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ASSISTED REPRODUCTION TECHNOLOGIES

Healthy live births from transfer of low-mosaicism embryos after preimplantation genetic testing for aneuploidy

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Abstract

Purpose This study evaluated the potential viability of embryos with low mosaicism level (< 50%) by comparing the clinical outcomes of single mosaic versus euploid blastocyst transfer. In addition, the live birth outcomes for various types of mosaicism with respect to abnormalities in chromosome structure and content were analyzed.

Methods This study included patients who underwent in vitro fertilization with preimplantation genetic testing for aneuploidy (PGT-A). The PGT-A cycles performed through next-generation sequencing with single euploid or mosaic embryo transfers were included. We collected 299 frozen single embryo transfer cycles—216 single euploid and 83 mosaic—between July 2016 and July 2018. This study analyzed clinical outcomes, including fetal karyotyping by using amniocentesis, gestational age at delivery, and live birth weight after single mosaic embryo transfer.

Results The average birth weight of infants in the euploid and mosaic blastocyst transfer groups was 3146.2 and 2997.7 g, respectively. The karyotyping results of prenatal diagnosis in all pregnant women were normal. Our study indicated that mosaic embryos can develop into euploid healthy infants with various levels or types of mosaicism. No significant difference was observed between infants from euploid and mosaic blastocyst transfers.

Conclusion If patients have no euploid embryos, mosaic embryos can be transferred as they have potential for implantation and development into euploid healthy infants. This study is invaluable for counseling clinical results after single mosaic embryo transfers.

Keywords Single embryo transfer · Mosaic embryo transfer · Healthy live births · Preimplantation genetic testing for aneuploidy

Introduction

Chromosome aneuploidy is a major cause of in vitro fertilization (IVF) failure; most embryos with aneuploidy result in implantation failure or first trimester miscarriage [1]. Retrospective studies have suggested that selecting euploid embryos for transfer is beneficial [2, 3]. Indeed, use of the preimplantation genetic test for aneuploidy (PGT-A) has been found to improve the live birth rate and significantly reduce the miscarriage rate following IVF treatment [4–6].

Unfortunately, PGT-A reveals no euploid embryos for some couples but a few embryos do have mosaicism. Blastocysts are classified as having mosaicism after the analysis of 5–10 trophectoderm cells. Mosaic blastocysts have lower implantation potential and result in higher risk of miscarriage than euploid blastocysts [7]. Mosaicism is a common phenomenon in preimplantation mammalian embryos that arises due to errors in mitosis during embryonic cleavage

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[8]. However, the effect of mosaicism on the babies born after mosaic embryo transfer is unknown. Therefore, most physicians and patients do not accept mosaic embryos for transfer in IVF cycles.

In 2015, a study first reported that mosaic embryo transfers resulted in pregnancy and even healthy infants when array comparative genomic hybridization (aCGH) was used [9]. Studies have since indicated that mosaic embryos can be viable [10, 11], with comparable implantation rate with that of euploid embryos. Blastocysts with mosaicism levels as high as 60% have resulted in successful pregnancy and live birth [10, 11]. These findings strongly suggest that mosaic aneuploidy does not necessarily indicate the end of developmental potential.

Reporting mosaic embryos as fully abnormal or not transferring them when euploid embryos are unavailable may lead to wastage of embryos that are viable. However, the paucity of long-term neonatal and childhood clinical data after mosaic embryo transfer makes the fate of these embryos uncertain. Although various societies, such as the Preimplantation Genetic Diagnosis International Society and American Society for Reproductive Medicine, have provided general recommendations regarding mosaic embryo transfer [12, 13], no guidelines have specifically addressed the mosaicism level of transferred embryos.

Although studies have reported some healthy babies following mosaic blastocyst transfer, the studies analyzing baby health are limited in number. No study has comprehensively analyzed the entire cycle involving a single mosaic embryo transfer. We speculated that single embryo transfers (SETs) using embryos with low mosaicism level (< 50%) can result in healthy euploid babies. However, the effect of various types of mosaicism with respect to abnormalities in chromosome structure and content on fetal development requires clarification. In this study, we analyzed the clinical outcomes of single low mosaicism embryo transfers and compared the clinical outcomes of various mosaicism types with respect to abnormalities in chromosome structure and levels mosaicism.

Materials and methods

This retrospective cohort study was approved by the Institutional Review Board (IRB) of Chung Shan Medical University Hospital (IRB: CS19039), Taichung, Taiwan. This study recruited couples undergoing PGT-A. There were 911 PGT-A cycles from July 2016 to July 2018 in our hospital. In this study, the indications for PGT-A include repeated implantation failure, advanced maternal age (AMA; ≥ 38 years), recurrent miscarriage, severe male factor infertility, gamete donation cycles, and long-term unexplained infertility. Oocyte donation cycles and repeat PGT-A cycles were excluded from this study. To analyze the clinical outcomes of mosaic embryo transfers, we included 299 couples who underwent PGT-A and SET. PGT-A was performed using next-generation sequencing (NGS) followed by SET with one euploid embryo or one mosaic embryo between July 2016 and July 2018. Data related to mosaic blastocysts—maternal age, mosaicism level, various mosaic structures, or mosaic contents of chromosomes—were reviewed. This study analyzed clinical outcomes, including the implantation rate, ongoing pregnancy rate, abortion rate, gestational age at delivery, and birth weight after single mosaic embryo transfer.

Ovarian stimulation, in vitro fertilization, embryo culture, trophectoderm biopsy, and frozen embryo transfer cycle

Ovarian stimulation

All women included in this study underwent controlled ovarian stimulation with a long protocol for GnRH agonist [14]. They were administered leuprolide acetate (Lupron, Takeda Chemical Industries, Osaka, Japan) in progress through the midluteal phase for downregulation. All women received injections of recombinant follicle stimulating hormone (Gonal-F; Serono, Bari, Italy) from the third day of the treatment cycle for ovarian stimulation until the dominant follicle had developed to a diameter > 18 mm; 250 μg of hCG (Ovidrel; Serono) was injected 36 h before oocyte retrieval.

In vitro fertilization and embryo culture

The retrieved oocytes were subjected to conventional insemination or intracytoplasmic sperm injection (ICSI) prior to which they were cultured in Quinn's Advantage Fertilization Medium (Sage BioPharma, Trumbull, CT, USA) containing 15% serum protein substitute (SPS, Sage BioPharma) in a low-oxygen environment of 5% CO_2, 5% O_2, and 90% N_2, which was prepared before oocyte retrieval. All embryos were further cultured in a cleavage medium (Sage BioPharma) containing 15% SPS. At 70 ± 2 h after insemination or ICSI, all cleaved embryos were group-cultured in microdrops of a blastocyst medium (Sage BioPharma) containing 15% SPS.

Trophectoderm biopsy and frozen embryo transfer cycle

Laser pulses were used to punch a small hole in the zona pellucida on day 3 or 4. Expanding and expanded blastocysts underwent trophectoderm biopsy on day 5 or 6. Immediately before trophectoderm biopsy, blastocyst quality was assessed as per the criteria reported by Gardner and Schoolcraft [15]. The blastocysts considered to be of desirable quality (4, 5, 6,

AB, BA, and BB) were biopsied. The blastocysts were fixed with a holding pipette (Humagen, Charlottesville, VA, USA), and laser pulses were used to punch a hole between cell junctions to accommodate the passage of several trophectoderm cells. Approximately 5–10 trophectoderm cells were aspirated into the biopsy pipette with smooth suction. The aspirated trophectoderm cells were detached from the blastocysts by using several laser pulses combined with smooth suction. The biopsied cells were washed approximately three times with phosphate-buffered saline and immediately placed in RNase/DNase-free polymerase chain reaction tubes. Re-expansion biopsied blastocysts were frozen using the Cryotech vitrification method (Repro-Support Medical Research Centre, Tokyo, Japan). Vitrification and warming were performed using Cryotech (Cryotech, Japan) according to the protocols described by Gutnisky et al. [14, 16].

All patients underwent an artificial cycle for endometrial preparation with estradiol and progesterone supplementation [14]. For a frozen embryo transfer (single euploid or mosaic blastocyst), the endometrial thickness should be ≥ 8 mm on day 18 of the menstrual cycle. In this study, the single good-quality embryo was thawed and transferred from the cohort of frozen embryos. The priority in embryo transfer was determined on the basis of the quality of euploid or mosaic blastocysts in each treatment cycle; that with the highest quality was transferred first. Mosaic embryo transfer also followed the PGDID guidelines: embryos with low-level mosaicism or that were single chromosome mosaic embryos were transferred. Euploid or mosaic blastocysts were selected for transfer, and warmed embryos were cultured in the blastocyst medium at 37 °C (5% CO_2 and 5% O_2) for 1 ± 2 h before the transfer. Embryo transfer procedures were performed as previously described [17]. To evaluate the clinical outcomes, the implantation rate was calculated by dividing the number of gestational sacs by the total number of blastocysts transferred. The ongoing pregnancy rate (OPR) was calculated by dividing the number of patients with live intrauterine pregnancies beyond 12 weeks of gestation (positive cardiac activity on ultrasound examination) by the total number of patients who underwent embryo transfer.

Laboratory procedure for PGT-A

Validation of mosaicism with mixing experiments

To determine mosaicism levels in trophectoderm biopsies, each laboratory needs to define a threshold for differentiating among several categories of mosaicism. First, we performed proof-of-principle mixing experiments to evaluate the sensitivity of the Illumina VeriSeq NGS platform in detecting mosaicism, as described [17, 18]. We obtained normal embryonic stem cell lines, 46XY [19], and aneuploid cell lines, including trisomy 16 (47XY + 16), with previously characterized karyotypes from our laboratory. The cells were thawed, and individual cells were observed under a dissecting microscope by micropipettes using a 130-mm capillary (Cook) and combined in different ratios, creating a mixture of six cell types in different proportions of the abnormal alleles of interest (0%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 100%). Proof-of-principle experiments were performed at least thrice, each time with the creation of a new cell mixture (Supplemental Fig. 1).

Determination of mosaicism levels (aneuploidy/euploidy ratio) by high-resolution NGS

Trophectoderm cell samples were lysed, and genomic DNA was randomly amplified using the SurePlex DNA System (Illumina) according to the manufacturer's protocol. The whole-genome amplified DNA product of each sample was processed to prepare genomic DNA libraries by using the VeriSeq PGS workflow (Illumina) at the Genetic Diagnosis Laboratory of Lee Women's Hospital (Taichung, Taiwan). The MiSeq Reagent Kit v.3 (Illumina) was used on a MiSeq System (Illumina). The generated bioinformatics data were analyzed using BlueFuse Multi Software v.4.4 (Illumina), and the mosaic chromosomes of each sample were checked by technicians [17, 20].

Statistical analysis

Clinical and pregnancy outcomes were compared using chi-square and *t* tests. A confidence level of $P < 0.05$ was considered statistically significant.

Results

Table 1 summarizes and compares the patient characteristics, including cause of infertility, hormone levels, number of retrieved oocytes, fertilization rate, blastocyst rate, and mosaicism rate. No significant differences were observed between the euploid and mosaic groups in infertility cause, hormones levels at baseline or on the day of ovulation trigger, number of oocytes retrieved, fertilization rate, blastocyst rate, and biopsy rate. The percentage of mosaicism after blastocyst biopsy in our genetic laboratory was 25.4% (381/1498). The mosaic rates were 21.7% (250/1153) and 38.0% (131/345) in the euploidy and mosaic groups, respectively (Table 1). Both the aneuploidy rate and mosaic rate in the mosaic group were significantly higher than those in the euploid group. The implantation rate (65.7% vs 51.8%) and OPR (64.8% vs 47.0%) in the euploid group were significantly higher than those in the mosaic group. Gestational age at delivery and birth weight were not significantly different between the groups. The OPRs among younger patients in the euploid group were

Table 1 Comparison of infertility causes, patient characteristics, embryo culture outcome, and PGT-A results between single euploid and mosaic embryo transfer groups

	Total SETs	Euploidy	Mosaic	*P* value
Total cycle no.	299	216	83	
Average age	36.5 ± 4.9	35.4 ± 3.8	37.0 ± 5.6	0.126
Rate of infertility causes %(no)				
Recurrent IVF Failure	40.5% (121)	40.7% (88)	39.7% (33)	0.66
Recurrent Miscarriage	19.4% (58)	21.8% (24)	13.2% (11)	0.09
Advanced women	42.1% (126)	39.4% (85)	49.4% (41)	0.12
Male factor	8.7% (26)	10.2% (22)	4.8% (4)	0.14
Combined with PGT-M	11.0% (33)	12.5% (27)	7.2% (6)	0.19
Unexplained infertility	9.6% (25)	8.8% (19)	7.2% (6)	0.66
Average level of hormone				
Basal FSH (mIU/ml)		6.4 ± 4.4[#]	8.3 ± 10.4	0.33
Basal LH (mIU/ml)		6.33 ± 6.7	8.3 ± 11.7	0.31
Basal E2 (pg/ml)		44.1 ± 33.3	56.3 ± 68.8	0.14
Basal P4 (ng/ml)		6.3 ± 6.8	6.9 ± 9.0	0.39
AMH (ng/ml)		4.5 ± 3.3	5.3 ± 6.1	0.17
Average hormone level on the day of ovulation trigger				
LH (mIU/ml)		2.5 ± 2.2	2.5 ± 2.4	0.87
E2 (pg/ml)		2803.5 ± 1920.6	2253.2 ± 2057.7	0.54
P4 (ng/ml)		1.0 ± 0.7	1.0 ± 0.7	0.88
Embryo culture				
Average number of oocytes retrieved		16.8 ± 10.0	14.8 ± 10.3	0.38
Fertility rate		66.2% (2412/3641)	66.4% (819/1234)	0.88
Blastocyst rate		47.8% (1153/2412)	42.1% (345/820)	0.005
Biopsied rate		100% (1153/1153)	100% (345/345)	–
Vitrification rate		100% (1153/1153)	100% (345/345)	–
Results of PGT-A				
Euploidy rate		41.2% (475/1153)	–	–
Aneuploidy rate		38.9% (449/1153)	62.0% (214/345)	< 0.0001
Mosaic rate		21.7% (250/1153)	38.0% (131/345)	< 0.0001

*There were one or more than one causes in some couples

[#] Mean ± standard deviation

No significant differences were discovered in *t* tests in age, infertility cause, average hormone level, average hormone level on the day of ovulation trigger, or average number of oocytes retrieved

The infertility cause, fertilization rate, blastocyst rate, biopsy rate, vitrification rate, aneuploidy rate, and mosaic rate between the single euploid and mosaic embryo transfer groups were compared using the chi-square test

significantly higher than that in the mosaic group (68.7% vs 50.0%). However, no significant intergroup differences were observed in the implantation and OPRs among older women (Table 2). In our analyses, maternal age did affect the clinical outcomes of babies delivered in each group.

Various types of mosaicism include various levels of mosaicism and various abnormalities of chromosome content and structures. OPRs were similar irrespective of the mosaicism level of the transferred embryo: 47.5% (28/59) and 45.8% (11/24) for 30% and 40% mosaicism levels, respectively, in the mosaic group (Table 3). Healthy euploid live births occurred from embryos of different levels of mosaicism. For women receiving embryos with 30% and 40% mosaicism, the average gestational age at delivery was 38.4 and 38.2 weeks,

Table 2 Clinical outcomes of single euploid and mosaic embryo transfers in different age groups

	Total SETs	Euploidy			Mosaic		
		Total	< 38 years	≧ 38 years	Total	< 38 years	≧ 38 years
Cycles (no.)	299	216	131	85	83	42	41
Age	36.5 ± 4.9	35.4 ± 3.8	33.4 ± 2.9	40.8 ± 2.8	37.0 ± 5.6	32.5 ± 3.1	41.6 ± 3.4
IR (*n*)	61.9% (185)	65.7% (142)[a]	68.7% (90)	61.2% (52)	51.8% (43)[a]	52.4% (22)	51.2% (21)
OPR(*n*)	59.9% (179)	64.8% (140)[b]	68.7% (90)[c]	58.2% (50)	47.0% (39)[b]	50.0% (21)[c]	43.9% (18)
AR (*n*)	11.2% (20)	12.9% (18)	12.2% (11)	14.0% (7)	5.1% (2)	0% (0)	11.1% (2)
Gestational age at delivery (*n*)	38.3 ± 1.7 (159)	37.6 ± 1.7 (120)	37.9 ± 1.9 (79)	38.2 ± 1.3 (42)	38.3 ± 1.7 (39)	38.6 ± 1.6 (21)	38.0 ± 1.7 (18)
Birth weight	3112.4 ± 464.4 (161)	3146.2 ± 450.0 (121)	3171.5 ± 440.3 (79)	3130.5 ± 469.0 (42)	2997.7 ± 501.1 (40)	3108.5 ± 474.0 (22)	2871.1 ± 518.1 (18)

Chi-square test; a, b, c; $P < 0.05$

No significance of AR gestational age at delivery and birth weight was observed

respectively, and the average birth weight was 3019.2 and 2927.1 g (Table 4). No significant differences were discovered between the various mosaicism groups in the implantation rate, OPR, gestational age at delivery, or birth weight. Maternal age did not affect the clinical outcomes when the mosaicism level was low (Table 3).

The type of mosaicism observed in the blastocysts was classified into three groups depending on the involvement of chromosome structures: whole chromosome, segmental, and complex mosaicism. Whole-chromosome mosaicism indicated a gain or loss of at least one entire chromosome to form monosomies or trisomies. Segmental mosaicism was defined as diploid–aneuploid mosaic ratios detected from gain or loss segments of ≥ 10 MB in size of individual chromosomes altered. Complex mosaicism was defined as a combination of whole-chromosome and segmental mosaicism in diploid–aneuploid mosaic samples. The OPRs and average birth weight in the whole-chromosome, segmental, and complex groups were 45.5% (5/11), 46.7% (28/60), and 50.0% (6/12) and 3280.0, 2951.0, and 3020.2 g, respectively (Table 3). The number of mosaic chromosomes in this study was categorized into 1, 2, and > 2, and the corresponding OPRs were 42.3% (22/52), 55.0% (11/20), and 55.6% (6/11), respectively (Table 3). No significant difference was observed in gestational age at delivery and live birth weight between these groups (Table 4). The abortion rate was not significantly different between participants stratified by mosaic level, chromosome structure, and chromosome content. The genotype and clinical outcome of each mosaic transfer are detailed Supplemental Table 1.

All karyotyping tests from amniocentesis were normal, and no congenital anomalies were found (Table 4). This implies that mosaic trophectoderms at the blastocyst stage might undergo self-correction or, at least, do not affect the chromosome status of the fetus. Healthy infants were born irrespective of the mosaicism level, chromosome structure, and content of transferred blastocysts. The clinical outcomes were not affected by these factors. Mosaic embryo transfers also resulted in euploid infants in our study.

Discussion

In PGT-A, trophectoderm cells that will develop into placental tissue are biopsied for testing. Gleicher and Orvieto [21] reported five essential assumptions supporting the hypothesis that PGT-A can eliminate aneuploid embryo transfer and improve IVF outcomes. Three of their assumptions were refuted by our data: (1) a single trophectoderm biopsy at the blastocyst stage was representative of the entire trophectoderm, (2) trophectoderm ploidy reliably represented the ICM (Inner Cell Mass), and (3) the ploidy did not change. Our results clearly demonstrated that mosaic embryos can develop into normal babies and that mosaic trophectoderm might not indicate the same genotype in the corresponding ICM. The mosaic trophectoderm ploidy may change or be corrected, leading to the development of a healthy euploid baby.

Their assumptions may hold true in cases of full aneuploidy (mosaicism level > 80%), but a gray area of chromosome complement identification (mosaicism level between 20% and 80%) exists in PGT-A results. In the present study, we analyzed the mosaicism level with trophectoderm biopsy by using an NGS platform. We used < 20% and > 80% levels to distinguish "mosaic embryos" from "euploid" and "aneuploid" following the technique of Munne et al. [10]. Classifying mosaic embryos into euploid or aneuploid is controversial because the association between mosaicism in trophectoderm cells and ICM cells remains unclear. Orvieto et al. reported different genotyping results of trophectoderm and ICM from the same embryo by using NGS [22].

Table 3 Clinical outcomes for mosaic embryos according to mosaicism type in the single embryo transfer group

	Cycles (no.)		Age	IR (*n*)	OPR (*n*)	AR (*n*)
Mosaicism total		83	37.0 ± 5.6	51.8% (43)	47.0% (39)	5.1% (2)
30%	Total	59	37.3 ± 5.8	52.5% (31)	47.5% (28)	3.6% (1)
	< 38 years	29	32.6 ± 3.0	48.3% (14)	48.3%(14)	0% (0)
	≧ 38 years	30	42.0 ± 3.7	56.7% (17)	46.7% (14)	7.1% (1)
40%	Total	24	35.9 ± 5.0	50.0% (12)	45.8% (11)	9.1% (1)
	< 38 years	13	33.0 ± 3.5	61.5% (8)	53.9% (7)	0% (0)
	≧ 38 years	11	40.4 ± 1.7	36.4% (4)	36.4% (4)	25.0% (1)
Mosaic type: mosaicism from different chromosome structures involved						
Whole	Total	11	34.2 ± 4.4	45.5% (5)	45.5% (5)	0% (0)
	< 38 years	9	32.6 ± 3.0	44.4% (4)	44.4% (4)	0% (0)
	≧ 38 years	2	41.0 ± 2.8	50.0% (1)	50.0% (1)	0% (0)
Segmental	total	60	37.4 ± 5.6	53.3% (32)	46.7% (28)	7.1% (2)
	< 38 years	27	32.3 ± 3.1	55.6% (15)	51.9% (14)	0% (0)
	≧ 38 years	33	41.5 ± 3.4	51.5% (17)	42.4% (14)	14.3% (2)
Complex	Total	12	37.4 ± 6.1	50.0% (6)	50.0% (6)	0% (0)
	< 38 years	6	32.6 ± 3.6	50.0% (3)	50.0% (3)	0% (0)
	≧ 38 years	6	42.2 ± 3.9	50.0% (3)	50.0% (3)	0% (0)
Mosaic type: no. of chromosomes involved						
1	Total	52	33.6 ± 3.3	46.1% (24)	42.3% (22)	4.6% (1)
	< 38 years	27	32.5 ± 3.2	51.9% (14)	48.2% (13)	0% (0)
	≧ 38 years	25	42.0 ± 3.6	40% (10)	36.0% (9)	11.1% (1)
2	Total	20	35.8 ± 4.7	65.0% (13)	55.0% (11)	0% (0)
	< 38 years	11	32.4 ± 3.3	54.6% (6)	54.6% (6)	0% (0)
	≧ 38 years	9	40.6 ± 1.8	77.8% (7)	55.6% (5)	0% (0)
> 2	Total	11	39.1 ± 6.1	54.6% (6)	54.6% (6)	16.7% (1)
	< 38 years	4	32.0 ± 3.7	50.0% (2)	50.0% (2)	0% (0)
	≧ 38 years	7	41.9 ± 3.7	57.1% (4)	57.1% (4)	25.0% (1)

No significant differences were observed in IR, OPR, or AR

IR implantation rate, *OPR* ongoing pregnancy, *AR* abortion rate

The present study provides some evidence that mosaic trophectoderm might change or be corrected during embryo development. Chromosomal mosaicism incidence, known to be significantly higher in IVF embryos than in later prenatal samples (chorionic villus sampling or amniocentesis), may contribute to errors in diagnosis [23, 24]. Euploid live birth from a mosaic embryo may result from abnormal cell apoptosis of mosaic trophectoderm cells during developmental processes or movement of abnormal ICM cells to the placenta. Birth weight depends on the elaborate interaction between maternal and fetal genotypes, placental function, maternal nutrition and lifestyle, and their effects on the epigenetic regulators of gene activity [25]. Confined placental mosaicism can also lead to clinically compelling intrauterine growth restriction or even intrauterine fetal death. By contrast, the clinical outcomes, including birth weight and gestational age at the delivery of euploid birth, were not significantly different from those for mosaic trophectoderm regardless of mosaicism level, chromosome structure, and number of mosaic chromosomes.

Women of more advanced age not only produce a high percentage of aneuploid embryos but also have a significantly lower pregnancy rate [26, 27]. A systematic review and meta-analysis of four randomized controlled trials (RCTs) indicated that PGT-A performed using the FISH or aCGH method to detect cleavage-stage embryos offered no benefits to AMA patients [28]. Although Orvieto et al. stated that until a proper, nonhypothetical RCT is conducted on its efficacy, PGT-A should be offered only under study conditions and with appropriate informed consent [29]. We did not design an RCT but offered counseling regarding PGT-A and mosaic embryo transfer data to couples undergoing IVF treatment. After a careful counseling process, we performed mosaic embryo transfer for couples without any euploidy embryos but at least one mosaic embryo available for PGT-A and embryo transfer; this indicated that the prognosis in such women may generally be better than that in those with AMA. Consequently, maternal age was not a major concern in this regard. Nonetheless, use of a high-resolution NGS platform to test the entire

Table 4 Comparison of obstetric outcomes of live birth from single euploidy or mosaic embryo transfers according to type of mosaicism

	Term development cycles (*n*)	Karyotyping normal % (*n*)	Congenital anomalies%*	Gestational weeks at delivery	Birth weight
Euploidy	120	100 (121)	0 (0)	38.4 ± 1.7	3146.2 ± 450.0
Mosaicism	36	100 (40)	0 (0)	38.3 ± 1.7	2997.7 ± 501.1
Mosaic type: low percentages of mosaicism					
30%	27	100 (29)	0 (0)	38.4 ± 1.6	3019.2 ± 520.3
40%	9	100 (11)	0 (0)	38.2 ± 1.8	2927.1 ± 461.0
Mosaic type: mosaicism from different chromosome structures involved					
Whole	5	100 (5)	0 (0)	39.4 ± 1.8	3280.0 ± 180.8
Segmental	25	100 (29)	0 (0)	37.9 ± 1.7	2951.0 ± 541.2
Complex	6	100 (6)	0 (0)	39.1 ± 0.9	3020.2 ± 462.5
Mosaic type: no. of chromosomes involved					
1	20	100 (24)	0 (0)	38.1 ± 1.8	2913.6 ± 463.6
2	11	100 (11)	0 (0)	38.8 ± 1.6	3102.3 ± 603.9
>2	5	100 (5)	0 (0)	38.0 ± 1.0	3189.0 ± 432.9

No significant differences were observed in any factor

*Congenital anomaly was defined as apparent defects in appearance, including nervous system, eye and face, cardiovascular disease, digestive system, urinary and renal system, muscle skeletal system, and chromosomal abnormalities

genome resulted in the avoidance of aneuploid embryo transfer and increased the pregnancy potential [30]. In the present study, no significant maternal age–related differences were observed in pregnancy or implantation rate after transferring a single euploid or mosaic blastocyst.

Kushnir et al. [31] reported that the degree of trophectoderm mosaicism was a poor predictor of ongoing pregnancy and miscarriage. They reanalyzed raw data from previous reports [10, 11] and did not discover significant predictability at any level of mosaicism between 20% and 80%. In our results, no significant difference in clinical embryo transfer outcomes between 30% and 40% mosaicism levels was observed. Thus, we propose that embryos with low mosaicism level (< 50%) can be considered for embryo transfers in PGT-A cycles and could result in a euploid live birth. Further research, with careful counseling, is nevertheless needed to explore the viability of embryos with high mosaicism level.

In the present study, clinical results were not different regardless of whether the transferred blastocysts had segmental or whole-chromosome mosaicism or 1, 2, or ≥ 2 mosaic chromosomes. By contrast, Victor suggested that for mosaic embryos, those with single-segmental types of mosaicism should be prioritized for transfer [31]. Munne et al. [10] reported that mosaicism type, structure, and chromosome number can affect implantation. The detectable segmental size involving loss or gain of chromosomal fragments in the present study was 10 Mb, which was the same as in the high-resolution data obtained by Munne et al. The resolution of VeriSeq NGS was validated by the manufacturer to be high enough to detect segmental (subchromosomal) aneuploidies of ≥ 20 Mb [32], although the detection of regions as small as 1.81 Mb has been reported using this platform [33]. The higher resolution not only reveals more abnormal segmental mosaicism but also causes more false-positive results.

In 2015, Gleicher et al. reported live birth following the transfer of mosaic and even aneuploid embryos [34]. The effect of mosaic chromosome abnormalities on ongoing pregnancy and spontaneous miscarriage was further examined [7]. The authors concluded that mosaic embryos have some capacity to produce normal infants. They discovered an unidentified mosaic embryo as one of the causes of early pregnancy loss after the transfer of embryos presumed to be euploid. No aneuploid ongoing pregnancy or birth has been reported following the transfer of mosaic embryos, but data on live birth and prenatal outcomes after mosaic embryo transfer remain lacking. Our study not only yielded results that are consistent with those of other studies regarding the lower implantation potential of mosaic embryos but also reported the largest number of live birth cases in the medical literature. Amniocentesis for karyotyping was performed in all patients, and no fetal chromosome anomalies were identified. The data indicate self-correction of trophectoderm mosaicism prior to amniocentesis. Another possibility is that the ICM cells were euploid even though the trophectoderm biopsy revealed mosaicism.

The potential for mosaicism is well documented even in natural pregnancy through data on sampling amniocentesis. In approximately 1–3% of pregnancies, although the fetus has normal chromosomes, the placenta either has a mosaic chromosome or is completely aneuploid [35]. Checking whether

the amniocentesis for a fetus is abnormal is vital during mosaic embryo implantation. Mosaic embryos help us understand the mechanism underlying fetal development of mosaicism.

Before the transfer of embryos with any type or level of mosaicism, patients should receive extensive genetics-related counseling to review the risks and benefits of the transfer of these embryos. The policy of prenatal screening for mosaic embryo transfer in our center included genetic karyotyping through amniocentesis, level 2 ultrasound for targeted fetal anatomical evaluation, and close monitoring of a pregnancy across all trimesters. Continued clinical assessment of infants into adolescence and adulthood is paramount.

A limitation of this study was its retrospective design. Therefore, this study was automatically biased because patients who underwent PGT-A were not necessarily identical to those who did not have their embryos tested. Second, we included patients with suspected infertility who were treated with PGT-A for analyzing mosaic embryos and compared all single mosaic embryo transfers with single euploidy embryo transfers. Therefore, we could not detect chromosomes of preimplantation embryos if couples did not provide consent for PGT-A. Chromosomal mosaicism is a relatively common finding in IVF-derived human embryos. Moreover, a significant difference in mosaicism among fertility clinics may also result from differences in the ART procedure [36, 37]. Mosaic embryo transfers raise concerns regarding pregnancy outcomes and have lower transfer priority than euploid embryos.

Varga et al. reported that PGT-A improved upon the conventional morphology-based selection in patients with AMA from 17.07 to 37.93% [38]. Sacchi et al. reported the effects of blastocyst-stage aneuploidy testing on clinical outcomes, and the pregnancy loss in their PGT-A and control groups was 3.6% and 22.6%, respectively [39]. In another study, no differences were noted in the miscarriage rate per number of clinical pregnancies irrespective of PGT-A: 14.3% versus 20.0% in the RPL group and 11.8% versus 0% in the RIF group [40]. In our study, a pregnancy loss of 12% with euploid embryos may appear slightly high, but most women had recurrent implantation failure and advanced age. We did not analyze the abortion rate with respect to infertility cause because some patients had complex infertility causes. Further research is warranted to elucidate the causes of infertility.

Our study indicated that embryos with low mosaicism level can develop into euploid healthy infants. These findings have implications for women who have only mosaic embryos and are valid clinical results. In our study, all fetuses derived from mosaic blastocyst transfer had normal karyotyping. This indicates that if PGT-A reveals (low level) mosaicism of embryos but no euploidy, the couple may consider choosing the mosaic embryos for transfer. Our findings are invaluable for understanding clinical results after SETs with mosaic embryos.

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