulation was performed using standard long gonadotropin-releasing hormone (GnRH) agonist, agonist microflare, or GnRH antagonist protocols using a combination of recombinant follicle-stimulating hormone (FSH, Follistim; Merck) and human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals). When two lead follicles had reached $\geq$18 mm in mean diameter, final oocyte maturation was triggered with either subcutaneous human chorionic gonadotropin (hCG: 5,000–10,000 units) or a combination of subcutaneous GnRH agonist (leuprolide acetate, 1 mg) and hCG (1,000 IU). Oocyte retrieval was performed 35.5 hours after trigger injection.

Cycles using both conventional insemination and intracytoplasmic sperm injection (ICSI) were included in the study. All embryos were cultured in sequential media and were routinely incubated until they reached blastocyst stage or until day 7. For oocytes undergoing conventional insemination, motile sperm at a concentration of 150,000–200,000/mL were coincubated overnight in Quinn's Fertilization media with 5% human serum albumin. After confirmation of fertilization, all embryos were then transferred into Quinn's Advantage Plus Cleavage medium.

For oocytes undergoing ICSI, mature oocytes were injected 5 to 6 hours after retrieval and subsequently cultured in Quinn's Advantage Plus Cleavage medium until day 3. All cleavage-stage embryos were then transferred into 15–30 μL of Quinn's Advantage Plus Blastocyst medium for group culture at 5% oxygen concentration and routinely incubated until day 5, 6, or 7. Embryos were graded based upon criteria set by Schoolcraft et al. (28) and determined to be ready for biopsy at the expanding blastocyst stage, when a clear distinction between the inner cell mass and TE can be observed.

Blastocysts were stabilized with a holding pipette, and a 20-μm biopsy pipette was then used to remove ~3–5 TE cells for biopsy with assisted cutting by the laser. Biopsied cells were washed with a washing buffer, placed in tubes with cell lysis buffer, and cryopreserved at −20°C before being sent for testing.

PGT-A Methods

Biopsied TE cells were analyzed for all 24-chromosomes using high-density oligonucleotide aCGH (Agilent Technologies)

by the testing laboratory (PacGenomics, Agoura Hills, California). Oligonucleotide aCGH used by the testing laboratory has been previously validated and has been shown to detect duplications and deletions as small as 1.8–2.4 Mb (Agilent) (29, 30).

In general, mosaic results were considered euploid by the testing laboratory if ≤40% mosaicism was present, while the decision to categorize a segmental mosaic embryo as euploid or aneuploid was individualized on a case by case basis. Notably, mosaicism ≤20% can be due to technical noise from the technology itself (29). Embryos that were found to have segmental mosaicism were then reanalyzed by NGS using a third-party reagent kit for library preparation and MiSeq (Illumina) sequencer, and analysis was performed with the Nexus 9.0 (Biodiscovery). Similar to aCGH, the mosaicism detection limit was set to 40%.

Data Analysis

The primary outcome was LBR in euploid embryos and embryos with unmasked segmental mosaicism. Secondary outcomes included CPR and spontaneous abortion (SAB) rate. Live birth was defined as a live birth of one or more neonates greater than or equal to 24 weeks' gestational age. Clinical pregnancy was defined as one or more fetal heart beats on ultrasound, and SAB was defined as a spontaneous pregnancy loss at less than 20 weeks' gestation. Complex segmental mosaics were defined as three or more segmental mosaics within the same embryo.

Statistical analysis was performed in SAS (SAS Institute). A generalized linear model was used to control for the impact of patient age and day of embryo biopsy on the chance of segmental mosaicism. Chi-square and Fisher's exact tests were used, when appropriate, to compare clinical outcomes including clinical pregnancy, live birth, and SAB between segmental mosaic and euploid embryos. A logistic regression analysis was performed to assess for associations between segmental mosaicism and pregnancy outcome while controlling for day of embryo biopsy and patient age. The data are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). $P<.05$ was considered statistically significant.

RESULTS

A total of 330 women underwent 380 frozen single euploid embryo transfers during the study time period. Of the 380 embryo transfers with PGT-A results retrospectively reexamined, 377 were included for analyses. Three embryos were excluded because they contained whole chromosome mosaicism. Of the 377 embryos, 357 embryos were designated euploid with no segmental mosaicism, and 20 embryos contained a segmental duplication and/or deletion. There were repeat patient cycles from the same patient (n = 46), but the data were analyzed to account for this and were found to be of no statistical significance. The average patient age and percentage of embryos biopsied on days 5, 6, and 7 were similar between the euploid and segmental mosaic embryos (Table 1).

The PGT-A results of the 20 segmental mosaic embryos are shown in Table 2 along with their clinical outcomes. Segmental mosaicism spanned multiple different chromosomes and locations, were of various sizes, and resulted in variable clinical outcomes. Fourteen embryos (70%) contained a single segmental mosaic chromosome, and six (30%) contained two or more segmental mosaic chromosomes. When assessing the incidence of duplications and deletions, we found that four embryos (20%) had segmental mosaic duplications and 13 (65%) embryos had segmental mosaic deletions. An additional three (15%) embryos possessed segmental mosaic duplication/deletions. The segmental mosaics were randomly distributed among the chromosomes.

The most common chromosomes affected were chromosome 2 and chromosome 8. Two embryos had a pure segmental mosaic deletion of a portion of chromosome 2, and a third embryo had a segmental deletion of chromosomes 2 and 16. Maternal age did not appear to have an effect on the incidence of segmental mosaicism in our cohort.

All embryo biopsies with segmental mosaicism were reanalyzed with aCGH. Of the 20 embryos classified as segmental mosaics by high-density aCGH, 11 (55%) had different results when analyzed with NGS. Eight patients were found to have more extensive chromosomal abnormalities than were seen with high-density aCGH. Five patients with previously diagnosed segmental mosaics had whole chromosome mosaicism noted on NGS, and three patients had an additional duplication and/or deletion that was identified by NGS. Of the three patients with less extensive findings on NGS, two patients had two segmental mosaics identified on aCGH and only one identified on NGS (patients 11 and 12), and one patient with a deletion on the terminal end of the short arm of chromosome 19 noted on aCGH had a normal chromosome 19 on NGS (patient 17). The results of reanalysis of the TE biopsies with differential findings between NGS and high-density aCGH are shown in Table 3.

Pregnancy outcomes resulting from the transfer of the segmental mosaic embryos are also shown in Table 2. All pregnancy outcomes occurred across all types of segmental mosaics. Notably, two live births resulted from the transfer of complex segmental mosaics (patient 7 and patient 12). None of the five patients subsequently found to have whole chromosome mosaicism after reanalysis with NGS went on

TABLE 1

Baseline characteristics between euploid and segmental mosaic embryos after unmasking preimplantation genetic testing for aneuploidy screening results.

Characteristic	Euploid embryos n (%)	Segmental mosaic embryos n (%)	*P* value[a]
Patient age (y)	38.3	39.1	NS
Day of embryo biopsy			
Day 5	263 (70)	16 (80)	NS
Day 6	87 (23)	4 (20)	
Day 7	7 (7)	0	

Note: $P<.05$ was considered statistically significant.
[a] General linear mixed model.

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TABLE 2

High-density oligonucleotide aCGH analysis of segmental mosaic embryo.

Patient no.	Patient age (y)	Chromosome number	Chromosome arm	Deletion or duplication	Size (Mb)	% Aneuploidy	Pregnancy outcome
1	40	1	q	Deletion	102	36.1	Biochemical
2	39	1	q (terminal)	Deletion	59.8	46.7	Biochemical
		12	q (terminal)	Deletion	13	55.2	
3	33	2	q (terminal)	Deletion	60.2	47.4	Live birth
4	41	2	q (terminal)	Deletion	110.9	34.7	Live birth
5	38	2	p (terminal)	Deletion	46.1	32.4	Negative
		16	p	Deletion	29.6	36	
6	37	3	p	Duplication	77.2	42.7	Biochemical
7	43	4	q	Duplication	100	59.9	Live birth
		4	q (terminal)	Deletion	7.5	109.8	
8	32	5	p (terminal)	Deletion	31.6	38.7	Negative
9	42	5	p	Deletion	45.9	46.7	SAB
10	31	6	q (terminal)	Duplication	62.9	45.7	Live birth
11	54	7	p (terminal)	Deletion	18.8	95.7	Biochemical
		22	q	Duplication	23.9	40.2	
12	42	7	q (terminal)	Deletion	50.7	53.1	Live birth
		19	p (terminal)	Deletion	19.5	41.7	
13	41	8	p (terminal)	Deletion	7.5	101.6	SAB
		8	p	Duplication	30.7	48.9	
		8	q	Deletion	98.3	36	
14	39	8	q (terminal)	Deletion	62.8	37.9	Negative
15	40	11	p (terminal)	Deletion	34.1	43.7	Negative
16	37	11	q (terminal)	Deletion	46.9	46.5	Live birth
17	30	19	p (terminal)	Deletion	5.8	43.6	Negative
18	36	19	q	Duplication	30.8	42.5	Negative
19	40	20	p	Deletion	9.1	83.6	Biochemical
20	39	21	q	Duplication	12.5	71.1	SAB

Note: Clinical pregnancy outcomes after embryo transfer are listed next to each embryo. aCGH = array comparative genomic hybridization; Mb = megabase; SAB = spontaneous abortion.

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to have a live birth. Of the additional three patients with additional duplications/deletions on NGS, two patients had a negative pregnancy, and one patient had a biochemical pregnancy. Of the three patients with less extensive chromosomal abnormalities on NGS, one patient had a biochemical pregnancy, one patient a live birth, and one patient a negative pregnancy result (Table 3).

A comparison of pregnancy outcomes between segmental mosaics and euploid controls is shown in Table 4. Segmental mosaic embryos had a statistically significantly lower LBR

TABLE 3

Comparison of embryos with differential results on high-density aCGH compared with NGS analysis.

Patient no.	Patient age	aCGH	NGS	Pregnancy outcome
1	40	1q del	Monosomy 1[a]	Biochemical
5	38	2p (terminal) del 16p del	2p (terminal) del Monosomy 16[a]	Negative
6	37	3p dup	Trisomy 3[a]	Biochemical
8	32	5p (terminal) del	5p (terminal) del 5q (terminal) del 6q (terminal) del 15q (terminal) dup	Negative
11	54	7p (terminal) del 22q dup	7p (terminal) del	Biochemical
12	42	7q (terminal) del 19 p (terminal) del	7q (terminal) del	Live birth
14	39	8q (terminal) del	8q (terminal) del 8p (terminal) del	Negative
17	30	19p (terminal) del	Normal chromosome 19	Negative
18	36	19q dup	Trisomy 19[a]	Negative
19	40	20p (terminal) del	20p (terminal) del 20q del	Biochemical
20	39	21q dup	Trisomy 21[a]	SAB

Note: aCGH= array comparative genomic hybridization; del = deletion; dup = duplication; NGS = next-generation sequencing; SAB = spontaneous abortion.

[a] Whole chromosome mosaicism.

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TABLE 4

Comparison of pregnancy outcomes between segmental mosaic embryos and euploid controls.

Outcome	Euploid embryos n (%)	Segmental mosaic embryos n (%)	Odds ratio (95% CI)[a]
CPR	215 (60)	8 (40)	0.41 (0.16–1.04)[b]
LBR	192 (53.8)	6 (30)	0.34 (0.13–0.92)[c]
Miscarriage rate	65 (18)	8 (40)	3.02 (1.18–7.76)[d]

Note: $P<.05$ was considered statistically significant. CI = confidence interval; CPR = clinical pregnancy rate; LBR = live-birth rate.
[a] Logistic regression with adjustment for age and day of trophectoderm biopsy.
[b] $P=.07$.
[c] $P=.04$.
[d] $P=.04$.

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compared to euploid controls (30% vs. 53.8%, $P=.04$), and a statistically significantly higher SAB rate (40% vs. 18%, $P=.04$). There was a trend toward reduced CPR in the segmental mosaic group compared with the euploid controls, (40% vs. 60%, $P=.07$; 95% CI, 0.16–1.04). Segmental mosaic embryos demonstrated statistically significantly poorer outcomes overall. Compared with the euploid controls, the segmental mosaic embryos demonstrated a 66% decrease in live birth (OR 0.34; 95% CI, 0.13–0.92) and a threefold higher instance of SAB (OR 3.02, 95% CI, 1.18–7.76).

DISCUSSION

The absence of euploid embryos to transfer can lead to great distress for patients undergoing IVF. This reality is increasingly being realized as the sensitivity of various platforms for PGT-A improves and a portion of embryos that were previously designated as euploid are now being identified as mosaic. The transfer of mosaic embryos is controversial, given the paucity of clinical data on long-term outcomes, and it is a continued area of research. Our study evaluates the impact of segmental mosaicism on CPR, SAB, and LBR. We demonstrate that these embryos have significantly reduced reproductive potential with increased miscarriage rates and decreased live-birth rates compared with euploid controls. However, despite the reduced reproductive potential, these embryos still have the potential to implant and progress into clinically viable pregnancies that can result in a live birth in approximately 30% to 40% of transfers (23, 24, 30).

Prior studies on outcomes after the transfer of mosaic embryos have shown reduced implantation rates and higher miscarriage rates compared with euploid embryos (23, 24, 31). A case series of 18 patients with mosaic embryos transferred resulted in a 33% LBR and a 12% miscarriage rate. Although all live births in this series were term deliveries with normal chorionic villus sampling results, no long-term neonatal data were reported (23). Another study retrospectively reanalyzed biopsies from previously transferred embryos presumed to be euploid by aCGH but that had slight deviations in their profiles that were too small to be characterized as aneuploid during the initial analysis. After reanalysis with NGS, 44 of the 150 embryos were determined to be mosaic. Compared with euploid controls, the mosaic embryos had reduced implantation rates (30.1% vs. 55.8%, $P=.038$) and higher miscarriage rates (55.6% vs. 17.2%, $P=.036$) (24).

Several studies have also assessed whether the percentage or complexity of mosaicism impacts embryonic reproductive potential. A recent prospective study found that embryos with >50% mosaicism using NGS have statistically significantly decreased IR (24.4% vs. 54.6%, $P=.0019$), CPR (15.2% vs. 46.4%, $P=.0013$), and LBR (15.2% vs. 46.6%, $P=.0013$) compared with euploid blastocysts. However, in embryos with mosaicism <50% had clinical outcomes similar to euploid controls (30).

A retrospective study by Munne et al. (31) specifically addressed the impact of the complexity of mosaicism on clinical outcomes and found that complex mosaic embryos had statistically significantly lower sustained implantation rates compared with single aneuploid, double aneuploid, or segmental mosaic embryos. They also noted that the overall fetal loss rate was statistically significantly higher and sustained implantation rate was statistically significantly lower in mosaic embryos compared with euploid controls (31). These studies suggest that embryos with a lower percentage of mosaicism and no complex aneuploidy should be given priority for transfer over other mosaic embryos if considering the transfer of a mosaic embryo.

Data are more limited regarding the transfer of segmental mosaic embryos. One study found that segmental mosaic embryos had similar ongoing implantation rates compared with single mosaic aneuploid and double mosaic aneuploid embryos (41%, 50%, and 45%, respectively) with a miscarriage rate of 33% in the segmental mosaic cohort; overall, the ongoing implantation rates of all mosaic embryos were decreased compared with euploid controls (40% vs. 63%, $P<.006$) (31).

In another retrospective study, 44 euploid blastocyst biopsies were reanalyzed by NGS, and 14 (32%) of the 44 the mosaic embryos identified were segmental mosaics. In the subset of segmental mosaics, the LBR of the was 57% and was not statistically decreased compared with euploid controls (24). In the latter study, the euploid controls consisted of only 51 embryos, which may explain why the investigators found no difference in LBR between the segmental mosaic cohort and the controls. Additionally, the use of aCGH, as opposed to high-density aCGH as in our study, may have led to suboptimal detection of smaller segmental mosaic embryos; these could have inadvertently been included in the euploid control group, thus potentially skewing results. Studies in segmental mosaics are both limited and conflicting. Our study findings will add to the limited knowledge that is available regarding the outcomes of these embryos.

Our study has several important strengths. The data come from a single, high-volume reproductive center experienced in TE biopsy with a diverse cohort of patients. All blastocysts were sampled by TE biopsy, and the results were from a single calendar year, thus minimizing bias associated with potential change in laboratory personnel and technique. Additionally,

PGT-A was performed in the same genetic screening laboratory using one approach, ensuring uniformity of technique. Finally, the inclusion of only single euploid embryo transfers resulted in less heterogeneity among the results, as multiple gestations and untested embryos can individually and collectively affect pregnancy rates.

Despite a number of strengths, our study was not without its limitations. There was a small sample size of segmental mosaic embryos, and this may explain why the CPR did not statistically significantly differ from euploid controls. Additionally, there was heterogeneity of segmental mosaic embryos and a lack of clearly defined criteria for designating a segmental mosaic embryo as euploid or aneuploid. At the time of our study, the standard laboratory cutoff to classify an embryo as euploid was assigned when >40% of the trophectoderm cells were abnormal. However, the final decision to label an embryo with segmental mosaicism as euploid was based on multiple factors, including patient cycle outcome, size of the duplication or deletion, percentage of segmental aneuploidy, and chromosome size and number. This subjective gray area is one of the inherent challenges with PGT-A as further data on outcomes are collected.

Furthermore, the use of aCGH for PGT-A in our study may be considered a limitation, as NGS may be more sensitive to detect mosaicism given the dynamic range across which it is able to read chromosomes. In our study, 11 of 20 patients had different results between aCGH and NGS, and eight patients had additional segmental mosaicism detected by NGS. One case-control study that reanalyzed trophectoderm biopsies with NGS from embryos designed euploid by aCGH before transfer found a previously undetected mosaicism rate of 31.8% in those transfers, resulting in a SAB versus 15.8% compared with those resulting in a live birth (10). However, another study found 98.4% concordance of segmental aneuploidies and 96% concordance for whole chromosome mosaicism between aCGH and NGS profiles, with a detection rate as small as 10 Mb (megabase). It remains to be shown whether these segmental aneuploidies are actually true abnormalities or a product of noise or artifact across all tests (20). Notably, our study used high-density oligonucleotide aCGH, which has been shown to detect duplications and deletions as small as 1.8–2.4 Mb (32, 33). This is significantly more sensitive than the 10 Mb limit of the more widely used bacterial artificial chromosome aCGH (22) and thus should be relatively equivalent to NGS.

Finally, despite the increasing sensitivity of newer PGT-A platforms, it remains to be seen whether a single TE biopsy is truly representative of the entire embryo. Johnson et al. (13) reassessed 51 embryos with aCGH and noted a 3.9% discordance rate between the inner cell mass (ICM) and TE biopsies; however, analysis of the paired TE biopsies from 29 of the embryos were 100% concordant. Huang et al. (34) also analyzed the ICM and three TE biopsies per embryo and found a 98% concordance rate between the ICM and at least one of the TE biopsies per embryo and an 84% concordance between the ICM and all three TE biopsies. Although these data show high concordance between the TE biopsy and ICM, it is clear that we cannot accurately determine whether a single TE biopsy is truly representative of the entire embryo.

Before the transfer of any mosaic embryo, including segmental mosaics, patients should receive extensive genetic counseling to review the possible risks and benefits regarding the transfer of these embryos. It should be emphasized that to date there are no long-term data on the impact to the offspring. Additionally, it is also recommended that invasive prenatal diagnostic testing with amniocentesis be performed after transfer of these embryos to confirm a normal fetal karyotype; it is possible that noninvasive prenatal testing and chorionic villus sampling are insufficient because they assess placental rather than fetal cells (31).

In conclusion, our results demonstrate that embryos containing segmental mosaicism have decreased clinical potential compared with euploid embryos. However, these embryos are still capable of resulting in live birth. Thus, we suggest that if they are noted after PGT-A, they should not be discarded but rather reported to clinicians. We acknowledge that there are limited data on the long-term outcomes in offspring of mosaic embryo transfers and that extensive genetic counseling is necessary for patients considering the transfer of these embryos. In the future, studies with a larger sample size of embryos as well as verification of these results with NGS, as well as long-term data on neonatal outcomes, will be paramount in our continued understanding of the reproductive potential and clinical phenotype of whole chromosome and segmental mosaic embryos.

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La transferencia de embriones con mosaicismo segmentario está asociada a una reducción significativa en la tasa de nacido vivo

Objetivo: evaluar el impacto del mosaicismo segmentario en los resultados de embarazo a partir de la transferencia de embriones designados previamente como euploides.

Diseño: Análisis retrospectivo de cohortes.

Lugar: Centro de fertilidad individual, privado y de gran volumen.

Paciente(s): Trescientas veintisiete mujeres que se sometieron a 377 transferencias de embrión único euploide congelado.

Intervención(es): Biopsia de trofoectodermo de embriones cultivados hasta la etapa de blastocisto, donde todos los embriones transferidos fueron designados como euploides por hibridación genómica comparativa de matriz de oligonucleótidos de alta densidad (aCGH); después de comprobar todos los resultados, se realizó una revaluación de los resultados de aCGH para evidenciar mosaicismos segmentarios (definido como mosaicismo en una porción de un cromosoma).

Principal(es) medida(s) de resultado: Tasa de nacido vivo y tasa de aborto espontáneo.

Resultado(s): De los 377 embriones transferidos, 357 fueron euploides sin mosaicismo, y 20 embriones tuvieron mosaicismo segmentario. Los embriones con mosaicos segmentarios tuvieron una tasa de nacido vivo menor y estadísticamente significativa comparada con los controles euploides (30.0% vs. 53.8%). Al controlar por edad y día de biopsia de Trofectodermo, las probabilidades de nacido vivo después de la transferencia de mosaicos segmentarios se redujeron en un 66% en comparación con controles euploides (0.34; intervalo de confianza del 95%, 0.13-0.92). La tasa de aborto espontáneo fue mayor y estadísticamente significativa después de transferencia de mosaicos segmentarios en comparación con los controles euploides (40.0% vs. 18.2%).

Conclusión(es): Los blastocistos con mosaicismo segmentario tienen potencial reproductivo reducido, pero conservan la capacidad de dar como resultado un nacido vivo. Estos resultados apoyan el informe del mosaicismo segmentario para optimizar la selección de un único embrión para transferir lo que maximizará la posibilidad de nacido vivo.