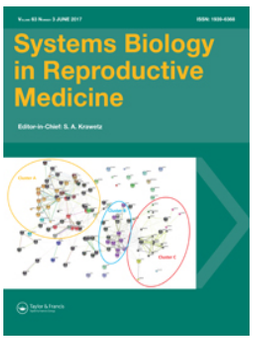

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Implantation potential of mosaic embryos

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ABSTRACT

Chromosomal mosaicism is a relatively common finding in human IVF embryos. However, the association between mosaicism in trophoectoderm and inner mass cells, the mechanisms involved, and its effects on implantation are far from established. We retrospectively reanalyzed array-CGH results from 1,362 trophoectoderm biopsies. We detected chromosomal mosaicism in 183 blastocysts (13.4%). A decrease in the clinical pregnancy rate when we compared the cycles where only mosaic embryos were transferred (26.9%) vs. euploid embryos were transferred (40.2%) was not statistically different ($p = 0.127$). Also a tendency to increase the biochemical miscarriage was reported (21.2% mosaic group vs. 12.3% euploid group; p=0.102). Our data suggests that the transfer of some mosaic embryos achieve full term pregnancies. Additional studies are needed to clarify how embryo mosaicism affects the outcomes of the IVF cycles.

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Comprehensive chromosome screening (CCS); embryo mosaicism; full term pregnancy

Introduction

Preimplantation genetic screening (PGS) is widely used to identify and transfer euploid embryos improving the clinical outcome of IVF [Scott et al. 2013]. Even though the use of PGS has led to an improvement of IVF outcomes some morphologically normal euploid blastocysts fail to implant and progress to delivery. Chromosomal mosaicism is a common phenomenon in IVF-derived embryos and has been considered as a feasible explanation for some failures after the transfer of euploid embryos [Taylor et al. 2014]. Although mosaicism in cleavage stage embryos has been reported [Chow et al. 2014], their true incidence at the blastocyst stage remains unclear. In addition, considering mosaic embryos as aneuploid embryos is a controversial topic [Scott and Galliano 2016], since the association between mosaicism in trophoectoderm cells and inner mass cells is unknown. Moreover, it seems that there is a mechanism by which mosaicism could be corrected [Bolton et al. 2016]. To clarify whether mosaic embryos should be discarded we compared the outcomes of transfer cycles with mosaic and euploid blastocysts.

Results and discussion

The array-CGH results from trophoectoderm biopsies of day 5 and 6 blastocysts (n = 1,362) were reanalyzed using the new bioinformatics method described in the material and methods section. We detected chromosomal mosaicism in 183 blastocysts (13.4%). Moreover, 50.5% of the analyzed embryos were euploid, 31.9% aneuploid, and the remaining 4.3 were without diagnosis. In the mosaic group, 52.5% were euploid embryos with mosaicism and 47.5% were also aneuploid. In the euploid-mosaic embryos, the frequency of embryos with mosaicism in only one mosaic chromosome was 57.3% vs. 42.7% for those with mosaicism in two or more chromosomes.

The outcomes of the cycles were compared between the cycles where only euploid-mosaic embryos were transferred and the cycles where euploid embryos were transferred (Table 1). Although the outcomes of transfer cycles seem to be lower among the mosaic group, the differences with regard to pregnancy rate (48.1% vs. 52.5%; $p = 0.572$), implantation rate (26.9% vs. 37.2%; $p = 0.224$), and miscarriage rate (7.1% vs. 18.1%; $p = 0.354$) were considered not statistically significant when they were analyzed using embryo quality as a confounding factor. A tendency could be observed in the biochemical miscarriage (21.2% in mosaic group vs. 12.3% in euploid group; $p = 0.102$) and clinical pregnancy rate (26.9 in mosaic group vs. 40.2% in euploid group; $p = 0.127$). The low statistical power and sample size could be the reason why statistical significance was not reached. In the mosaic group, according to the number of chromosomes with mosaicism, no significant differences were reported, however lower outcomes were shown in the cycles where embryos carrying two or more mosaic chromosomes were transferred (Table 1).

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Table 1. Clinical outcome of cycles from euploid-mosaic and euploid embryos.

	MOSAIC	EUPLOID	*p*	1-chromosome mosaic	≥2 chromosome mosaic	*p*
FEMALE AGE (y)	31.0	30.6	0.687	31.6	30.3	0.531
CYCLES WITH TRANSFER	52	322		29	23	
TRANSFERRED EMBRYOS	52	382		30	24	
POSITIVE PREGNANCY RATE (%)	48.1	52.5	0.572	58.6	34.8	0.099
BIOCHEMICAL MISCARRIAGE (%)	21.2	12.3	0.102	24.1	17.4	0.622
CLINICAL PREGNANCY (%)	26.9	40.2	0.127	34.5	17.4	0.061
IMPLANTATION RATE (%)	26.9	37.2	0.224	36.7	20.8	0.089
MISCARRIAGE RATE (%)	7.1	18.1	0.354	10	0	0.999
HEALTHY BABIES	10	54		6	4	
BIRTH WEIGHT (Kg) (Mean±SD)	2.9±0.6	2.8±0.5		2.6±0.3	3.2±0.7	
DURATION OF PREGNANCY (weeks) (Mean±SD)	37.4±2.0	37.4±3.5		36.6±2.2	38.5±1.2	

Logistic regression using embryo quality as confounding factor.

This data suggests that the transfer of mosaic embryos could affect the outcomes of the IVF cycles. Mosaic embryos are usually compromised by the presence of aneuploid cells and have reduced developmental potential. Even so, the mosaic embryos can yield IVF success and therefore they should not be discarded in couples that do not have euploid embryos for transfer. If detected during routine comprehensive chromosome screening (CCS) cycle treatment, mosaic embryos should not necessarily be excluded, but they should be given a lower priority for transfer than those that appear to be fully euploid, as the likelihood of producing a child could be reduced.

An important concern about the transfer of mosaic embryos is its clinical consequences. Table 2 summarizes the clinical outcome of the cycles where euploid mosaic embryos were transferred. No patterns of mosaicisms were detected between mosaic embryos and the outcome of the cycle. The clinical effects of mosaicism depend on a variety of factors: the moment when the error occurs during the development and if the error can continue to propagate. The prevalence and consequences of mosaicism are much more pronounced when it is detected during the cleavage-stage than during the blastocyst or prenatal testing. This would indicate a selection mechanism against mosaicism in the later stages of development. It is evident that the effects of the mosaicism depend greatly on the location of the mosaic cell line along with what chromosome(s) is involved [Taylor et al. 2014].

Our results add to the literature regarding the incidence of blastocyst mosaicism and birth after the transfer of mosaic embryos [Greco et al. 2015]. Establishing the prevalence of embryo mosaicism is required to calculate the CCS error rate. These errors may be the result of a biological error because the mosaicism remained undetected in the analysis [Munne et al. 2016]. It has been suggested that anaphase lag leading to chromosome loss is the most common mechanism causing mosaicism. Larger sample size studies could help us to determine if differences in mosaicism vary in different subpopulations of patients, such as advanced maternal age, recurrent implantation failure, repeated pregnancy loss, and male factor. Moreover, these data may also be useful for obstetricians counseling those couples considering CCS and these patients once they become pregnant. Pregnancies established after transfer of mosaic embryo should be subjected to prenatal testing to confirm the absence of aneuploidies in the fetus.

Table 2. Chromosomal constitution and outcome of cycles from euploid-mosaic.

No. Embryo(s)	Chromosomal constitution	Mosaicism (%)	Sex	Clinical Outcome	Birth Weight (Kg)	Duration of pregnancy (weeks)
1	arr 3(q12.1q27.1)x1	25-37	XX	Baby healthy at birth	2.3	36
2	arr(17)x3,(19)x3	25-37	XX	Baby healthy at birth	4.2	40
3	arr(13)x1,(17)x3	25-37	XX	Baby healthy at birth	2.5	37
4	arr(20)x3	25-37	XY	Baby healthy at birth	2.9	36
5	arr 4(q21.3q35.1)x3	25-37	XX	Baby healthy at birth	2.7	40
6	arr 17(q12q25.2)x3	37-50	XY	Baby healthy at birth	2.3	34
7	arr(5)x3,(8)x1,(18)x3	37-50	XX	Baby healthy at birth	3.3	38
8	arr(1)x3	25-37	XY	Baby healthy at birth	2.7	37
9	arr(4)x1,(7)x1,(17)x3,(19)x3,(22)x3	25-37/37-50	XX	Baby healthy at birth	2.9	39
10	arr 9(q12q21.1)x1	25-37	XY	Baby healthy at birth	3.0	38
11	arr 9(q12q21.1)x1	37-50	XX	Ongoing pregnancy		
12	arr(20)x1	37-50	XY	Ongoing pregnancy		
13	arr(9)x1	25-37	XY	Ongoing pregnancy		

Material and methods

We retrospectively reanalyzed array-CGH results from trophoectoderm biopsies of day 5 and 6 blastocysts (from January 2014 to December 2015). CCS was performed with couples who attended the Instituto Bernabeu with advanced maternal age, abnormal sperm FISH, and/or a history of recurrent miscarriage or implantation failure. All the couples gave their written informed consent for the procedure. This study involved only retrospective analysis of anonymous medical records and was approved by the Instituto Bernabeu Institutional Review Board. A total of 1,362 embryos were included. Whole Genome Amplification was performed using Picoplex kit (Rubicon Genomics®, Ann Arbor, MI, USA) according to the manufacturer's instructions. Array-CGH analysis was performed using Agilent SurePrint G3 8x60K (Agilent Technologies®, Palo Alto, CA, USA) CGH microarrays with previous whole genome amplification of genomic DNA (Picoplex, Rubicon Genomics®). Previous euploid diagnosis for a given chromosome was assigned considering log2ratio lower than ±0.3. We reanalyzed the results using a new bioinformatics method in the Cytogenomics v2.5 software (Agilent Technologies®). This method allowed us to identify mosaic embryos when the log2ratio was between 0.17 and 0.3 (percentage of aneuploid cells ≥25%). To validate the algorithm a sample of mosaic embryos were evaluated and the percentage of aneuploidy cells determinate by Next Generation Sequencing (VeriSeq Illumina®, San Diego, CA, USA). One hundred and eighty-three embryos were diagnosed as mosaic. Fifty-two euploid-mosaic embryos and 382 euploid embryos were transferred and had a known clinical outcome. Regarding the number of chromosomes with mosaicism of the transferred embryos, 30 euploid-mosaic embryos carried one mosaic chromosome, while 24 embryos carried two or more mosaic chromosomes. The main outcome measures were implantation rate, positive pregnancy rate, biochemical and clinical miscarriage rates, and clinical pregnancy rate. The differences between groups were evaluated using the logistic regression statistical test (SPSSv20.0). The embryo quality showed significant differences between the groups and was included as a confounding factor in the statistical analysis.

Declaration of interest

The material contained in the manuscript is original, has not been published, has not been submitted or is not being submitted elsewhere. The authors report no conflicts of interest.

Notes on contributors

Study design: BL, RM, JAO; Sample collection: JT; Laboratory experiments: BL, HB, JAO, RM; Analysis: BL, HB, JAO, RM; Drafting manuscript: BL, JL, JT, RB; Critical discussion: BL, HB, JAO, RM, JL, JT, RB.

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