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Clinical outcomes after the transfer of blastocysts characterized as mosaic by high resolution Next Generation Sequencing- further insights

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ABSTRACT

Objective: To determine the pregnancy outcome potential of euploid, mosaic and aneuploid embryos.
Design: Retrospective study.
Setting: Reference genetics laboratories.
Patient(s): 2654 PGT-A cycles with euploid characterized embryo transfers, 253 PGT-A cycles with transfer of embryos characterized as mosaic, and 10 PGT-A cycles with fully abnormal embryo transfers.
Intervention(s): Blastocysts were assessed by trophectoderm (TE) biopsy followed by PGT-A via array CGH or NGS.
Main outcome measure(s): Implantation, miscarriage, ongoing implantation rates (OIR), and karyotype if available, were compared between different embryo groups, and between the two PGT-A techniques.
Results: The Ongoing Pregnancy Rate (OPR)/transfer was significantly higher for NGS-classified euploid embryos (**85%**) than for aCGH ones (**71%**) ($p < 0.001$), but the OPR/cycle was similar (**63%** vs **59%**). NGS-classified mosaic embryos resulted in **37%** OPR/cycle ($p < 0.001$ compared to euploid). Mosaic aneuploid embryos with < 40% abnormal cells in the TE sample had an OIR of **50%** compared to **27%** for mosaics with 40–80% abnormal cells in the TE, and **9%** for complex mosaic embryos. All the karyotyped ongoing pregnancies (n = 29) were euploid. Transfers of embryos classified as aneuploid via aCGH (n = 10) led to one chromosomally abnormal pregnancy.
Conclusion(s): NGS-classified euploid embryos yielded higher OIRs but similar OPRs/cycle compared to aCGH. NGS-classified mosaic embryos had reduced potential to reach term, compared to euploid embryos. If they did reach term, those with karyotype results available were euploid. Embryos carrying uniform aneuploidies affecting entire chromosomes were mostly unable to implant after transfer, and the one that implanted ended up in a chromosomally abnormal live birth.

1. Introduction

Mosaicism, or the presence in an embryo of several cell lines each one possessing a different chromosome constitution was extensively investigated using FISH in studies examining entire embryos. During these studies either all or the vast majority of cells were analyzed (Delhanty et al., 1993; Munné et al., 1993, 1994; Coonen et al., 1994). Analysis of entire cleavage-stage embryos indicated that in contrast to meiotically derived aneuploidy, which increases with advancing maternal age but not with dysmorphism (defined as the presence of multinucleation, fragmentation, unevenness) (Munné et al., 1995; Márquez et al., 2000), mosaicism arises through post-zygotic malsegregation and increases with cleavage-stage dysmorphism, but not with advancing maternal age. Exception to these findings, are mosaic embryos, which

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are also fully aneuploid for another chromosome type (Munné et al., 1995, 2002, 2006, 2007; Magli et al., 2007; Munné, 2006; Colls et al., 2007). There have been fewer studies on mosaicism at the blastocyst stage. Such studies show slightly lower rates of mosaicism at the final stage of embryo development before implantation, and similar to the findings at the cleavage stage, confirm it as being independent of female age (Evsikov and Verlinsky, 1998; Bielanska et al., 2002; Sandalinas et al., 2001; Fragouli et al., 2011; Capalbo et al., 2013; Greco et al., 2015; Johnson DSCinnioglu et al., 2010; Munné and Wells, 2017).

Robust Preimplantation Genetic Testing for Aneuploidy (PGT-A) strategies involve molecular techniques capable of assessing all biopsied cells from a TE biopsy as a group, and not individually. Of these molecular techniques, such as aCGH, qPCR, SNP arrays and high resolution Next Generation Sequencing (hr-NGS), only hr-NGS, which has a higher dynamic range and resolution, can detect more mosaicism events than aCGH, or other molecular PGT-A techniques. Various studies have suggested that hr-NGS can identify chromosome abnormalities when present in 20–80% of the TE biopsy, (Greco et al., 2015; Maxwell SM et al., 2016; Fragouli et al., 2017; Munné et al., 2017a). In general, a TE biopsy has approximately 5 cells. Hence, hr-NGS can identify between 1 and 4 mosaic cells in a 5 cell TE biopsy. Using hr-NGS 22% of blastocysts were found to be mosaic in recent investigations by our group (Munné and Wells, 2017; Maxwell SM et al., 2016; Munné et al., 2017a; Wells et al., 2014; Fiorentino et al., 2014; Kung et al., 2015; Ruttanajit et al., 2016; Munné et al., 2016), compared to just 4.8% detected by aCGH (Greco et al., 2015).

There are several NGS platforms with different resolutions (Maxwell SM et al., 2016; Munné et al., 2017a; Fiorentino et al., 2014; Munné et al., 2016; Spinella et al., 2018), and not all of them can detect mosaicism to the same extent. The platform we use for hr-NGS is based on Illumina's VeriSeq NGS strategy (Munné et al., 2017a; Spinella et al., 2018). Our work demonstrated that hr-NGS is a much more sensitive method, compared to aCGH, and can accurately identify the presence of mosaicism at similar levels as FISH did when analyzing embryos cell by cell (Maxwell SM et al., 2016; Fragouli et al., 2017). However, some confusion remains since there is still scant data about the formation mechanisms, and clinical implications associated with these embryos.

Critics of hr-NGS argue that low level mosaicism is indistinguishable from technical background noise (Capalbo et al., 2016), or that mosaicism detection is flawed because if the same proportion of trisomic and monosomic cells are present in a TE biopsy, then a euploid result might be obtained (Scott and Galliano, 2016). However, mounting clinical evidence suggests that this group of embryos have a distinct clinical outcome, compared to euploid embryos, miscarrying more and implanting less (Maxwell SM et al., 2016; Fragouli et al., 2017; Munné et al., 2017a; Spinella et al., 2018; Grifo J et al., 2015; Rodriguez-Purata et al., 2016), although some can reach term and be karyotypically normal (Greco et al., 2015). We have argued before (Munné and Wells, 2017; Munné et al., 2016) that mosaic embryos should be considered as an intermediate group with lower potential of reaching term than euploid embryos but in the absence of euploid ones, could be considered for transfer. This new paradigm should result in fewer misdiagnoses but should not be confused as support for the transfer of aneuploid embryos. On the contrary, with hr-NGS euploid and aneuploid diagnoses are more definitive, compared to other methodologies.

Three of these studies, albeit small, analyzed the pregnancy outcome of mosaic embryos based on different parameters, such as % of abnormal cells in the TE sample, chromosomes involved in mosaic errors, and type of mosaicism. The obtained results suggested that some mosaic embryos have an appreciable implantation ability (Fragouli et al., 2017; Munné et al., 2017a; Spinella et al., 2018), whereas complex abnormal mosaics and those with a high percentage of abnormal cells in the TE sample perform worse. Moreover, the type of chromosome involved in the mosaic error did not seem to affect the implantation potential of the corresponding embryo. This is in contrast with prenatal diagnosis data showing that fetuses with mosaicism for chromosomes 8, 9, 13, 16, 18, 21, and XY survive to term more frequently than those involving other chromosomes (Grati et al., in press). A larger data set is needed to further assess the trends observed in the abovementioned smaller investigations. Therefore, the purpose of this study was to analyze the pregnancy outcome of transferred mosaic embryos from two large reference laboratories (CooperGenomics and Genoma) and one in-house laboratory (Clinica Dexeus) to determine which mosaic embryos have higher chances of implanting. In addition, this dataset was compared to another one consisting of euploid and aneuploid embryos transferred, in an attempt to determine the causes of discrepancy between PGT-A and PND/postnatal diagnosis results and calculate positive and negative predictive values.

2. Methods

2.1. Patients and embryos

This study includes several series of patients. Series 1 consists of all PGT-A patients undergoing transfers of euploid embryos at any of the fertility centers referring such samples to a reference PGD laboratory (CooperGenomics, Livingston, NJ), and for which the infertility center provided pregnancy follow up information to CooperGenomics. This data was collected between 3/1/14 and 4/30/17, and included cycles performed using PGT-A by array CGH or hr-NGS. Data was used only from 36 fertility centers that provided substantial pregnancy follow up results that could be linked to PGT-A results.

Because the average maternal age of Series 1 differed significantly between cycles using aCGH and those using NGS, we selected chronologically the most recently performed aCGH cycles, as well as a number of cycles identical to those performed with NGS for the age groups “egg donor (average age 25.5), < 35 years old, 35–39 and 40 and older. This way enabled us to obtain an identical maternal age in the aCGH and NGS group of euploid embryos replaced. This patient group will be referred as “reduced Series 1”.

Series 2 included patients undergoing transfers of mosaic embryos with the TE PGT-A either performed at CooperGenomics, Genoma or Dexeus. Some of these embryos and patients were previously published by Munné et al. (2017a) or Genoma (Spinella et al., 2018), but other embryos were transferred after those publications and some were unpublished data from Dexeus. Some fertility centers requested mosaicism reported while others not. For the ones that did not, mosaic embryos were classified as euploid if they had < 40% or < 50% abnormal cells (depending on the PGT lab). Mosaic embryos were selected for transfer either due to lack of euploid blastocysts in the patient's cycle, or due to them having < 50% abnormal cells in the TE biopsy and therefore being characterized as euploid when the fertility center did not wanted mosaicism reported.

Series 3 included a group of aneuploid characterized embryo transfers. PGT-A for these patients and their embryos was performed by CooperGenomics. The patients in this series had no euploid or mosaic embryos for transfer and decided to replace abnormal embryos with single monosomies or a combination of monosomies and trisomies, with the assumption that if the diagnosis was correct these embryos will not implant, but if it was not, then their transfer could produce a viable pregnancy (Esfandiari et al., 2015). CooperGenomics was not involved in this decision, and its policy is not to recommend transfer of embryos identified to be abnormal via PGT-A.

2.2. PGT-A analysis

Array CGH was performed as described previously (Colls et al., 2012; Gutiérrez-Mateo et al., 2011) with some minor modifications, as reported by Munné et al. (2017b).

The hr-NGS platform that used by all genetic laboratories involved

in the study was the VeriSeq (Illumina, USA), while the cytogenetic analysis was performed with the BlueFuse Multi v3 software, as described previously (Munné et al., 2017a). This platform can detect the presence of mosaicism with a resolution of 20–80% in a biopsied TE sample, as demonstrated previously by mixing ratios of normal to abnormal cell lines (Munné et al., 2017a). The average TE biopsy contains approximately 5 cells. Hence, the VeriSeq NGS platform can detect between 1 and 4 mosaic cells in a 5 cell TE biopsy. Embryos with chromosome errors observed in part of the corresponding TE biopsy were classified as mosaic, embryos with chromosomally normal NGS results in the corresponding TE biopsy were classified as euploid, and embryos with chromosome abnormalities present in all the cells of the corresponding TE biopsy were classified as aneuploid. Diagnostic decisions were made based on specific CNVs. A chromosome with 3 copies received a value of 3 (trisomic), a chromosome with 2 copies received a value of 2 (euploid), and a chromosome with 1 copy received a value of 1 (monosomic). Values between these numbers were classified as mosaic. Specifically, samples with values between 2 and 3 were classified as mosaic trisomies and those between 1 and 2 were classified as mosaic monosomies. TE biopsies with a segmental (or partial) aneuploidy had either a section of a chromosome with a value of 1 (partial monosomy) or a value of 3 (partial trisomy). This hr-NGS platform can detect extra or missing chromosome segments as small as 1.5–6 Mb in size depending on the quality of the biopsy. We used our previous classification for mosaic segmental (segmental abnormalities in mosaic form) and mosaic complex abnormalities (mosaics affecting 3 or more chromosomes) (Munné et al., 2017a).

All laboratories used the same platform and software. Mosaicism lower than 20% (< 1/5 cells) or higher than 80% (> 4/5 cells) was considered not differentiable from technical noise, therefore, < 20% mosaics were classified as euploid and > 80% as aneuploid.

2.3. Determination of discrepancies and further actions

We considered for this study that a discrepancy event occurred when follow up of a PGT-A cycle reported that transfer of a euploid characterized embryo resulted to a mosaic or aneuploid conception. We considered only discrepancies within the group of cycles for which follow up information could be obtained, and not spurious discrepancies with no other follow up from that center.

Once a discrepancy was reported, the following steps took place:

1) The amplified DNA remaining after completion of the PGT-A test was re-examined with the same PGT-A platform, to determine if clerical or human errors occurred in the PGD laboratory.
2) If aCGH had been used, the remaining DNA from the PGT-A procedure was retested also by hr-NGS in order to identify the possible presence of mosaicism, as aCGH is not capable of identifying mosaicism with great accuracy.
3) DNA from the POC, prenatal diagnosis sample, or the liveborn infant was requested. This DNA was analyzed again by hr-NGS to verify that the laboratory performing the analysis of the pregnancy did not make any mistakes, due to e.g. the presence of maternal contamination in a karyotype analysis, or false positive results during NIPT testing.
4) DNA from the POC, prenatal diagnosis sample or liveborn infant was genotyped and compared with the fingerprinting results of the remaining amplified DNA of the embryo to determine that the embryo and fetus were the same. If not, this could indicate a mix-up of embryos, or in vivo conception.
5) DNA from the parents was also requested, to determine in the above situation, if the amplified DNA of the embryo matched the DNA of the parents and fetus.

It was not possible to follow these steps in a few cases, due to inability to obtain the POC or prenatal DNA due to them being discarded by the reference laboratory, prior to notification of the discrepancy. Also, some couples were reluctant to provide their own DNA or that of the infant.

2.4. Statistical analysis

Implantation was defined as the presence of a fetal sac (detected at 6–10 weeks by ultrasound). The variables of interest were implantation rate (IR) (sacs detected/embryos replaced), fetal Loss Rate (FLR) (embryos lost/embryos implanting), ongoing implantation rate (OIR) (IR minus FLR), Pregnancy rate per cycle (PR-C) (presence of at least one sac/cycle with a biopsy), Pregnancy rate per transfer (PR-T)(presence of at least one sac/cycle with a transfer), Miscarriage rate (MR)(complete loss of pregnancy/pregnancies), Ongoing pregnancy rate per cycle (PR-C minus MR/cycles with a biopsy), and Ongoing pregnancy rate per transfer (PR-C minus MR/cycles with a transfer). Being a reference PGD center we had no record of PGT-A cycles started that did not result to a biopsy. Two variables were considered significantly different when $p < 0.025$.

In our previous study (Munné et al., 2017a) we performed a multivariable analysis to determine which variables were significant regarding types of mosaics with higher chances of implanting. Since those variables were already identified previously, we decided this analysis was not necessary again. Therefore, a single variable study was performed using Chi square and ANOVA when needed. Also, we calculated a regression line between OIR and % of mosaicism.

2.5. IRB and consenting of patients

All patients included in this study had consented to PGT-A. We used the Aspire Institutional Review Board (IRB) HIPAA waiver of authorization protocol #PGSP 201. The study was also considered to be exempt from approval according to the common rule 45 CFR 46.101(b) (Munné et al., 1994), which states that '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 manner that subjects cannot be identified, directly or through identifiers linked to subjects'. Retrospective chart reviews were performed using secure electronic medical records of patients with embryos that underwent PGT-A.

The decision to transfer a mosaic or abnormal embryo did not involve the intervention of the reference PGD laboratory and was taken after the referring fertility center had discussed and counseled the patients receiving such embryos for transfer. The PGD laboratories involved in this study only found out retrospectively about such transfers, after obtaining pregnancy follow up information, unless stated otherwise (i.e. part of series 2 was directly counselled by one of the scientists of one of the reference PGD laboratories as stated in Munné et al. (2017a)). The couples from the reference PGD laboratory of Genoma were initially seen by a clinical geneticist. Genetic counselling consisted of a review of the couple's clinical history, followed by an explanation of the PGT-A process. A calculation of the possible genetic outcomes, the likely success rates, the possibility of no euploid embryos available for transfer, and the risk of misdiagnosis were also discussed. Finally, the possible risks related with the transfer of mosaic embryos were specified and confirmatory prenatal diagnosis for any ensuing pregnancy was recommended. Amniocentesis was suggested as ideal follow-up in place of chorionic villus sampling (CVS), in order to overcome the confined placental mosaicism issue related with the latter invasive prenatal testing procedure.

After genetic counselling, patients were referred to the collaborating IVF clinic to arrange the clinical aspects of the treatment.

3. Results

Series 1 included a total of **6805** cycles of PGT-A by aCGH and **1490**

Table 1
Pregnancy outcome of euploid embryos diagnosed by aCGH or hr-NGS from the same fertility centers (series 1).

TOTAL	aCGH euploid (a)	NGS euploid (b)	p value
Cycles biopsied	2456	1327	
Age	**36.5**	**34.1**	
Cycles with transfer	1796	973	
Embryos replaced	2503	1338	
Embryos implanted	1678	1107	
cycles with + sac	1458	891	
Cycles lost	118	60	
Sacs and fetus lost	209	82	
Sacs ongoing	1469	1025	
cycles ongoing	1340	831	
IR (%)	**67%**	**83%**	$p < 0.025$
PR/transfer (%)	81%	92%	
PR/cycle (%)	59%	67%	
Miscarriage rate (%)	**8%**	**7%**	$p < 0.05$
OIR (%)	**59%**	**77%**	$p < 0.001$
OPR/Transfer	75%	85%	
OPR/cycle	**55%**	**63%**	$p < 0.001$

cycles of PGT-A by hr-NGS, all with pregnancy follow up information. These cycles took place in 36 different fertility centers. Of these, 24 centers used only aCGH, 3 used only hr-NGS, and 9 used both methods.

To determine pregnancy outcome differences in cycles with transfer of euploid embryos between both techniques (aCGH and hr-NGS), we used the subset of cycles from the 9 centers using both aCGH and hr-NGS for the purposes of PGT-A. These cycles constituted reduced series 1. Specifically, reduced series 1 included **2456** cycles of PGT-A by aCGH (average maternal age of 36.5 years) and **1327** cycles of hr-NGS (average maternal age of 34.1 years). This data is shown in Table 1. The results show that OPR per cycle was significantly higher when using hr-NGS (**62%**) than aCGH (**54%**) ($p < 0.001$), but that was partially due to differences in maternal age between groups. Hr-NGS's superior ability to identify euploid embryos, compared to aCGH, could also have contributed to the improved OPR.

To avoid the female age bias, we used the "reduced series 1" of **1327** cycles of aCGH and **1327** cycles of hr-NGS. The average female age for both groups of patients in this reduced group of cycles was identical and was **34.1** years. This data is shown in Table 2. The OPR per cycle was not different between aCGH (**59%**) and NGS (**63%**), although the OPR per transfer was (**71%** vs **85%**, $p < 0.001$), since fewer embryos were replaced ($p < 0.001$) in the hr-NGS group but those had a higher OIR (**63%** vs **76%**, $p < 0.001$). These results further confirmed that hr-NGS is able to distinguish euploid embryos from those with mosaic errors with improved sensitivity. Although there were more cycles without a transfer with NGS (354 with hr-NGS vs. 229 with aCGH), mosaic embryos by NGS could still be replaced if no euploid were available. However, if that happened, they were not included in this Table.

Table 2
Comparison of aCGH and NGS results in "reduced series 1" cycles.

TOTAL	aCGH euploid (a)	NGS euploid (b)	p value
Cycles biopsied	1327	1327	
Age	34.08	34.10	
Cycles with transfer	1098	973	$P < 0.001$
Embryos replaced	1423	1338	
Embryos implanted	997	1107	
cycles with + sac	854	891	
Cycles lost	75	60	
Sacs and fetus lost	104	82	
sacs ongoing	893	1025	
cycles ongoing	781	831	
IR (%)	**70%**	**83%**	$p < 0.001$
PR/transfer (%)	78%	92%	**$p < 0.001$**
PR/cycle (%)	64%	67%	NS
Miscarriage rate (%)	9%	7%	NS
OIR (%)	**63%**	**77%**	**$p < 0.001$**
OPR/Transfer	**71%**	**85%**	**$p < 0.001$**
OPR/cycle	59%	63%	NS

Series 2 included clinical outcome data after the replacement of mosaic embryos from 14 centers, three of which were also included in series 1.

To determine differences between pregnancy outcome of cycles with transfer of euploid and characterized mosaic embryo we compared the "reduced series 1" data with the series 2 data. As shown in Table 3, the replacement of embryos characterized by hr-NGS as mosaic resulted in a **25%** miscarriage rate, compared to **7%** for embryos characterized as euploid by hr-NGS ($p < 0.001$), and an OIR of **37%** per cycle of mosaic-characterized embryo transfer, compared to **77%** after euploid characterized embryo transfer ($p < 0.001$).

The mosaic-characterized embryos of Table 3 come from 5 reference PGT labs (see supplemental data), while the euploid-characterized embryos come from a single center (center 4, in supplemental data). To determine differences in outcomes between mosaic embryos from PGT lab 4 versus the other labs, we calculated the OIR just for PGT lab 4. We found 52 mosaic-characterized embryos from PGT Lab 4. OIR was 38% (20/52) for PGT lab 4 and 37% for all mosaics (Table 3). Also segregating 20–40% from > 40% abnormal mosaic-characterized embryos, the OIR rates were 52% and 29%, respectively for PGT lab 4, compared to 50% and 27%, respectively for all mosaics (Table 4). Thus we conclude that the comparison of the NGS euploid-characterized group (PGT lab 4) can be compared to the total of series 3 for the purpose of this study.

Series 3 consisted of 10 fully aneuploid embryos classified as such by aCGH, which were also transferred. Of those, there were **8 carrying** monosomies, and **2** identified to be monosomic for one chromosome and trisomic for another. The transfer of these embryos resulted in **one** ongoing chromosomally abnormal pregnancy. The transferred embryo was diagnosed to be monosomic for chromosome 7, and this led to the birth of a baby who was mosaic for trisomy 5 (but normal for chromosome 7). The baby unfortunately died six weeks after birth (Table 3). The Positive Predictive Value of aCGH, for full aneuploidies was therefore near 100% (10/10 did not make a viable euploid pregnancy). Although the monosomy 7 diagnosis should be characterized as a double misdiagnosis, the PPV of aCGH remains 10/10. In our experience, this could have been a high grade mosaic monosomy 7/trisomy 5 for which only the monosomy 7 was present in the biopsy and aCGH classified it as full monosomy 7 due to the limitations of aCGH detecting mosaicism.

Mosaic embryos were further analyzed following the categories determined in a previous but smaller dataset to be significantly different (Munné et al., 2017a). Specifically, the implantation potential of mosaic embryos was examined in relation to the type of chromosome abnormality present in the analyzed TE sample (whole or segmental), the number of chromosome abnormalities in the TE sample (single, double, or complex), and the proportion of chromosomally abnormal cells in the TE sample (< 40% abnormal cells or 40–80%).

As shown in Table 4, aneuploid mosaic embryos with < 40% abnormal cells had an OIR of **50%**. This OIR was significantly higher than the **27%** observed after the transfer of mosaic embryos with 40–80% abnormal cells and the **9%** seen after the transfer of complex mosaics. There was no difference between monosomic (**42%**), trisomic (**42%**) or mosaics carrying 2 chromosome errors, (**43%**) or between whole chromosome mosaicism (**42%**) and segmental mosaicism (**40%**). Additional details of the chromosome constitution of each mosaic-characterized embryo transferred can be found in the Supplemental Table 1. A regression line was calculated for % OIR/transfer (Y) and % of mosaicism (X), which was equal to: $Y = -0.6366*X + 61.93$. although there was a trend it was not statistically significant ($p = 0.0622$).

The chromosomes affected by mosaic errors in the samples analyzed

Table 3
Comparison of aCGH and hr-NGS pregnancy outcomes from all centers.

	aCGH euploid (a)	NGS euploid (b)	NGS mosaic (c)	aCGH or NGS aneuploid (c)[a]	p value
Cycles biopsied	1327	1327	253	10	
Age	34.1	34.1	36.6	43.1	
Cycles with transfer	1098	973	253	10	
Embryos replaced	1423	1338	253	10	
Embryos implanted	997	1107	125	1	
cycles with + sac	854	891	125	1	
Cycles lost	75	60	31	1	
Sacs and fetus lost	104	82	31**	1	
sacs ongoing	893	1022	94	0	
cycles ongoing	781	831	941	0	
IR (%)	70%	83%	49%	10%	a vs c, b vs c: < 0.001
PR/transfer (%)	78%	92%	49%	10%	a vs b,a vs c, b vs c: < 0.001
PR/cycle (%)	75%	83%	49%	10%	a vs c, b vs c: < 0.001
Miscarriage rate (%)	**9%**	**7%**	**25%**	**100%**	a vs c, b vs c: < 0.001
OIR (%)	**63%**	**77%**	**37%**	0%	a vs b,a vs c, b vs c: < 0.001
OPR/Transfer	71%	85%	37%	0%	a vs b,a vs c, b vs c: < 0.001
OPR/cycle	**59%**	**63%**	**37%**	**0%**	a vs c, b vs c: < 0.001

[a] Instead of ongoing implantation or pregnancy here we considered viable euploid pregnancy. The only implanting embryo resulted in a non-euploid baby that died after birth. ** One terminated but euploid, with necrosis suggestive of PKHD.

are shown in Supplemental Table 1. A recent study by Grati et al. (in press) reported that among fetuses identified as mosaic by prenatal diagnosis, those with abnormalities affecting chromosomes 8, 9, 13, 16, 18, 21, and XY are the ones with the highest chance of reaching term. We therefore compared the implantation potential of mosaic-characterized embryos containing single abnormalities affecting these chromosomes to that of the rest of the group of mosaic-characterized embryos. As shown in Table 4, there were no differences between these two groups regarding their OPR.

The clinical outcomes after the transfer of embryos characterized as mosaic for chromosome abnormalities that could reach term, i.e. trisomies 13, 18, 21, X/Y and monosomy X were as follows: the transfer of a total of 4 embryos characterized as mosaic monosomy X led to one ongoing pregnancy, two miscarriages and a failed implantation; the transfer of two embryos with mosaic segmental monosomy X led to two ongoing pregnancies, and the transfer of one embryo mosaic for trisomy X and two embryos mosaic for trisomy 18 led to failed implantations (see supplemental data Table 1). Additionally, there were a few characterized mosaic embryos with abnormalities that could lead to UPD (trisomies 7, 14, and 15) replaced. Specifically, the transfer of 2 embryos with mosaic trisomy 7 resulted to one ongoing pregnancy and a failed implantation, the transfer of an embryo with mosaic trisomy 14 led to one ongoing pregnancy, and the transfer of 9 embryos mosaic for trisomy 15 led to 3 failed implantations, one miscarriage and 5 ongoing pregnancies. Unfortunately, we were unable to collect karyotyping information for most miscarriages, ongoing pregnancies or live born babies.

It is possible that mosaic embryos that were replaced blindly, because the IVF center requested mosaicism not to be reported, their implantation potential was better than for embryos identified as mosaic, since the later could be of lower morphologic and developmental competence. To determine if that was true we compared those two groups and we found no differences (Table 5).

Among the cycles with follow up after the transfer of embryos identified to be euploid (series 1), **25** of the pregnancies established after aCGH analysis and **7** of the pregnancies established after hr-NGS resulted in chromosomally abnormal conceptions. Of these **32** discrepancies, **2** were below the resolution of aCGH and **one** of NGS, and in **one** the DNA of the embryo did not match the DNA of the POC. Of the **28** remaining discrepancies, **6** were mosaic after reanalysis by NGS, **7** mosaics by Prenatal Diagnosis, **14** were unexplained, and one was a 6.5 Mb deletion missed by hr-NGS (Tables 6 and 7). As such, 22 **(0.6%)** aCGH and **6 (0.6%)** NGS discrepancies were classified as having a potential technical origin. Overall the chance of a euploid characterized embryo resulting to a chromosomally abnormal conception was calculated to be **0.6%** and to a live birth **0.2%**. It is possible that the 0.2% could be lower since three of these abnormalities were detected by NIPT, but we were unable to obtain further confirmation by invasive prenatal diagnosis.

Two of the abnormal fetuses were trisomic for chromosome 21 and

Table 4
OIR per type of mosaic.

Mosaic type	% abnormal or subtype	replaced	+sacs	miscarried	ongoing	OIR/Transfer	p
Complex mosaics (a)	any	35	6	3	3	9%	a vs b: $p < 0.001$, b vs c: $p < 0.025$,
Aneuploid mosaics (by sub-type)	monosomic mosaic	64	35	8	27	42%	a vs d:
	trisomic mosaic	38	20	4	16	42%	$p < 0.001$,
	double mosaic	51	27	5[a]	22	43%	a vs e: $p < 0.005$
Aneuploid mosaics (by chromosome type)	8, 9, 13, 16, 18, 21, and XY	50	28	7	21	42%	
	others	203	97	24	73	36%	
Aneuploid mosaics (by % abnormal)	20–40% (b)	105	63	10	53	50%	
	> 40% (c)	48	19	6	13	27%	
Total Aneuploid mosaics (d)	any	153	82	17	65	42%	
Segmental mosaics (e)	any	65	37	11	26	40%	
Mosaics embryos (f)	**20–80%**	**253**	**125**	**31**	**94**	**37%**	f vs g, b vs g: $p < 0.001$
			49%	25%	37%		
Euploid embryos (g)	**< 20%**	**1328**	**1107**	**82**	**1025**	**77%**	
			83%	7%	77%		

[a] One terminated but euploid, with necrosis suggestive of PKHD.

Table 5
No differences in OPG between embryos reported or not as mosaic.

mosaicism reported?	% abnormal	% mosaic Average	replaced	+sacs	miscarried	ongoing	OIR/Transfer
no	> 40	48%	17	7	1	6	35%
	20–40	30%	102	55	12	43	42%
yes	> 40 (a)	56%	67	24	8	16	24%
	20-40 (b)	35%	67	39	9	30	45%

a vs b: $p < 0.025$.

another one had a mosaic trisomy 21, that is 3/4411 pregnancies (0.068%) or a risk of trisomy 21 of 1/1470, compared with the estimated risk of 1/270 for women 35 years old, or > 10X lower risk (Table 7).

Of the mosaic embryos that produced an ongoing pregnancy, **29** had a prenatal or postnatal karyotype, while the rest were not available for several reasons, the main one being the referring clinic not wanting the reporting of mosaicism. In such cases all embryos with low % of abnormal cells in the TE biopsy were reported as normal. Others were lost to follow up. Most miscarriages also were not karyotyped, but among the ones which underwent cytogenetic analysis, **one** was abnormal and the other **two** normal (Table 8).

4. Discussion

Recent studies published by our group determined that the widely used NGS VeriSeq platform (Illumina) can detect the presence of mosaic chromosome abnormalities in biopsied TE samples with greater sensitivity, compared to aCGH (22% vs. 5%, respectively) (Greco et al., 2015; Munné et al., 2017a). The higher sensitivity and dynamic range of hr-NGS should therefore enable a better selection of euploid embryos with good implantation potential during PGT-A, resulting in higher OIR than aCGH. Indeed Table 2 shows that although there were fewer transfers after NGS than with aCGH, OIR/transfer was higher for NGS than aCGH. This is probably because some aCGH were most probably mosaics but detected as euploid by aCGH, and these mosaic embryos have lower implantation rates than truly euploid embryos. However, OPR/cycle were essentially the same between both methods, because for the purpose of this study, to assess the clinical outcome of mosaics, NGS mosaics were excluded in the NGS column Table 2, but still have some implantation potential. If the goal of the patient is to maximize fastest time to viable pregnancy when there are available euploid embryos, then NGS is a better method. If there are no NGS euploid embryos, mosaics still have some potential to implant albeit less than euploid embryos and have higher risk of miscarriage.

An exception to NGS being better than aCGH might be at older ages, were many more embryos are reported as fully aneuploid. Embryos characterized as “mosaic only” actually decrease with advancing maternal age (they become mosaic for a chromosome and fully aneuploid for another)[20] and thus the advantage of NGS over aCGH may not exist above certain maternal age.

It should be noted, however, that many IVF clinics have been generally reluctant to transfer mosaic embryos, due to the possible risks associated with the presence of the chromosome abnormality in part of the biopsied TE sample. Since recent studies (Greco et al., 2015; Munné and Wells, 2017; Fragouli et al., 2017; Munné et al., 2017a; Spinella et al., 2018; Friedenthal, Maxwell, Munné, Kramer, McCulloh, McCaffrey, Grifo) are indicating that some mosaic embryos have the ability to implant in certain cases the blanket exclusion of all mosaic embryos from transfer is bound to have a negative effect on pregnancy rates/retrieval in PGT-A cycles. An alternative approach to manage mosaic embryos is therefore needed. Such an approach should ensure that embryos with a good implantation potential are not discarded. It could be conceivable, for example, that embryos characterized as clear euploid after NGS are prioritized for transfer, but if no pregnancy is established, certain types of mosaic embryos could also be considered for transfer.

The main issue with this alternative approach is the limited results available associated with the developmental fate of mosaic embryos. The current investigation therefore aimed to further expand our understanding on this area. The available data on the developmental ability of mosaic embryos indicates that they miscarry more, implant less, but 37% produce a viable pregnancy (this study, and ref. 20). This was further confirmed in the current group of mosaic embryos under investigation. Specifically, it was evident from our results that mosaic embryos implant less frequently than embryos classified as clear euploid by hr-NGS (**49% vs 83%).** They also miscarry more (**25% vs 7%)** and fewer of them result in an ongoing pregnancy (**37% vs 63%**). Prenatal or post-natal analysis of pregnancies and birth established after the transfer mosaic embryos has shown that the resulting babies have normal karyotypes (this study and ref. 18).

In our previous work, we demonstrated for the first time that the implantation potential of various types of mosaic embryos was different (Munné et al., 2017a; Spinella et al., 2018). The present study increases

Table 6
Discrepancies between PGS and prenatal/postnatal genetic diagnosis.

		Array		NGS	
date range		3/1/14 - 4/30/17		3/1/14 - 4/30/17	
Procedures performed in data range		6805		1490	
Average age		37.4		37.4	
Procedures pregnant		3461	51%	950	64%
Pregnancy loss		366	**10.6%**	51	**5.4%**
DISCREPANCIES:		total	ongoing	total	ongoing
Non- technical discrepancies	below resolution	2	1	1	0
	embryo-fetus DNA mismatch	1	0	0	0
Human error	missed segmental	0	0	1	1
Mosaic	Mosaic by NGS	6	0	0	0
	Mosaic by prenatal, poc	7	0	0	0
Undetermined	mosaic, error, other	9	4	5	1
Total discrepancies		**25**	**5**	**7**	**2**
Technical discrepancies		**22**	**4**	**6**	**2**
% technical discrepancies		**0.6%**	**0.12%**	**0.6%**	**0.21%**

Table 7
Detailed description of discrepancies between PGS and prenatal/postnatal genetic diagnosis.

#	PGS diagnosis	PGS test	Discrepancy	Test used	PGS reanalysis by NGS	Preg ongo-ing?	match DNA parents?	reanalysis POC	Misdiagnosis cause
1	46,XX	Array	46,XX, add(20)(p12)	POC	46,XX	No	yes	no match	POC does not match
2	46,XX	Array	mosaic 21, 9q33.1dup	child	46, XX	yes	n/a	n/a	Not detectable
3	46,XX	Array	1.4 Mb deletion 15q26.2	POC	46,XX	No	n/a	n/a	Not detectable
4	46,XX	NGS	92,XXXX	POC	not detectable	No	n/a	n/a	Not detectable
5	46,XY	NGS	6.48 Mb deletion 10q25.2q25.3	amnio	46,XY	yes	n/a	n/a	missed
6	46,XX	Array	mosaic Trisomy 12 and 13	POC	46,XX	No	n/a	n/a	Mosaicism
7	46, XX	Array	47,XX + 5[3]/46,XX[17]	POC	46,XX	No	n/a	n/a	Mosaicism
8	46,XY	Array	Mosaic Trisomy 21	POC	n/a	No	n/a	n/a	Mosaicism
9	46,XY	Array	Mosaic 47,XY, + 2[4]/46,XY[16]	POC	46,XY	No	n/a	n/a	Mosaicism
10	46,XX	Array	Trisomy 11	POC	Mos T 11	No	n/a	n/a	Mosaicism
11	46,XY & 46,XY	Array	Trisomy 16	POC	# 1 mos T16, #2 Normal	No	n/a	n/a	Mosaicism
12	46,XY	Array	Trisomy 13	POC	Mosaic T13, Mosaic partial M2pterp24.1	No	n/a	n/a	Mosaicism
13	46,XY	Array	46,XY (no discrepancy)	POC	mosaic partial M1, mosaic M8	No	n/a	n/a	Mosaicism
14	46,XY	Array	Trisomy 8	POC	mosaic T8	No	n/a	n/a	Mosaicism
15	46,XX	Array	Mosaic Trisomy 13	POC	46,XX	No	n/a	n/a	Mosaicism
16	46,XX	Array	Mosaic Trisomy 9	POC	46,XX	No	n/a	n/a	Mosaicism
17	46,XY	Array	45,X	POC	46,XY	No	match	Mosaic 45,X/ 46,XY	Mosaicism
18	46,XY	Array	46,XX (no discrepancy)	POC	mosaic T4, mosaic partial T10	No	n/a	n/a	Mosaicism
19	46,XY & 46,XY	Array	47XY + 14,der (14; 14)(10; q10)	POC	n/a	No	n/a	n/a	undetermined
20	46,XX & 46,XX	Array	Trisomy 6	POC	46,XX & 46,XX	No	n/a	match	undetermined
21	46,XX & 46,XX	Array	Trisomy 15	POC	46,XX	No	n/a	n/a	undetermined
22	46,XY	Array	Trisomy 8	POC	46,XY	No	match	match	undetermined
23	46,XX	Array	Trisomy 21	POC	46,XX	No	match	match	undetermined
24	46,XY	NGS	46,XY,del(11)(q23)	POC	46,XY	No	n/a	n/a	undetermined
25	46,XX	NGS	45,X	POC	46,XX	No	match	match	undetermined
26	46,XY	NGS	48,XXY, + 17	POC	46,XY	No	n/a	n/a	undetermined
27	46,XY	NGS	Trisomy 7	POC	46,XY	No	n/a	n/a	undetermined
28	46,XY	Array	Trisomy 21	live birth	46,XY	yes	n/a	match	undetermined
29	46,XY	Array	Trisomy 18	amnio	46,XY	yes	n/a	n/a	undetermined
30	46,XX	Array	45,XO	NIPT	46,XX	yes	n/a	n/a	undetermined
31	46,XX	Array	Trisomy 13	NIPT	46,XX	yes	n/a	n/a	undetermined
32	46,XY	NGS	Trisomy 13	NIPT	46,XY	yes	n/a	n/a	undetermined

Table 8
Karyotype results.

	miscarried	terminated[c]	ongoing	total
N/A [a]	27	0	49	76
pending	0	0	16	16
euploid	2	1	29	32
abnormal[b]	1	0	0	1
	30	1	94	125

[a] Lost to follow up or not performed.
[b] del(15q14.1-q15.1).
[c] Necrosis suggestive of PKHD.

the number of mosaic embryos replaced allowing, in this way, further clarification of trends. As in our previous studies, the OIR of embryos that had multiple mosaic errors (complex mosaic) was significantly lower (**9%**) compared to mosaic embryos carrying one or two whole chromosome or segmental aneuploidies (**40–42%**) or euploid embryos (77%). An investigation involving the dissection of mosaic blastocysts into their ICM and several different TE parts (Garrisi J et al., 2016) showed very few euploid ICM cells in embryos classified as complex mosaic, compared to the rest of the mosaic embryos which had approximately 50% normal ICM cells. It should be noted that embryos classified as clear aneuploid via hr-NGS had no normal ICM cells.

In the current study we observed a significant difference in OIR between embryos with high (> 40%) and low (20–40%) rates of abnormal cells (**27% vs 50%, $p < 0.025$**) in the biopsied TE sample analyzed. This trend was also seen in one of our previous investigations (Munné et al., 2017a), and similar results were obtained in a recent study by Spinella et al. (2018). It is to be expected that with a larger dataset, more nuanced differences might be found and one or more cut-offs or different cut-offs be determined. We are basing 20%, 40% and 80% cut offs on the recommendation of 5-cells being biopsied, and therefore these cut-offs imply 1/5, 2/5 and 4/5 abnormal cells in the TE biopsy.

These different sets of data are in agreement with the mouse model described in the Bolton et al. (2016) investigation. During that investigation, chimeras of euploid and reversine-induced highly abnormal cells, equivalent to human complex mosaic embryos, were created in 1:1 to 1:3 ratios. In the 1:1 chimeric embryos the normal cells eventually took over and produced normal offspring, but the 1:3 chimeric embryos produced fewer pups (Bolton et al., 2016). Live-embryo imaging and single-cell tracking showed that these complex abnormal cells had a growth disadvantage in the TE, while those in the ICM were eliminated by apoptosis, thus suggesting that chromosomally abnormal cells tend to divide slower than normal cells. It can, therefore, be postulated that if the number of chromosomally normal cells in a mosaic diploid aneuploid characterized embryo is larger compared to the aneuploid ones, then the normal cells are likely to take over. On the other hand, if there are only a few chromosomally normal cells present in the embryo, and the vast majority has one or more errors, then the embryo is likely to fail (Bolton et al., 2016). Interestingly, Bolton and colleagues

observed that the distribution of abnormal cells was not clonal but was even throughout the TE and ICM parts (Bolton et al., 2016). This model is not optimal since it does not consider what would happen with mosaic aneuploidies involving just one chromosome. Moreover, the "mosaic" mouse embryos investigated were artificially constructed. It is therefore possible that the mechanisms of aneuploid cell elimination proposed by Bolton et al. (2016) do not accurately represent processes occurring in human mosaic diploid aneuploid embryos. A mechanism of active self-correction was proposed by Bazgar et al. (Bazrgar et al., 2013) and involved the loss of the extra chromosome in trisomic cells, leading in this way to occasional cases of UPD. It should be noted, however, that UPD is extremely rare and to date has not been observed in mosaic diploid aneuploid embryos (Spinella et al., 2018). The preferential allocation of chromosomally abnormal cells to the TE of a mosaic embryo has also been suggested, leading to confined placental mosaicism (CPM) in the resulting fetus, but this was also not seen when examining CVS samples obtained from pregnancies established after the transfer of mosaic embryos (Spinella et al., 2018).

In agreement with our previous findings (Munné et al., 2017a), we did not observe any influence of a specific chromosome abnormality on the implantation ability of the current group of mosaic embryos. This observation is contradictory to what has been reported from the analysis of either POCs or prenatal samples, as far as pure and mosaic aneuploidies are concerned (Grati et al., in press). However, the present data set is small and PGDIS guidelines still recommend caution similar to the recommendations of Grati et al. (in press).

Considering all the above, as well as this study findings that all blastocyst classified by PGT as mosaics so far are euploid, we could hypothesize the presence of two distinct mechanisms leading to the genesis of mosaicism: the first mechanism is active during early preimplantation development, it is the result of cleavage stage chromosome instability (Bean et al., 2001) and seems to affect embryo survival; the second mechanism is active during later gestational stages and may be associated with tissue invasion (Bolton et al., 2016; Weier et al., 2005).

We also confirmed in this larger study that mosaic monosomies and mosaic trisomies have similar OIRs. It is possible that the sampling of 5 cells only captures a majority of trisomic or monosomic cells, i.e. if there is a mixture of 1 monosomic and 4 trisomic cells, the average result would be 2.6, and presenting itself as a mosaic trisomy during NGS analysis. Indeed, data obtained from the analysis of cleavage and blastocyst stage embryos with the use of FISH studies suggested that most mosaic chromosome abnormalities were caused by mitotic nondisjunction, and only 5% by anaphase lag (Munné et al., 2002). Alternatively, aneuploid mosaic embryos may start with a 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. Recent guidelines on transferring mosaic embryos suggesting preferential selection of monosomic mosaic embryos over trisomic ones may therefore need to be changed (PGDIS, 2016; COGEN).

Overall the chance of a euploid characterized embryo resulting in a chromosomally abnormal conception was calculated to be **0.6%** and the chance of a euploid characterized embryo resulting in a chromosomally abnormal baby was calculated to be **0.2%**. This risk is much reduced in relation to some recently published mathematical models (Paulson, 2017). Such models and associated assumptions should be considered with caution and should be second to actual clinical data. The 0.6% risk should be viewed as an estimate of the negative predicted value of aCGH and NGS.

The Positive predictive value (PPV) of PGT-A, could be calculated as the % of embryos characterized as abnormal that do not implant or which result in a chromosomally abnormal pregnancy. We were able to calculate the PPV for aCGH but not for NGS. Ten fully aneuploid embryos, all diagnosed by aCGH, were replaced. One aneuploid characterized embryo resulted in a mosaic trisomy 5 affected baby that died shortly after birth. Thus, the positive predictive value for whole chromosome abnormalities, calculated as the % of embryos not producing a euploid baby, was near 100% (10/10). We found another dataset of embryos from Rubino et al. (2016) reporting the transfer of 13 monosomic embryos classified as such by aCGH, one of which made a healthy baby. The combination of both datasets leads to a predictive value for aCGH of 96% (22/23), identical to the 96% reported by Scott et al. (2012) with qPCR. Since one of the abnormal embryos transferred resulted in an affected pregnancy, and because of early reports using FISH demonstrated that monosomic embryo could contain trisomic cell lines (Pabon et al., 2005), we consider that replacing monosomic embryos, as suggested by some (Esfandiari et al., 2015), to be riskier than anticipated.

Two of the abnormal fetuses were trisomic for chromosome 21, and one was mosaic for trisomy 21. This can be interpreted as **3/4411** pregnancies (**0.07%**) or **1/1470**, compared with the estimated risk of **1/270** for women 35 years old, or six times lower than the risk of trisomy 21 in women 35 years of age. This risk is either similar or lower than the risk of losing a pregnancy after amniocentesis. The predicted risk of trisomy 21 conception in this cohort is probably grossly overestimated since many pregnancies were lost to follow up. It should be noted that centers not reporting pregnancy follow up, would still report misdiagnoses to us. There is still a small age independent risk of trisomic offspring after PGT-A, and for this reason we would recommend prenatal testing, after transfer of mosaic characterized embryos. This prenatal testing can be either invasive (CVS or amniocentesis) or non-invasive (NIPT).

For cycles involving the transfer of mosaic embryos we would recommend performing amniocentesis, since CVS would examine the placenta derived from the TE and not the fetus itself. So far not a single mosaic embryo replaced has resulted in a pregnancy with full trisomy, although the numbers are small considering that only 0.6% would after a euploid transfer. It is questionable that current NIPT tests would detect mosaicism, especially abnormalities affecting segments of chromosomes. Another aspect of transferring mosaic embryos that remains unresolved is whether there is an associated increased risk of congenital abnormalities. We currently have follow up information for 81 pregnancies only, so it is difficult to address this question with this knowledge.

Our recommendation for the clinical management of mosaic embryos, according to the results we have to date, is to classify them into a high and a low risk groups. The high-risk group would include embryos characterized as complex mosaic, as wells as those with mosaic abnormalities present in > 40% of the biopsied TE cells. The low risk group would include embryos with 1–2 mosaic abnormalities in the analyzed TE sample and those with mosaic abnormalities present in < 40% of the biopsied TE cells. During a PGT-A IVF cycle, embryos characterized as clearly euploid by NGS would be prioritized for transfer, followed by those "low grade" mosaic embryos, and then by the "high grade" mosaic embryos, provided that the patient is well informed about the associated risks of miscarriage and abnormal live births. These guidelines should always be adjusted to fit the needs of a patient, as these would differ depending on the indication of PGT-A (e.g. a young repeated pregnancy loss patient may prefer to go through another PGT-A cycle rather than have a transfer of a mosaic embryo, whereas a patient of advanced female reproductive age may not have time to try again) These guidelines, mostly coincide with earlier guidelines (PGDIS, 2016; COGEN) except for deprioritizing mosaic transfers according to chromosome type, such as those compatible with live births in pure aneuploid form (X, Y, 13, 18, 21), uniparental disomy (UPD) (Magli et al., 2007; Bielanska et al., 2002; Sandalinas et al., 2001) or intrauterine growth restriction (Fragouli et al., 2011). According to this larger set of results, all such embryos have similar chances to produce a viable offspring. It is likely that these conservative guidelines will require further adjustments, as our insight into the developmental potential of mosaic embryos becomes more detailed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2019.103741.

References

Bazrgar, M., Gourabi, H., Valojerdi, M.R., Yazdi, P.E., Baharvand, H., 2013. Self-correction of chromosomal abnormalities in human preimplantation embryos and embryonic stem cells. Stem Cells Dev. 22, 2449–2456.

Bean, C.J., Hunt, P.A., Millie, E.A., Hassold, T.J., 2001. Analysis of a malsegregating mouse Y chromosome: evidence that the earliest cleavage divisions of the mammalian embryo are non-disjunction-prone. Hum. Mol. Genet. 10, 963–972.

Bielanska, M., Tan, S.L., Ao, A., 2002. High rate of mixoploidy among human blastocysts cultured in vitro. Fertil. Steril. 78 (6), 1248–1253.

Bolton, H., Graham, S.J.L., Van der, A.N., Kumar, P., Theunis, K., Fernandez Gallardo, E., et al., 2016. Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. Nat. Commun. 7, 11165.

Capalbo, A., Wright, G., Elliott, T., Ubaldi, F.M., Rienzi, L., Nagy, Z.P., 2013. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. Hum. Reprod. 28 (8), 2298–2307.

Capalbo, A., Ubaldi, F.M., Rienzi, L., Scott, R., Treff, N., 2016. Detecting mosaicism in trophectoderm biopsies: current challenges and future possibilities. Hum. Reprod. https://doi.org/10.1093/humrep/dew250. (in press).

COGEN. COGEN Position Statement on Chromosomal Mosaicism Detected in Preimplantation Blastocyst Biopsies. (submitted).

Colls, P., Escudero, T., Cekleniak, N., Sadowy, S., Cohen, J., Munné, S., 2007. Increased efficiency of preimplantation genetic diagnosis for infertility using "no result rescue. Fertil. Steril. 88 (1), 53–61.

Colls, P., Escudero, T., Fischer, J., Cekleniak, N., Ben-Ozer, S., Meyer, B., Damien, M., Grifo, J., Hershlag, A., Munné, S., 2012. Validation of array comparative genome hybridization for diagnosis of translocations in preimplantation human embryos. Reprod. Biomed. Online 24, 621–629.

Coonen, E., Harper, J.C., Ramaekers, F.C.S., Delhanty, J.D.A., Hopman, A.H.N., Geraedts, J.P.M., et al., 1994. Presence of chromosomal mosaicism in abnormal preimplantation embryos detected by fluorescence in situ hybridisation. Hum. Genet. 94 (6), 609–615.

Delhanty, J.D.A., Griffin, D.K., Handyside, A.H., Harper, J., Atkinson, G.H.G., Pieters, M.H.E.C., et al., 1993. Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation, (FISH). Hum. Mol. Genet. 2 (8), 1183–1185.

Esfandiari, N., Ryan, E.A.J., Gotlieb, L., Casper, R., 2015. Case report: successful pregnancy following transfer of embryos from oocytes with abnormal zona pellucida and cytoplasm morphology. Reprod. Biomed. Online 11, 620–623.

Evsikov, S., Verlinsky, Y., 1998. Mosaicism in the inner cell mass of human blastocysts. Hum. Reprod. 13 (11), 3151–3155.

Fiorentino, F., Biricik, A., Bono, S., Spizzichino, L., Cotroneo, E., Cottone, G., et al., 2014. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil. Steril. 101 (5), 1375–1382.

Fragouli, E., Alfarawati, S., Daphnis, D.D., Goodall, N.N., Mania, A., Griffths, T., et al., 2011. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. Hum. Reprod. 26 (2), 480–490.

Fragouli, E.A.S., Spath, K., Tarozzi, N., Borini, A., Wells, D., 2017. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. Hum. Genet. 136, 805–819.

Friedenthal J, Maxwell SM, Munné S, Kramer Y, McCulloh DH, McCaffrey C, Grifo JA. Next-generation sequencing for preimplantation genetic screening improves pregnancy outcomes compared to array comparative genomic hybridization in single thawed euploid embryo transfer cycles. Fertil. Steril. , (in press).

Garrisi J, W.R., Bauckman, K., Mendola, R., Colls, P., Munné, S., 2016. Discordance among serial biopsies of mosaic embryos. Fertil. Steril. 106 (Suppl. l), e151.

Grati FG, Gallazzi G, Branca L, Maggi F, Simoni G, Yaron Y. An Evidence-Based Scoring System for Prioritizing Mosaic Aneuploid Embryos Following Preimplantation Genetic Screening (PGS) Reprod Biomed Online, (in press).

Greco, E., Minasi, M.G., Fiorentino, F., 2015. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N. Engl. J. Med. 373 (21), 2089–2090.

Grifo J, C.P., Ribustello, Escudero, Liu, E., Munné, S., 2015. Why do array-CGH (aCGH) euploid embryos miscarry? Reanalysis by NGS reveals undetected abnormalities which would have prevented 56% of the miscarriages. Fertil. Steril. 104 (Suppl. e14).

Gutiérrez-Mateo, C., Colls, P., Sánchez-García, J., Escudero, T., Prates, R., Wells, D., Munné, S., 2011. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. Fertil. Steril. 95, 953–958.

Johnson DS, J., Cinnioglu, C., Ross, R., Filby, A., Gemelos, G., Hill, M., et al., 2010. Comprehensive analysis of karyotypic mosaicism between trophectoderm and inner cell mass. Mol. Hum. Reprod. 16 (12), 944–949.

Kung, A., Munné, S., Bankowski, B., Coates, A., Wells, D., 2015. Validation of next-generation sequencing for comprehensive chromosome screening of embryos. Reprod. Biomed. Online 31 (6), 760–769.

Magli, M.C., Gianaroli, L., Ferrareti, A.P., Lappi, M., Ruberti, A., Farfalli, V., 2007. Embryo morphology and development are dependent on the chromosomal complement. Fertil. Steril. 87 (3), 534–541.

Márquez, C., Sandalinas, M., Bahçe, M., Alikani, M., Munné, S., 2000. Chromosome abnormalities in 1255 cleavage-stage human embryos. Reprod. Biomed. Online 1 (1), 17–26.

Maxwell SM, C.P., Hodes-Wertz, B., McCulloh, D.H., McCaffrey, C., Wells, D., Munné, S., et al., 2016. Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. Fertil. Steril. 106, 1414–1419.

Munné, S., 2006. Chromosome abnormalities and their relationship to morphology and development of human embryos. Reprod. Biomed. Online 12 (2), 234–253.

Munné, S., Wells, D., 2017. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. Fertil. Steril. 107, 1085–1091.

Munné, S., Tang, Y.X., Weier, H.U.G., Stein, J., Filkenstein, M., Grifo, J., et al., 1993. Sex distribution in arrested precompacted human embryos. Zygote 1 (2), 155–162.

Munné, S., Weier, H.U.G., Grifo, J., Cohen, J., 1994. Chromosome mosaicism in human embryos. Biol. Reprod. 51 (3), 373–379.

Munné, S., Alikani, M., Tomkin, G., Grifo, J., Cohen, J., 1995. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil. Steril. 64 (2), 382–391.

Munné, S., Sandalinas, M., Escudero, T., Marquez, C., Cohen, J., 2002. Chromosome mosaicism in cleavage-stage human embryos: evidence of a maternal age effect. Reprod. Biomed. Online 4 (3), 223–232.

Munné, S., Ary, J., Zouves, C., Escudero, T., Barnes, F., Cinioglu, C., Ary, B., 2006. Cohen J Wide range of chromosome abnormalities in the embryos of young egg donors. Reprod. Biomed. Online 12 (3), 340–346.

Munné, S., Chen, S., Colls, P., Garrisi, J., Zheng, X., Cekleniak, N., et al., 2007. Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. Reprod. Biomed. Online 14 (5), 628–634.

Munné, S., Grifo, J., Wells, D., 2016. Mosaicism: "survival of the fittest" versus "no embryo left behind. Fertil. Steril. 105 (5), 1146–1149.

Munné, S., Blazek, J., Large, M., Martinez-Ortiz, P.A., Nisson, H., Liu, E., Tarozzi, N., Borini, A., Becker, A., Zhang, J., Maxwell, S., Grifo, J., Babariya, D., Wells, D., Fragouli, E., 2017a. Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected by high resolution next generation sequencing. Fertil. Steril. 107, 1113–1119.

Munné, S., Alikani, M., Ribustello, L., Colls, P., Martínez-Ortiz, P.A., Referring Physician Group*, McCulloh, D.H., 2017b. Euploidy rates in donor egg cycles significantly differ between fertility centers. Hum. Reprod. 32, 743–749.

Pabon, J.E., Harton, G., Seabaugh, A., Maitta, R., Pabon, V., Srivastava, R.K., 2005. Successful implantation and ongoing pregnancy of a single monosomy 16 preimplantation embryo: case report. Fertil. Steril. 84 (Suppl. 1), S333.

Paulson, R.J., 2017. Preimplantation Genetic Screening: what is the clinical efficiency? Fertil. Steril. 108, 228–230.

PGDIS, 2016. PGDIS Position Statement on Chromosome Mosaicism Andpreimplantation Aneuploidy Testing at the Blastocyst Stage. Newsletter.

Rodriguez-Purata, J.L.J., Whitehouse, M., Duke, M., Grunfeld, L., Sandler, B., Copperman, A., et al., 2016. Reproductive outcome is optimized by genomic embryo screening, vitrification, and subsequent transfer into a prepared synchronous endometrium. Assist. Reprod. Genet. https://doi.org/10.1007/s10815-016-0647-y. (in press).

Rubino, P., Dearden, L., Guan, L., Ruiz De Assin, R., Mazmanian, K., Kolb, B.A., Nelson, J., Norian, J.M., Wilcox, J., Tan, T., 2016. Healthy baby after intrauterine transfer of monosomic embryos. Fertil. Steril. 106 (Suppl. l), e160 (P-145).

Ruttanajit, T., Chanchamroen, S., Cram, D.S., Sawakwongpra, K., Suksalak, W., Leng, X., et al., 2016. Detection and quantitation of chromosomal mosaicism in human blastocysts using copy number variation sequencing. Prenat. Diagn. 36 (2), 154–162.

Sandalinas, M., Sadowy, S., Alikani, M., Calderon, G., Cohen, J., Munné, S., 2001. Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. Hum. Reprod. 16 (9), 1954–1958.

Scott Jr., R.T., Galliano, D., 2016. The challenge of embryonic mosaicism in preimplantation genetic screening. Fertil. Steril. 105 (5), 1150–1152.

Scott Jr., R.T., Ferry, K., Su, J., Tao, X., Scott, K., Treff, N.R.T., 2012. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil. Steril. 97, 870–875.

Spinella, F., Fiorentino, F., Biricik, A., Bono, S., Ruberti, A., Cotroneo, E., Baldi, M., Cursio, E., Minasi, M.G., Greco, E., 2018. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. Fertil. Steril. 109 (1), 77–83.

Weier, J.F., Weier, H.U.G., Jung, C.J., Gormley, M., Zhou, Y., Chu, L.W., et al., 2005. Human cytotrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype. Dev. Biol. 279 (2), 420–432.

Wells, D.K.K., Rico, A., Grifo, J., Anderson, S., Sherlock, J., Taylor, J.C., et al., 2014. Clinical utilization of a rapid low-pass whole-genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation. J. Med. Genet. 51, 553–562.