

Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing

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Objective: To determine the pregnancy outcome potential of mosaic embryos, detected by means of preimplantation genetic screening (PGS) with the use of next-generation sequencing (NGS).

Design: Retrospective study.

Setting: Genetics laboratories.

Patient(s): PGS cycles during which either mosaic or euploid embryos were replaced.

Intervention(s): Blastocysts were biopsied and processed with the use of NGS, followed by frozen embryo transfer. Trophectoderm (TE) biopsies were classified as mosaic if they had 20%–80% abnormal cells.

Main Outcome Measure(s): Implantation, miscarriage rates, and ongoing implantation rates (OIRs) were compared between euploid and types of mosaic blastocysts.

Result(s): Complex mosaic embryos had a significantly lower OIR (10%) than aneuploidy mosaic (50%), double aneuploidy mosaic (45%), and segmental mosaic (41%). There was a tendency for mosaics with 40%–80% abnormal cells to have a lower OIR than those with <40% (22% vs. 56%). However, few embryos ($n = 34$) with a mosaic error in 40%–80% of the TE sample were replaced. There was no difference between monosomic and trisomic mosaics or between entire chromosome mosaicism or segmental mosaicism. Implantation rates were significantly higher (70% vs. 53%), miscarriage rates lower (10% vs. 25%), and OIRs higher (63% vs. 40%) after euploid embryo transfer than after mosaic embryo transfer.

Conclusion(s): Forty-one percent of mosaic embryos produced an ongoing implantation. Complex mosaic blastocysts had a lower OIR than other mosaics. Mosaic monosomies performed as well as mosaic trisomies and mosaic segmental aneuploidies. The results suggest that embryos with >40% abnormal cells and those with multiple mosaic abnormalities (chaotic mosaics) are likely to have lower OIRs and should be given low transfer priority. (Fertil Steril® 2017;108:62–71. ©2017 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)).

Key Words: Mosaicism, PGD/PGS, next-generation sequencing, pregnancy outcome

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Mosaicism has been described in human embryos since the beginning of preimplantation genetic screening (PGS) for aneuploidy (1–4). Although meiotically derived aneuploidy was found to increase with advancing maternal age, there was no clear relationship with other embryologic factors, such as dysmorphism (defined as presence of multinucleation, fragmentation, and unevenness) (5, 6). Conversely, other types of chromosome abnormalities, such as mosaicism occurring due to post-zygotic malsegregation, polyploidy, and haploidy, were found to increase with dysmorphism, but not with maternal age, with the exception of aneuploid embryos carrying a combination of meiotic and mitotic (mosaic) errors (5,7–12). Whole cleavage-stage embryo analysis, with strict criteria to classify an embryo as mosaic if at least two of its cells are abnormal, showed that ~30% of them were mosaic (12). On the other hand, lower rates of mosaicism have been detected in blastocysts (13–17).

With the advent of molecular techniques (array comparative genome hybridization [aCGH], single-nucleotide polymorphism array, quantitative polymerase chain reaction [qPCR], next-generation sequencing [NGS]), the DNA of all of the cells in the biopsy is analyzed as a single entity and compared to a control DNA sample. This has therefore precluded the analysis of mosaicism. In addition, some of these techniques do not have enough sensitivity to detect the presence of mosaicism in a biopsied embryo sample. Array CGH, the most widely used of these methods, can not detect mosaicism with a great degree of accuracy. The software used for aCGH analysis (Bluefuse; Illumina) has not been validated for mosaicism detection, and a noisy profile or a mosaic profile could look alike. With perfect profiles, we would be comfortable detecting 40%–60% mosaicism. Below 40% we would classify it as normal and above 60% as abnormal. Recently Greco et al. (18) reported a 4.8% rate of mosaicism (mosaicism range 35%–50%), compared with 15%–37% with the use of fluorescence in situ hybridization (FISH) (15–17,19).

In the past few years another technique, NGS, was validated and then implemented for PGS (20–23). NGS has a higher dynamic range compared with aCGH, and it can detect the presence of ~20%–80% abnormal cells in a blastocyst biopsy (21, 24, 25). There are several different NGS platforms, and not all of them can detect mosaicism to the same extent, because they have different resolutions. The platform we use is based on Illumina's Veriseq NGS strategy (high-resolution NGS [hr-NGS]) can detect mosaicism when aneuploidy is present in 20%–80% of TE cells biopsied from a blastocyst. Considering that a blastocyst biopsy has on average 5–10 cells, we are able to detect mosaicism being present in 1/5 (20%) to 4/5 (80%) of the TE cells. With the use of hr-NGS, we reported 21% of embryos to contain euploid/aneuploid cell lines and ~10% aneuploid/aneuploid (but with different aneuploid cell lines) (26). These findings are similar to the historical data obtained with the use of FISH when analyzing all cells individually. In addition, and similarly to the FISH data, we did not observe an increase of mosaicism rates with advancing maternal age (26). This preliminary work demonstrated that hr-NGS is a much more sensitive method, compared with aCGH and can

accurately identify the presence of mosaicism (24, 27). Another NGS platform, CNV-Seq (23), can also detect 20%–80% mosaicism, but in that study only 13% of embryos were classified as mosaic. Lower-resolution NGS, such as Embryvu, has not reported the detection of mosaicism.

The ability of hr-NGS to accurately identify mosaicism in TE samples has led to significant disagreement and confusion regarding the biologic meaning of these findings. A recent investigation has even suggested that low-level mosaicism is indistinguishable from technical background noise (28). Another opinion paper (29) suggested that mosaicism detection is flawed, because if the same amount of trisomic and monosomic cells are present in a TE biopsy, then the average result will be of euploidy. However, a preliminary study (30) in which several biopsies from the same embryo were analyzed with the use of hr-NGS found that only two out of 28 embryos had monosomic and trisomic cell lines for the same chromosome abnormality, suggesting that this event is rare, because by the time the embryo reaches the blastocyst stage one of the abnormal cell lines has taken over, or compartmentalization is unlikely to produce that result.

There is also scant information on the clinical implications of replacing embryos classified as mosaic by means of hr-NGS. After replacing 44 mosaic blastocysts classified as such by means of hr-NGS but euploid by means of aCGH, 38% (17/44) implanted but 29% (5/17) of those miscarried, compared with 57% (29/51) implanting and 24% (5/29) miscarried for a well matched control euploid embryo group (27). This is similar to 38% implantation rate after replacing mosaic embryos identified by means of aCGH (18). The difference between these two studies is that hr-NGS detects many more mosaic blastocysts than aCGH (29% vs. 5%).

In another study, reanalysis by means of hr-NGS of surplus DNA from embryos classified as euploid by means of aCGH that ended up in miscarriage showed that 46% were euploid and the rest mosaic or polyploid (31). Using the same approach, we also found significantly more mosaic embryos in lost pregnancies than in ongoing pregnancies ($P=.0062$) (24). The miscarriage rate after replacing embryos classified as euploid by means of NGS has been determined to be 6%, compared with 13% for aCGH (32) and 20% for qPCR (33).

Thus the evidence suggests that embryos classified as being mosaic by means of NGS miscarry more and implant less, but ~40% of them can still result in a viable pregnancy. To date there is little information about which of these NGS mosaics have the highest chance to make a viable pregnancy. Therefore the purpose of the present study was to retrospectively analyze the pregnancy outcomes of replaced mosaic embryos and compare them to the type of mosaicism seen according to several different criteria, such as percentage of abnormal cells, chromosome abnormality type (segmental, trisomy, monosomy, complex abnormal), and chromosomes involved.

METHODS

Patients and Embryos

This study included patients that received transfer of mosaic embryos only and for which the clinical outcome was known. Forty-four of these embryos (series 1) were included in a

previous investigation by Fragouli et al. (27): 10 were double-embryo transfers (DETs) and 34 single-embryo transfers (SETs). Of the DETs, one transfer resulted in both mosaic embryos producing ongoing pregnancies, and the other four transfers did not lead to a pregnancy. The other 99 embryos (series 2), were generated in three different centers; these embryos were all replaced in SETs.

Comparison Group

The four centers participating in the study were asked to provide control groups of transferred euploid embryos, which were previously analyzed by means of PGS with the use of hr-NGS. The patients generating the control-group embryos were well matched for female age to the patients generating the mosaic embryos. Patients in test and control groups were undergoing IVF treatment in combination with PGS during the same period of time with the test group patients.

Determination of Mosaicism Detection and Types of Mosaic Blastocysts

The platform that we used during this study, which we are characterizing as hr-NGS, was the Veriseq NGS (Illumina). The Veriseq NGS strategy interrogates ~24 million sequence reads, so with a loss of 30%–40% reads per run, and analyzing 24 samples per experiment, it provides 600,000–900,000 reads per sample and can detect the presence of mosaicism with a resolution of 20%–80% in a biopsied sample. The cytogenetic analysis of biopsied samples was performed with the Bluefuse Multi v3 software for NGS, which provided copy number counts for each chromosome pair. Although it is true that the software will automatically highlight abnormal chromosomes when such errors are present in >50% of the TE sample, internal validation demonstrates a technical accuracy down to 10% resolution. Therefore, diagnostic decisions were made based on specific copy number variations. A chromosome with two copies received a value equal to 2 (euploid), a chromosome with one copy (monosomy) received a value of 1, and a chromosome with three copies (trisomic) received a value of 3. Values between these numbers were considered to represent mosaic abnormalities, with samples with values between 1 and 2 being considered mosaic monosomies, and those between 2 and 3 mosaic trisomies. An embryo biopsy with a segmental (or partial) aneuploidy had either a piece of a chromosome having a value of 1 (partial monosomy) or of 3 (partial trisomy). The Veriseq NGS technique can detect extra or missing chromosome segments as small as 1.5 Mb in size. Values for segmental abnormalities between 1 and 2 were classified as mosaic partial monosomies and those between 2 and 3 as mosaic partial trisomies (combined we call them mosaic segmental). TE samples that had two mosaic abnormal chromosomes were classified as double mosaic (regardless of whether these errors were a combination of two whole-chromosome events, two segmental events, or one segmental and one whole-chromosome event), and those with three or more mosaic chromosomes were characterized as complex mosaic.

Using the numeric values given by the software, which have been validated first through mixing experiments

(see below), we were able to determine the percentage of abnormal cells in the TE biopsies analyzed.

The Reprogenetics position is that mosaicism <20% or >80% can not be differentiated from technical noise, therefore, <20% mosaics should be classified as euploid and >80% as aneuploid. For this study we grouped embryos in 20%–40% and >40%–80% groups to determine if low and high rates of abnormal cells have an impact on ongoing implantation rates (OIRs).

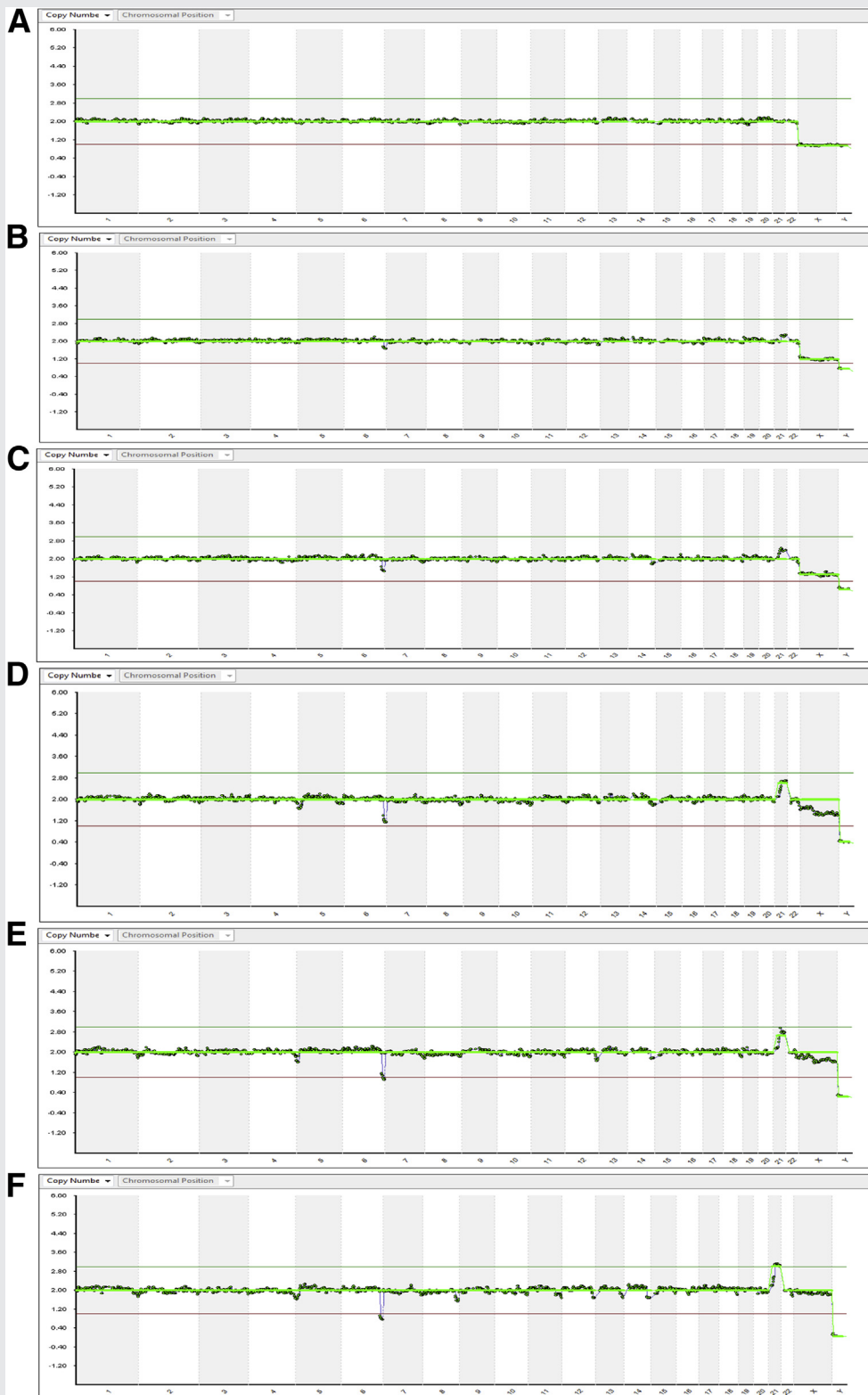
We used our electronic medical records database (eIVF; Practicehwy.com), which provided us with some of the follow-up information obtained for the study, and the rest was provided by direct feedback from the centers not using eIVF.

Samples for centers 1 and 2 were processed by Reprogenetics (US Labs), samples for center 3 by Genesis Genetics, and samples for center 4 (series 1) by Reprogenetics UK. All three companies are subsidiaries of Cooper Genomics.

Mixing Experiments for Validation of Mosaicism

We recently published a series of dilution experiments involving the mixture of euploid and aneuploid cell lines (24, 27). The aneuploid cells were isolated from fibroblast cell lines obtained from the National Institute of General Medical Sciences Human Genetic Cell Repository at the Coriell Institute for Medical Research (USA). These were combined with euploid male (46,XY) lymphocytes in different ratios, i.e., 0 aneuploid cells and 5 euploid cells, 1 aneuploid cell and 4 euploid cells (20% abnormal), 2 aneuploid cells and 3 euploid cells (40% abnormal), 3 aneuploid cells and 2 euploid cells (60% abnormal), 4 aneuploid cells and 1 euploid cell (80% abnormal), and 5 aneuploid cells (100% abnormal). Gains affecting entire chromosomes were examined with the use of two male cell lines with trisomy 13 (47,XY,+13: GM00526) and trisomy 18 (47,XY,+18: GM01359) and an XXY trisomy 21 cell line (48,XXY,+21: GM04965). Losses affecting entire chromosomes were examined with the use of a 45,X0 cell line (45,X0: GM00857). Partial chromosome losses and gains were examined with the use of two male and one female cell lines carrying a partial loss of 10p [46,XY,del(10)(p14p12): GM03047], a partial loss of 13q [46,XX,del13(pter>q14): GM00509], and an unbalanced translocation between chromosomes 21 and X [46,XX,der(21)(21qter>21p11::Xqter): GM01730]. The mixing experiments were repeated three times, and each time a different amplified DNA product resulting from mixing normal and aneuploid cells was investigated. The aneuploid cell lines carrying the segmental errors affecting chromosome 13 (GM00509) and chromosome X (GM01730) did not grow very well in culture. We were therefore able to analyze samples isolated from these two cell lines only once. In addition, DNA amplified from embryos with four different structural abnormalities, 46,XX; del(6)(q25.3-qter), dup(21)(q21.2-qter), 46,XX; dup(14)(q31.3-qter), del(18)(q22.1-qter), 46,XY; del(14)(q31.3-qter), dup(18)(q22.1-qter), and 46,XY; dup(13)(pter-q22.3), del(18)(pter-q12.2), were mixed in the same above ratios with euploid DNA in quantities equivalent to 5 cells. Figure 1 shows different ratios of 46,XY to 46,XX; del(6)(q25.3-qter), dup(21)(q21.2-qter) cells.

FIGURE 1



Ratio of 46,XY to 46,XX; del(6)(q25.3-qter), dup(21)(q21.2-qter) cells: (A) 5:0 (100%:0%), (B) 4:1 (80%:20%), (C) 3:2 (60%:40%), (D) 2:3 (40%:60%), (E) 1:4 (20%:80%), (F) 0:5 (0%:100%).

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.

TABLE 1

Center comparison of mosaic and control embryos.

Parameter	Center 1		Center 2		Center 3		Center 4 (series 1)		Total		Difference among centers
	Mosaic	Euploid	Mosaic	Euploid	Mosaic	Euploid	Mosaic	Euploid	Mosaic	Euploid	
Age (y)	37.7	35.8	39.0	39.2	34.1	35.2	37.3	37.9	35.8	37.3	
Embryos replaced, n	13	201	11	478	75	315	44	51	143	1,045	
Implanted, n	5	142	9	355	47	210	15	29	76	736	
Implantation rate, %	38	71	82	74	63	67	34	57	53	70	
P value	NS		NS		NS		NS		NS		NS
Embryos lost, n	4	11	2	28	10	31	3	5	19	75	
Fetal loss rate, %	80	8%	22	8	21	15	20	17	25	10	
P value	.004		NS		NS		NS		.002		NS
Ongoing implantations, n	1	131	7	327	37	179	12	24	57	661	
Ongoing implantation rate, %	8	65	64	68	49	57	27	47	40	63	
P value	.02		NS		NS		NS		.006		NS

Note: NS = not significant.

Munné. *Pregnancy outcome of mosaic blastocysts. Fertil Steril 2017.*

Statistical Analysis

The variables of interest were the implantation rate (IR; embryos implanting/embryos replaced), with implantation defined as the presence of a fetal sac (detected during the 6–10-week ultrasound), fetal loss rate (FLR; embryos lost/embryos implanting), and OIR (IR – FLR).

Two types of statistical analyses were performed. A global one was used to determine which variables were globally significant to explain IR (with value of 1 if embryo resulted in a pregnancy, 0 if not), FLR (with value of 1 if embryo resulted in a fetal loss, 0 if not), and OIR (with value of 1 if embryo resulted in an

ongoing pregnancy, 0 if not). Each one of these variables was fitted to a general linear mixed model (GLMM) with a logit link function to study the effect of the center random factor and all the fixed effects considered in the study, such as maternal age (x_1), highest mosaic percentage (x_2), mosaic group (x_3), and percentage of abnormal cells (x_4). After checking that the effect of this random variable was not significant, there was no need of creating a GLMM model, so we proceeded to adjust the data with the use of a simpler logistic model. This model included only the aforementioned fixed effects. The analytic expression of the model for OIR variable was:

TABLE 2

Pregnancy outcome of mosaic embryos according to mosaicism type, percentage abnormal cells, and chromosomes involved.

Mosaic type	% Abnormal	No. of cycles	Implanted		Lost		Ongoing		P value
			n	%	n	%	n	%	
Complex	20–40	17	2	12	0	0	2	12	NS
	>40–80	4	0	0	0	0	0		
Double	20–40	22	11	50	2	18	9	41	NS
	>40–80	7	5	71	1	20	4	57	
Monosomic	20–40	28	20	71	6	30	14	50	NS
	>40–80	6	2	33	0	0	2	33	
Trisomic	20–40	17	11	65	0	0	11	65	NS
	>40–80	3	1	33	1	100	0	0	
Segmental	20–40	25	17	68	7	41	10	40	NS
	>40–80	14	7	50	1	14	6	43	
	20–40, all	109	61	56	15	25	46	42	
Single aneuploid	>40–80, all	34	15	44	3	20	12	35	.059
	20–40	45	31	69	6	19	25	56	
	>40–80	9	3	33	1	33	2	22	
Complex, all		21	2	10	0	0	2	10	<.005
Double, all		29	16	55	3	19	13	45	
Aneuploid, all		54	34	63	7	21	27	50	
Segmental, all		39	24	62	8	33	16	41	
No. of chromosomes involved									<.005
1		93	58	62	15	26	43	46	
2		29	16	55	3	19	13	45	
≥3		21	2	10	0	0	2	10	
Total		143	76	53	18	24	58	41	

Note: NS = not significant.

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TABLE 3

Chromosome abnormalities detected by means of next-generation sequencing by Reprogenetics (US Labs).

Abnormality	n (%)
Euploid	12,516 (42.87)
Aneuploid	5,751 (19.70)
Complex abnormal	3,477 (11.91)
Triploid	246 (0.84)
Full segmental	837 (2.87)
Mosaic aneuploid	2,789 (9.55)
Mosaic segmental	2,143 (7.34)
Complex mosaic	1,436 (4.92)
Total embryos	29,195

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$$\text{logit}(P(OIR = 1)) = \log\left(\frac{P(OIR = 1)}{P(OIR = 0)}\right) = \alpha + \sum_{i=1}^4 \beta_i \cdot x_i$$

Where α is the interception and β_i the coefficient for each fixed variable x_i .

Analogous models were estimated for the other two dependent variables, IR and FLR.

A second statistical analysis consisted of a single-variable analysis to study differences at different scales. This single-variable study was performed with the use of chi-square and analysis of variance (ANOVA) when it was needed.

Institutional Review Board and Consenting of Patients

Patients undergoing PGS in the United States signed informed consents stating the risks of replacing embryos with mosaicism. For purposes of PGS analysis of collected and deidentified data, we used the Aspire IRB HIPAA waiver of authorization protocol PGSP 2015. Patients undergoing PGS at Reprogenetics UK were consented as described in Fragouli et al. (27).

Patients included in this study had consented to PGS with the use of aCGH or NGS. We considered the study to be exempt from Institutional Review Board approval. According to the common rule 45 CFR 46.101(b) (4), exemptions include “research involving the collection or study of existing data, documents, records, pathologic specimens if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects can not be identified, directly or through identifiers linked to subjects.”

A retrospective chart review was performed with the use of a secure electronic medical record (eIVF) of patients with embryos that underwent PGS.

RESULTS

Mixing Experiments

Supplemental Table 1 presents the results of mixing aneuploid and euploid cells in groups of 5:0 cells (100% abnormal), 4:1 cells (80%), 3:2 cells (60%), 2:3 cells (40%), and 1:4 cells (20%) to mimic diploid-aneuploid mosaicism.

(Supplemental Tables 1–5 are available online at www.fertstert.org.) The results obtained matched the expected ratios and provided an SD. These experiments were further complemented with DNA equivalent of 5 cells mixed in the same ratios of euploid and a structural abnormality, as presented in Supplemental Table 2. Again, the experiments detected ratios similar to what would be expected from these ratios.

Clinical Outcomes

A total of 143 mosaic embryos from six centers. The participating patients had an average maternal age of 35.8 years. The outcome of each embryo, cytogenetic results, and other pertinent information is provided in the Supplemental Table 3. Overall, they resulted in a 53% (76/143) IR, 24% (18/76) FLR, and 41% (58/143) OIR.

These results are compared in Table 1 with a set of matched controls. Overall, the IR seemed to be lower for mosaic compared with euploid embryos (53% vs. 71%) but this difference was not significant ($P > .05$). However, the FLR was significantly higher (24% vs. 10%; $P = .002$) and OIR lower (41% vs. 63%; $P < .006$) after the transfer of mosaic embryos.

The pregnancy outcome results of replaced mosaic embryos were further stratified according to type of mosaic abnormality scored, number of chromosomes involved, percentage of abnormal cells, and chromosome types involved. Table 2 summarizes those results. In summary, only complex abnormal mosaics, those with three or more chromosomes involved, had a significantly ($P < .001$) reduced OIR (10%), compared with mosaic single aneuploid (mosaic trisomic + mosaic monosomic: 50%), double mosaic (45%), and mosaic segmental (41%).

As a reference, there were 4.92% complex abnormal embryos in the Reprogenetics (US Labs) database of NGS-analyzed embryos (Table 3).

Within each of the mosaic type categories, there was no significant difference between embryos with 20%–40% abnormal cells and embryos with >40% abnormal cells. Overall, embryos with 20%–40% abnormal cells had an OIR of 42% and those with >40% abnormal cells had an OIR of 35%, but owing to the small sample size these differences were not significant ($P = .1$). Similarly, there was no difference between mosaic monosomy or mosaic trisomy regarding OIR. However, complex mosaics had a significantly lower OIR (10%) than the other mosaic embryos ($P < .005$). We did not see significant differences between single-chromosome mosaic embryos (46% OIR) and double-chromosome mosaic embryos (45% OIR).

Because there was no difference between embryos with a single mosaic monosomy or a single mosaic trisomy, we analyzed the subgroup of aneuploid mosaic embryos as a whole, subdividing it into those with 20%–40% abnormal cells, which had an OIR of 56%, and those with >40% abnormal cells, which had an OIR of 22%, a nonsignificant difference ($P = .142$) owing to the small size of the >40% group.

Regarding the chromosome number involved (Supplemental Table 4), we grouped chromosomes 1–12 in

one group and 13–22 in another, based on chromosome size. The OIR was similar, 45% and 43%, respectively. The only difference was that chromosomes 1–12 involved more segmental mosaic events (56%) than the smaller chromosomes (21%; $P < .0001$).

Of the chromosome abnormalities that could reach term as full aneuploidies (chromosomes 13, 18, 21, X/Y) or be involved in uniparental disomy (UPD; chromosomes 7, 14, 15), few trisomies or monosomy X were replaced (Supplemental Table 4): Three monosomy X mosaics were replaced, resulting in one ongoing and two miscarried pregnancies; three monosomy 21 mosaics and one trisomy 21 mosaic were replaced, resulting in two lost pregnancies; two monosomy 13 mosaics resulted in one ongoing and one miscarried pregnancy, four monosomy 18 mosaics resulted in two ongoing pregnancies; one monosomy 7 mosaic resulted in an ongoing pregnancy; two monosomy 15 mosaics resulted in one ongoing pregnancy, two monosomy 14 mosaics resulted in one ongoing pregnancy; and one trisomy 14 mosaic resulted in an ongoing pregnancy (Supplemental Table 3). Unfortunately we were unable to collect karyotyping information on the miscarriages, ongoing pregnancies, or live-born babies.

We also analyzed differences among the four centers. Table 1 shows that there were no significant differences seen between these four centers regarding IR, FLR, and OIR. This is in agreement with the results of the GLMM model including the variable center as a random factor which turned out to be nonsignificant ($P = .877$).

Maternal age was not related to the percentage of abnormal cells, with embryos with 20%–40% abnormal cells having an average maternal age of 36.6 years and embryos with >40% abnormal cells having an average age of 35.7 years. Embryos with one chromosome abnormality had an average age of 35.4 years, those with two 36.1 years, and those with three 36.8 years, but these differences were not statistically significant ($P = .915$; ANOVA).

The multivariable analysis led to the conclusion that only the mosaic type variable is significant for explaining IR ($P = .013$). However, none of the fixed effects that were considered turned out to be significant for explaining the dependent variable OIR, except a new dichotomic variable x_5 that simply specifies if a mosaic is complex ($x_5 = 1$) or not ($x_5 = 0$). This added variable turns out to be the only significant variable in the logistic model for OIR ($P = .009$). It would be interesting to perform this analysis again when we have more data, because, although the mosaicism type is not significant as far as explaining OIR is concerned, it currently shows a slight tendency ($P = .068$) that led us to postulate about a possible effect of the mosaicism type. However, as already mentioned, the data are insufficient at this stage to draw more definite conclusions. As far as the FLR is concerned, no logistic model was obtained owing to the small number of samples with FLR = 1. Supplemental Table 5 presents the estimated coefficients for each level of the significant independent variables in the logistic model. As we can see in that table, the odds of OIR for a sample classified as noncomplex is 7.3 times larger than for another sample classified as complex. As far as the IR is concerned, we can conclude from Supplemental Table 5 that implantation is 17.4 times more probable for an

embryo with a mosaic monosomy than for a complex one. Likewise, embryos with two or more errors, as well as partial mosaics, are, respectively, 11.7, 14.3 and 9.0 times more likely to implant than a complex ones.

DISCUSSION

There is limited evidence about the developmental fate and implantation ability of mosaic embryos. Recent studies using comprehensive chromosome screening methodology indicate that they miscarry more (24, 27, 31) and implant less (27), but ~40% of them can still result in a viable pregnancy (18, 27). Our investigation of a much larger data set supports earlier observations that 41% of mosaic embryos can result in ongoing pregnancy if they are transferred. Additionally, we demonstrate for the first time that the implantation potential differs markedly among the various types of mosaic blastocysts. Specifically, our results indicate that embryos that have multiple mosaic errors (complex mosaic) implant significantly less than embryos with one or two chromosomes in mosaic form.

The observation that complex mosaic embryos result in very few ongoing pregnancies coincides with preliminary data from another study in which mosaic blastocysts were dissected into inner cell mass (ICM) and several different TE parts (30). The results showed that complex mosaic embryos had very few euploid ICM cells, whereas the rest of the embryos had ~40% normal ICMs. In contrast, fully abnormal nonmosaic embryos had no normal ICMs (30). Of all the embryos in the Reprogenetics (US Labs) database, 4.5% were characterized as complex mosaic. Such embryos are likely to have a very low chance of reaching term.

Another important observation of this study is that mosaic monosomies and mosaic trisomies have similar OIRs. This particular finding suggests that the recently published Preimplantation Genetic Diagnosis International Society (PGDIS) guidelines recommending that “embryos showing mosaic euploid/monosomic embryos are preferable to euploid/trisomic ones, given that monosomic embryos (except 45, X) are not viable” (34) should be changed. Although anaphase lag could be a mechanism of mitotic mosaicism, early FISH mechanistic studies on mosaic embryos showed that only 5% (28/556) of aneuploid mosaics were caused by anaphase lag; the rest were due to nondisjunction (8). This means that most aneuploid mosaic embryos start with normal, monosomic, and trisomic cell lines, and either one of them is sampled more often or one of the cell lines proliferates more than the others. According to the dissection experiments from Garrisi et al. (30), the later mechanism is probably more common, because only one of the embryos dissected contained two complementing mosaic lines (a monosomic and a trisomic one) whereas the others had only one line per mosaic chromosome.

Experiments performed on mice demonstrated that some cell lines grow faster and displace the slower ones (35). In that mosaic model, highly abnormal cells (i.e., equivalent to complex mosaic embryos) were combined in 1:1 and 1:3 ratios. In those with a 1:1 ratio, the abnormal cells developed slower, and the normal cells eventually took over and produced

normal offspring. Those with a 1:3 ratio of normal:abnormal cells produced fewer pups (35). These experiments suggest that abnormal cells are not capable of self-correcting but have a tendency to divide more slowly than normal cells, and the normal cells, if the proportion is large enough, take over. In addition, these experiments showed that the distribution of abnormal cells was not clonal, but that they distributed evenly and allocated to both TE and ICM (35). One would expect similar results in the human and a correlation between abnormal cell load in mosaic embryos and OIRs. However, owing to our small sample size, we were unable to see a correlation between percentage of abnormal cells and OIR ($P=.14$). A large number of abnormal cells present in the entire embryo is likely to be indicative of a lower chance of ongoing implantation. It is unknown, however, if a large number of abnormal cells in a single 5–10-cell TE biopsy can accurately represent the chromosome constitution of the remaining of the embryo. This could explain why some embryos with >40% mosaic errors in the TE biopsies are capable of implanting.

Our study also clarified that there is no correlation between the type of chromosome affected by mosaicism and OIRs. Almost all chromosome types in mosaic form were compatible with ongoing pregnancy (except chromosome 17), and large chromosomes had an OIR identical to small chromosomes. It should be noted that large chromosomes had significantly more segmental mosaic abnormalities ($P<.0001$) than smaller ones. This was expected, because chromosome size could play a role in the mechanisms leading to breakage and segmental anomalies (36).

Of the chromosome abnormalities that could reach term as full aneuploidies (chromosomes 13, 18, 21, X/Y) or be involved in UPD (chromosomes 7, 14, 15), few trisomies were replaced, probably because of the associated risk. Specifically, embryos carrying 19 monosomies and four trisomies for those chromosomes were replaced resulting in nine ongoing pregnancies and three miscarriages. Follow-up for these pregnancies is continuing.

The multivariate analysis confirmed that female age had no influence on OIR. This is consistent with observations from the replacement of chromosomally normal embryos in which IR and FLR were not affected by advancing maternal age (37).

On the other hand, mosaicism rates could be fertility center related. We previously reported that mosaicism can be caused by different culture conditions (38). More recently it has been postulated that changes in culture media can lead to different rates of mosaicism (39). Indeed, mosaicism rates in egg donors, a good group by which to compare centers, varied greatly from 17% to 47% ($P<.001$) (40). However, the referring fertility center was not identified as a variable that affects pregnancy outcome. The differences that were observed between centers were due to other variables, such as the type of mosaicism and the percentage of abnormal cells (in the case of implantation).

Study Limitations

There are several limitations to this study. First, it was not sufficiently powered to detect differences between some

subgroups of mosaic embryos. We are in the process of collecting more data, which will enable us to provide a more nuanced analysis of the potential pregnancy outcome of different mosaic embryo types.

Approximately 20% of blastocysts are characterized as mosaic after NGS (26). Moreover, in their investigation, Maxwell et al. (24) observed that 9% of all transferred blastocysts leading to ongoing pregnancies were mosaic. These observations are similar to our data ($20\% \times 41\% \text{ OIR} = 8\%$ of ongoing pregnancies originating from a mosaic embryo). The proportion of mosaic blastocyst stage embryos is five times higher than that observed in chronic villus sampling (CVS; 1.84%) (41). However, CVS analysis does not routinely take place with methodology as sensitive as NGS (42). Moreover, we were not able to determine if and how, similarly to the mouse data (35), the aneuploid cell line resolves itself in a mosaic blastocyst, which leads to a normal ongoing pregnancy.

Mosaicism in fetuses and babies can result in congenital abnormalities, autism, and mental retardation. Every chromosome in a mosaic form has been described to be associated with an abnormal phenotype. The spectrum of these phenotypes ranges from normal development to severely affected, and genotype-phenotype correlations are difficult to be established. This is further complicated by different rates of abnormal cells between mosaic individuals as well as tissues affected (36).

A critical, yet unresolved, question is whether preimplantation mosaicism results in a higher chance of congenital abnormalities and affected children. To determine if replacing mosaic blastocysts would result in a higher frequency than the <2% of mosaic fetuses observed by means of CVS, >700 pregnancies should be karyotyped. So far, data from <200 ongoing pregnancies after replacement of mosaic embryos has been collected (18, 27). Unfortunately, this information could not be collected for the present study, either because some of the centers did not report mosaicism (center 3), the data was based on reanalysis with the use of NGS of biopsied embryos after previous analysis by means of aCGH and the embryos were deidentified (series 1), or the majority of patients had not yet delivered, did not undergo amniocentesis, or were lost to follow-up.

Ideally, all ongoing pregnancies and babies resulting after the transfer of mosaic embryos should be karyotyped and followed for years through the child's development. For this reason, we recommend, as well as the guidelines of PGDIS (34) and Controversies in Preconception, Preimplantation, and Prenatal Genetic Diagnosis (CoGen) (43), that prenatal diagnosis with the use of amniocentesis is performed in pregnancies resulting from the transfer of mosaic embryos. Noninvasive prenatal testing and CVS are inadequate because they test the TE (the placenta) and not the ICM (the fetus). Nevertheless, even a normal amniocentesis does not rule out mosaicism in untested tissue types.

Recommendations for Replacement of Mosaic Embryos

Some laboratories are using an artificial cutoff to classify all mosaicism events as normal or abnormal (usually 40% or 50%

abnormal cells). In our opinion, this is likely to cause false positives (discarding potentially viable embryos) and false negatives (resulting in misdiagnoses and potential associated complications). Instead, our opinion, first expressed earlier (25) and then incorporated in the PGDIS (34) and CoGen (43) guidelines and supported by others (44), is that mosaics need to be classified as a third category and deprioritized for transfer after euploid embryos. Depending on the needs of the patient, they could be considered for transfer if there are no euploid embryos available and the patient is counseled about the risks. This decision is patient dependent, because it may be less appropriate, for example, for recurrent pregnancy loss patients (higher risk of miscarriage) and patients not wishing to do another PGS cycle.

The present study corrects some of the recommendations recently published (34, 43, 44) in that there is no difference in implantation rates after transferring trisomic, monosomic or segmental mosaics. A notably poorer clinical outcome was observed only with the transfer of complex mosaic embryos, which have very little potential to implant. The rest of the recommendations stand, such as deprioritizing their transfer after euploid embryos, classifying them as mosaic and not as euploid or aneuploid, and prioritizing embryos with 20%–40% mosaicism over those with >40% abnormal cells. Complex mosaic embryos could potentially be considered for transfer in cases of women of advanced age (>40 years) with a very poor reproductive history. Transfer of complex mosaic embryos may give such patients a small (10% in our data set) chance of achieving an ongoing pregnancy. As already mentioned, a transfer of a mosaic embryo (simple or complex mosaic) should always take place after detailed counseling to ensure that the patient is aware of and understands all associated risks.

The rest of the guidelines focus on which chromosomes affected by mosaicism should be avoided for transfer, coinciding with those at risk of being incompatible with life in pure aneuploid form (chromosomes X, Y, 13, 18, 21), UPD (chromosomes 7, 14, 15), or intrauterine growth restriction (chromosome 16). Currently, however, there is no clear link established between mosaicism at the blastocyst stage and mosaicism seen during prenatal diagnosis, with some evidence showing that the two are the result of independent mechanisms (35, 45). Therefore, these recommendations might be shown in the future to be too conservative. The ever increasing use of NGS for the purposes of PGS along with appropriate data collection and pregnancy follow-up will hopefully provide a more detailed insight in the developmental ability of mosaic blastocysts.

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SUPPLEMENTAL TABLE 1**Dilution experiments to validate detection of mosaicism by hr-NGS.****Average chromosome copy number**

Ratio aneuploid: euploid cells (% abnormal)	Trisomy 13 + euploid cells (N = 3)/SD	Trisomy 18 + euploid cells (N = 3)/SD	45XO + euploid cells (N = 3)/SD	Trisomy 21 + euploid cells (N = 3)/SD	Trisomy XXY + euploid cells (N = 3)/SD	-10p14-10p21 euploid cells (N = 3)/SD
5:0 (100%)	3.05/0.03	3.02/0.05	0.99/0.01	2.94/0.05	1.96/0.04	1.12/0.03
4:1 (80%)	2.93/0.06	2.78/0.05	1.11/0.02	2.71/0.07	1.84/0.05	1.21/0.02
3:2 (60%)	2.78/0.07	2.65/0.09	1.32/0.1	2.66/0.07	1.66/0.12	1.39/0.05
2:3 (40%)	2.45/0.05	2.41/0.05	1.6/0.02	2.43/0.04	1.52/0.1	1.57
1:4 (20%)	2.3/0.02	2.2/0.01	1.76/0.03	2.16/0.3	1.22/0.01	1.89/0.18

Note: N = number of experiments.

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.

SUPPLEMENTAL TABLE 2

Dilution experiments to validate detection of mosaicism by hr-NGS.

Euploid	Translocation	%	Fragment size (MB)		% Mosaicism	
			del(6)(q25.3-qter)	dup(21)(q21.2-qter)	del(6)(q25.3-qter)	dup(21)(q21.2-qter)
46,XY	46,XX; del(6)(q25.3-qter), dup(21)(q21.2-qter)	0	0	0	0	0
		20	11	21	35	25
		40	11	22	50	35
		60	12	19	85	65
		80	12	23	95	75
100	12	21	100	100		
Euploid	Translocation	%	dup(14)(q31.3-qter)	del(18)(q22.1-qter)	dup(14)(q31.3-qter)	del(18)(q22.1-qter)
46,XY	46,XX; dup(14)(q31.3-qter), del(18)(q22.1-qter)	0	0	0	0	0
		20	0	12	0	8
		40	20	12	35	40
		60	19	12	50	70
		80	19	13	65	80
100	19	12	70	100		
Euploid	Translocation	%	del(14)(q31.3-qter)	dup(18)(q22.1-qter)	del(14)(q31.3-qter)	dup(18)(q22.1-qter)
46,XX	46,XY; del(14)(q31.3-qter), dup(18)(q22.1-qter)	0	0	0	0	0
		20	17	12	20	20
		40	18	13	40	25
		60	20	12	65	30
		80	19	12	80	65
100	19	12	100	80		
Euploid	Translocation	%	dup(13)(pter-q22.3)	del(18)(pter-q12.2)	dup(13)(pter-q22.3)	del(18)(pter-q12.2)
46,XX	46,XY; dup(13)(pter-q22.3), del(18)(pter-q12.2)	0	0	0	0	0
		20	51	30	25	25
		40	53	29	50	35
		60	53	28	60	50
		80	53	28	90	80
100	54	28	100	100		
Total		%			Average	Standard Dev
		0			0.0	0.0
		20			19.0	11.6
		40			37.1	7.6
		60			59.3	17.7
		80			77.1	10.4
		100			92.9	12.5

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.

SUPPLEMENTAL TABLE 3

List of each embryo characteristics.

Embryo #	Center	Maternal age, y	Mosaicism description	% Mosaic	Mosaic type	Pregnancy outcome
1	3	35	Mos. Mono. 2 (20%), Mos. Tris. 5 (40%), Mos. Tris 20 (45%)	45	Complex	No pregnancy
2	4	34	Mos. partial Tris. 5q (20%) / Mos. Tris. 14 (50%) / Mos. partial Mono. 10q (20%)	50	Complex	No pregnancy
3	3	29	Mos. Tris. 4 (50%), Mos. Tris. 6 (40%), Mos. Mono 11 (35%), Mos Tris. 20 (35%)	50	Complex	No pregnancy
4	4	40	Mos partial Tris. 10q (50%) / Mos. partial Mono. 1p (20%) / Mos. partial Mono. 4p (20%)	50	Complex	No pregnancy
5	4	33	Mos. Trisomies 7/ Mos. Tris 15 / Mos. Mono. 18	20	Complex	No pregnancy
6	4	40	Mos. partial mono 20p / Mos. Mono. 14 / Mos. Mono. 21	20	Complex	No pregnancy
7	4	36	Mos. monosomy 5 / Mos. partial monoso 17q / Mos. Partial mono. 19q	20	Complex	No pregnancy
8	4	40	Mos. partial trisomy 17q / Mos. Mono. 15 / Mos. Mono. 21	20	Complex	No pregnancy
9	4	36	Mos. Mono 2 (20%) / Mos. partial Mono. 8p(20%) / Mos. Partial Mono. 9p (30%)	30	Complex	No pregnancy
10	4	36	Mos. Tris. 5 (20%) / Mos. Tris 22 (20%) / Mos Tris 17 (20%) / mos Tris 21 (30%)	30	Complex	No pregnancy
11	4	38	Mos. partial tris. 12p / Mos. Tris 18/ Mos. Tris. 19 / Mos. Tris. 20/ Mos. Tris. 21	30	Complex	No pregnancy
12	4	40	Mos. Tris 5 (20%) / Mos. Mono 4(30%) / Mos. Mon. 6 (10%) / Mos. Mono. 14(20%) / Mos. Mono. 19 (20%)	30	Complex	No pregnancy
13	4	42	Mos. Tris 8 (30%) / Mos. Tris 9 (20%) / Mos Tris. 12 (40%) / Mos Tris. 16 (40%) / Mos tris. 20 (20%) / mos tris. 21 (30%) / Mos tris 22 (30%) / Mos. mono 1 (20%) / Mos. Mono 17 (20%)	30	Complex	No pregnancy
14	3	41	Mos. Tris 11 (35%), Mos Tris 14 (35%), Mos. Mono 15 (25%)	35	Complex	No pregnancy
15	3	35	Mos Tris 5 (30%), Mos. partial mono. 8pter-q23.1 (35%), Mos Tris 13 (30%)	35	Complex	No pregnancy
16	4	39	Mos. trisomy 2 (20%) / Mos. partial mono. 10q (20%) / Mos. mono 13q (40%)	40	Complex	No pregnancy
17	4	39	Mos. Mono 4 (20%) / Mos. Tris 8 (40%) / Mos. Tris 21 (40%)	40	Complex	No pregnancy
18	4	38	Mos. Tris 7 / Mos. Tris. 17 / Mos. Mono 11	40	Complex	No pregnancy

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.

SUPPLEMENTAL TABLE 3

Continued.

Embryo #	Center	Maternal age, y	Mosaicism description	% Mosaic	Mosaic type	Pregnancy outcome
19	4	32	Mos.Tris. 5 / Mos.Tris. 9 / Mos.Tris. 15	20	Complex	No pregnancy
20	3	30	Mos. Tris 13 (25%), Mos. Tris 14 (25%), Mos. Tris 21 (25%)	25	Complex	No pregnancy
21	4	40	Mos.Mono 4 (20%) / Mos. Mono 16 (20%) / Mos. Mono 21 (30%) / Mos. partial Tris. 3q / Mos. partial tris 16p (20%) / Mos. Tris. 18 (20%)	30	Complex	No pregnancy
22	1	33	Mos. Partial Mono. 18pter-p11.21 / Mos. Trisomy 19	80	Double	No pregnancy
23	4	42	Mos. partial Tris. 11q (50%) / Mos. partial Tris. 16q (20%)	50	Double	No pregnancy
24	3	27	Mos. Partial mono 2q33.1-qter (50%) / Mos. Mono 14 (30%)	50	Double	Ongoing
25	1	39	Mos. Partial Mono. 7q11.22-qter / Mos. Partial Mono. 9q21.11-qter	50	Double	Ongoing
26	4	35	Mos. partial Tris 9q (50%) / Mos. Partial Tris. Xq (40%)	50	Double	Ongoing
27	3	35	Mos. Trisomy 7 (25%), Mos. Trisomy 8 (45%)	45	Double	Ongoing
28	4	35	Mos partial Mono. 7p (50%) / Mos Mono 11 (40%)	50	Double	Miscarried
29	3	33	Mos. Trisomy 14 (30%) / Mos. partial mono 21q22.21-qter (40%)	40	Double	No pregnancy
30	4	43	Mos. monosomy 5 / Mos. trisomy 19	20	Double	No pregnancy
31	4	36	Mos. trisomy 5 / Mos. monosomy 21	20	Double	No pregnancy
32	4	36	Mos. monosomy 21 / Mos. trisomy 22	20	Double	No pregnancy
33	4		Mos. monosomy 3 (20%) / Mos. trisomy 11 (30%)	30	Double	No pregnancy
34	4	35	Mos. partial mono. 5q (40%) / Mos. mono. 9 (20%)	40	Double	No pregnancy
35	3	34	Mos. Monosomy 15 (40%), Mos. Monosomy 18 (30%)	40	Double	No pregnancy
36	3	37	Mos. Partial mono. 8pter-q24.12 (35%) / Mos. partial mono. 17pter-q21.33 (35%)	35	Double	No pregnancy
37	3	27	Mos. Trisomy 6 / Mos. Trisomy 16	30	Double	No pregnancy
38	4	33	Mos. Tris. 9 / Mos. Tris. 20	20	Double	No pregnancy
39	4	42	Mos. Tris. 5 / Mos. Tris. 9	20	Double	No pregnancy
40	3	43	Mos. Mono 6 (35%) / Mos. Partial Mono 13q32.2-qter (25%)	35	Double	Ongoing
41	3	41	Mos. Mono 7 (30%) / Mos. Tris. 20 (35%)	35	Double	Ongoing
42	4	36	Mos.partial tris. 5q / Mos.partial tris.9q	30	Double	Ongoing
43	3	35	Mos. Monosomy 14 (35%), Mos. Monosomy 16 (30%)	35	Double	Ongoing
44	4	30	Mos.Tris. partial Mono. 10q / Mos. partial Tris. 14q	20	Double	Ongoing

Munné. Pregnancy outcome of mosaic blastocysts. Fertil Steril 2017.

SUPPLEMENTAL TABLE 3

Continued.

Embryo #	Center	Maternal age, y	Mosaicism description	% Mosaic	Mosaic type	Pregnancy outcome
45	4	43	Mos.partial tris. 3p (20%) / Mos.partial tris. 5q (30%)	30	Double	Ongoing
46	4	42	Mos.partial tris. 4p (40%) / Mos.partial Mono. 5p (20%)	40	Double	Ongoing
47	4	35	Mos. partial Mono. 2q /Mos. partial Mono. 10q	40	Double	Ongoing
48	3	35	Mos. Trisomy 5 (40%) / Mos. Trisomy 19 (30%)	40	Double	Ongoing
49	3	32	Mos. Monosomy 8 (40%) / Mos. Monosomy 11(40%)	40	Double	Miscarried
50	4	37	Mos. trisomies 20 / Mos. Tris. 21	40	Double	Miscarried
51	1	32	Mos. Monosomy 22	50	Mono	No pregnancy
52	1	43	Mos. Monosomy 16	80	Mono	No pregnancy
53	2	41	Mos. Monosomy 16	80	Mono	No pregnancy
54	2	42	Mos. Monosomy 19	80	Mono	No pregnancy
55	2	42	Mos. Monosomy 6	80	Mono	Ongoing
56	3	41	Mos. Monosomy 17	40	Mono	No pregnancy
57	4	38	Mos. monosomy 21	20	Mono	No pregnancy
58	3	25	Mos. Monosomy 18	25	Mono	No pregnancy
59	3	36	Mos. Monosomy 3	30	Mono	No pregnancy
60	3	32	Mos. Monosomy 13	30	Mono	No pregnancy
61	3	33	Mos. Monosomy 22	35	Mono	No pregnancy
62	3	37	Mos. Monosomy 18	20	Mono	Ongoing
63	3	26	Mos. Monosomy 1	25	Mono	Ongoing
64	3	35	Mos. Monosomy 6	25	Mono	Ongoing
65	3	36	Mos. Monosomy 11	25	Mono	Ongoing
66	3	34	Mos. Monosomy 13	25	Mono	Ongoing
67	3	36	Mos. Monosomy 18	25	Mono	Ongoing
68	3	37	Mos. Monosomy X	25	Mono	Ongoing
69	3	40	Mos. Monosomy 5	30	Mono	Ongoing
70	3	25	Mos. Monosomy 6	30	Mono	Ongoing
71	3	32	Mos. Monosomy 15	30	Mono	Ongoing
72	3	33	Mos. Monosomy 19	35	Mono	Ongoing
73	4	43	Mos. Monosomy 7	20	Mono	Ongoing
74	2	40	Mos. Monosomy 18	40	Mono	Ongoing
75	3	37	Mos. Monosomy 13	25	Mono	Miscarried
76	3	20	Mos. Monosomy X	25	Mono	Miscarried
77	3	36	Mos. Monosomy X	30	Mono	Miscarried
78	3	42	Mos. Monosomy 20	35	Mono	Miscarried
79	3	37	Mos. Monosomy 21	35	Mono	Miscarried
80	4	37	Mos. monosomy 17	20	Mono	Miscarried
81	4	37	Mos. Monosomy 19	20	Mono	No pregnancy
82	3	34	Mos. Monosomy 4	30	Mono	No pregnancy
83	3	38	Mos. Partial monosomy 3pter-p22.3	45	Partial	No pregnancy
84	3	28	Mos. partial monosomy 13q21.33-qter	45	Partial	No pregnancy
85	1	40	Mos. Partial Trisomy 2q24.1-qter	50	Partial	No pregnancy
86	1	41	Mos. Partial Monosomy 3pter-p12.2	50	Partial	No pregnancy
87	1	38	Mos. Partial Monosomy 7q11.21-qter	50	Partial	No pregnancy
88	4	41	Mos. partial monosomy 6q	80	Partial	No pregnancy
89	1	38	Mos. Partial Trisomy 6q22.2-qter	60	Partial	Miscarried
90	3	31	Mos. Partial monosomy 2q	45	Partial	Ongoing
91	3	29	Mos. Partial monosomy 14q24.3-qter	45	Partial	Ongoing
92	3	31	Mos. Partial monosomy 5q35.1-qter	50	Partial	Ongoing
93	2	34	Mos. Partial Trisomy 20	50	Partial	Ongoing
94	2	42	Mos. Partial monosomy 9	80	Partial	Ongoing

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.

SUPPLEMENTAL TABLE 3

Continued.

Embryo #	Center	Maternal age, y	Mosaicism description	% Mosaic	Mosaic type	Pregnancy outcome
95	3	34	Mos. partial monosomy 1q	45	Partial	No pregnancy
96	3	35	Mos. Partial monosomy 3pter-p22.2	25	Partial	No pregnancy
97	3	33	Mos. Partial monosomy 2q	30	Partial	No pregnancy
98	3	34	Mos. Partial Monosomy 12pter-p11.2	30	Partial	No pregnancy
99	3	36	Mos. Partial monosomy 9q21.33-qter	40	Partial	No pregnancy
100	4	38	Mos. partial monosomy 8p	20	Partial	No pregnancy
101	1	30	Mos. Partial Monosomy 4pter-p11	30	Partial	No pregnancy
102	4	37	Mos. partial monosomy 6p	30	Partial	No pregnancy
103	3	35	Mos. Partial Trisomy Xp11.22-qter	25	Partial	Ongoing
104	3	37	Mos. Partial monosomy 5q14.1-qter	30	Partial	Ongoing
105	3	40	Mos. Partial monosomy 14q32.12-qter	35	Partial	Ongoing
106	3	43	Mos. Partial Monosomy 2pter-p23.3	40	Partial	Ongoing
107	3	25	Mos. Partial monosomy 7q21.12-qter	40	Partial	Ongoing
108	3	46	Mos. Partial Monosomy 11pter-p11.2	40	Partial	Ongoing
109	3	38	Mos. Partial Trisomy Xpter- p22.1	40	Partial	Ongoing
110	2	42	Mos. Partial monosomy 12	30	Partial	Ongoing
111	4	35	Mos. partial trisomy 14q	30	Partial	Ongoing
112	4	33	Mos. partial monosomy 2p	40	Partial	Ongoing
113	3	28	Mos. Partial Trisomy 8q23.3- qter	25	Partial	Miscarried
114	3	34	Mos. Partial Trisomy 9q	25	Partial	Miscarried
115	3	25	Mos. Partial Monosomy 1p34.2-pter	30	Partial	Miscarried
116	3	29	Mos. Partial monosomy 15q14.1-q15.1	35	Partial	Miscarried
117	3	25	Mos. partial monosomy 4q21.21-qter	35	Partial	No pregnancy
118	3	38	Mos. Trisomy 10	45	Tris	No pregnancy
119	1	41	Mos. Trisomy 22	80	Tris	Ongoing
120	1	41	Mos. Monosomy 19	80	Tris	Miscarried
121	3	38	Mos. Trisomy 3	30	Tris	No pregnancy
122	3	33	Mos. Trisomy 6	30	Tris	No pregnancy
123	3	37	Mos. Trisomy 6	30	Tris	No pregnancy
124	3	32	Mos. Trisomy 16	30	Tris	No pregnancy
125	3	30	Mos. Trisomy 19	35	Tris	No pregnancy
126	4	40	Mos. trisomy 10	20	Tris	No pregnancy
127	4	26	Mos. Trisomy 14	20	Tris	Ongoing
128	3	33	Mos. Trisomy 3	25	Tris	Ongoing
129	3	37	Mos. Trisomy 5	25	Tris	Ongoing
130	3	38	Mos. Trisomy 6	30	Tris	Ongoing
131	3	31	Mos. Trisomy 8	30	Tris	Ongoing
132	3	32	Mos. Trisomy 16	30	Tris	Ongoing
133	3	38	Mos. Trisomy 16	35	Tris	Ongoing
134	3	40	Mos. Trisomy 7	40	Tris	Ongoing
135	3	38	Mos. Trisomy 19	40	Tris	Ongoing
136	3	36	Mos. Trisomy 22	40	Tris	Ongoing
137	4	35	Mos. trisomy 20	30	Tris	Ongoing
138	1	30	Mosaic Partial Trisomy 4pter- p15.2	60	Partial	No pregnancy
139	1	44	Mosaic Partial Trisomy 10pter-p11.21	30	Partial	Miscarried
140	2	40	Mos. Monosomy 18	30	Mono	Ongoing
141	2	32	Mos. Partial mono 12	30	Partial	Miscarried
142	2	32	Mosaic partil tri 14	30	Partial	Miscarried
143	2	42	MOS. MONOSOMY 20	60	Mono	Delivered

Munné. Pregnancy outcome of mosaic blastocysts. Fertil Steril 2017.

SUPPLEMENTAL TABLE 4

Pregnancy outcome of embryos with one or two mosaic chromosomes. Per chromosome including only embryos with 1 or 2 abnormalities.

Per chromosome	Monosomic events	Trisomic events	Segmental events	Total	Preg	Lost	Ong preg	% ong preg
1	1	0	2	3	2	1	1	33
2	0	0	7	7	5	0	5	71
3	2	3	4	9	2	0	2	22
4	1	0	4	5	1	0	1	20
5	2	4	6	12	8	0	8	67
6	4	3	4	11	5	0	5	45
7	3	2	3	8	7	1	6	75
8	1	2	3	6	4	2	2	33
9	1	2	6	9	5	1	4	44
10	0	2	3	5	2	1	1	20
11	4	0	2	6	4	2	2	33
12	0	0	3	3	2	1	1	33
13	3	0	2	5	3	1	2	40
14	2	2	5	9	8	1	7	78
15	2	0	1	3	2	1	1	33
16	3	4	1	8	3	0	3	38
17	2	0	1	3	1	1	0	0
18	6	0	1	7	4	0	4	57
19	3	6	0	9	4	1	3	33
20	2	4	1	7	6	2	4	57
21	2	2	1	5	2	1	1	20
22	2	3	0	5	2	0	2	40
X	3	0	3	6	6	2	4	67
Total	49	39	63	151	88	19	69	46
1-12	19	18	47	84	47	9	38	45
13-22	27	21	13	61	35	8	26	43
			$P < .0001$					NS

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.

SUPPLEMENTAL TABLE 5

Coefficients, significance and OR for the variables of the logistic model for the dependent variables implantation rate (IR) and ongoing implantation state (OIR). For each variable, the complex mosaic type is the reference category.

Dependent variable	Independent variable	Coefficient		OR	
		β_i	P value		
IR	x_4		.013		
	Mono	2.857	.001	17.4	
	Double	2.459	.003	11.7	
	Tris	2.657	.002	14.3	
OIR	x_5	Partial	2.200	.007	9.0
		Non-complex	1.987	.009	7.3

Munné. Pregnancy outcome of mosaic blastocysts. *Fertil Steril* 2017.