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### Preimplantation genetic screening: a reappraisal

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# Chapter 4

## Chromosomal mosaicism in human preimplantation embryos: a systematic review

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## **Abstract**

### **Background**

Although chromosomal mosaicism in human preimplantation embryos has been described for almost two decades, its exact prevalence is still unknown. The prevalence of mosaicism is important in the context of preimplantation genetic screening in which the chromosomal status of an embryo is determined by the analysis of a single cell from that embryo.

### **Methods**

Here we report a systematic review and meta-analysis of studies on the chromosomal constitution of human preimplantation embryos. In 36 studies, out of 2117 citations that met our search criteria, data was provided extensively enough to allow classification of each analysed embryo with pre-specified criteria for its chromosomal makeup. The main outcome of this classification was the prevalence of chromosomal mosaicism in human preimplantation embryos.

### **Results**

A total of 815 embryos could be classified. Of these, 177 (22%) were diploid, 599 (73%) were mosaic, of which 480 (59% of the total number of embryos) were diploid-aneuploid mosaic and 119 (14% of the total number of embryos) were aneuploid mosaic, and 39 (5%) contained other numerical chromosomal abnormalities. The distribution of the embryos over these categories was associated with the developmental stage of the embryos, the method used for analysis and the number of chromosomes analysed.

### **Conclusions**

Diploid-aneuploid mosaicism is by far the most common chromosomal constitution in spare human preimplantation embryos after in vitro fertilisation. This undermines the reliable determination of the ploidy status of a cleavage stage embryo based on the analysis of a single cell. Future research should determine the origin and developmental potential of mosaic embryos.

The introduction of human in vitro fertilisation (IVF) into clinical practice made it possible to study human embryos in the earliest stages after conception and it was rapidly discovered that numerical chromosome abnormalities, i.e. aneuploidies, exist in human preimplantation embryos (Steptoe and Edwards, 1978; Angell et al., 1983). In 1993 chromosomal mosaicism, the phenomenon that not all cells in an embryo have the same chromosomal content, was described in human preimplantation embryos for the first time (Delhanty et al., 1993). Since then many studies have been published on this topic, with mosaicism rates varying between 15 percent (Harper et al., 1995) and more than 90 percent (Daphnis et al., 2005).

One of the reasons for these varying rates of mosaicism in the literature are the different definitions of mosaicism that have been used. For example, in many studies embryos were classified as 'diploid' or 'normal' and not as 'mosaic' despite the presence of a certain percentage (up to 50%) of aneuploid blastomeres in these embryos (e.g. (Munne et al., 1995; Ziebe et al., 2003; Baart et al., 2006)). The reason provided by these authors to classify such embryos as diploid is that they consider these embryos to be viable and therefore a low percentage of aneuploid cells in an otherwise diploid embryo would be clinically irrelevant.

The prevalence of mosaicism is highly relevant for preimplantation genetic screening (PGS) in which selection of embryos for transfer into the uterus is often based on the chromosomal analysis of one aspirated blastomere. A recent meta-analysis of randomized controlled trials showed that PGS fails to improve live birth rates after IVF (Mastenbroek et al., 2011). One of the possible causes for this may be mosaicism, particularly diploid-aneuploid mosaicism, where an embryo consists of both diploid and aneuploid cells.

In view of the varying mosaicism rates reported so far, we undertook a systematic review and meta-analysis of studies on the chromosomal constitution of human preimplantation embryos. We used a pre-specified set of classification criteria to combine the outcomes of these studies, to determine the exact prevalence of mosaicism in human preimplantation embryos, regardless of its consequences for viability. We used the outcomes of this review to discuss mosaicism in relation to PGS efficacy.

## Methods

PubMed ([www.pubmed.gov](http://www.pubmed.gov)) was searched using the following search criteria: "(mosaic OR mosaic OR aneuploidy OR aneuploidies) AND (embryo OR embryos)" with the following limits: Language: English, Publication Date: 1980/01/01 to 2010/01/01 and Humans or animals: Humans. The resulting titles and abstracts were scanned for relevancy independently by two authors (JvEA / BSR) and reference lists were cross-checked for other potentially relevant studies. All studies analysing the chromosomal constitution of human preimplantation embryos were considered. Reviews, letters, editorials and congress abstracts were excluded. After retrieval of full text articles, studies were excluded if: (1) the study dealt with embryos of which only a single biopsied cell was analysed, (2) there was no or incomplete information

on the embryos that were analysed or on the individual cells per embryo analysed, (3) the information was based on preimplantation genetic diagnosis for chromosomal abnormalities other than aneuploidies, (4) the study reported on tripronuclear embryos only, (5) data was overlapping with another publication, (6) embryos were developed through techniques not commonly used in IVF or (7) the study reported only on embryos consisting of less than three cells.

For each embryo of the included studies the following data was retrieved: origin (a spare embryo from a regular IVF cycle or a spare embryo from a PGD cycle), developmental history (a developing or an arrested embryo), timing of analysis (day of preimplantation development), developmental stage (cleavage stage or blastocyst stage), method of analysis (FISH, CGH, PRINS, PCR, array), number of chromosomes analysed, and number of cells with a result.

Subsequently, the same two authors categorized independently each embryo of the included studies according to the pre-specified criteria (Table 1). In addition, the percentage of diploid cells in each diploid-aneuploid mosaic embryo was noted. Any disagreement was resolved by a third author (SM). From CGH and array analyses only numerical chromosome aberrations were retrieved.

**Table 1. Classification criteria for the chromosomal makeup of human preimplantation embryos.**

Chromosomal makeup	Criteria*	FISH examples for X,Y and 18**
Diploid	All cells contain two chromosomes for each chromosome pair tested	XX,1818[7] X,Y,1818[7]
Mosaic	Not all cells contain the same chromosomal makeup	
Diploid-aneuploid mosaic	A mosaic embryo with one or more diploid cells	XX,1818[5]/XX,18[2] XY,1818[3]/XY,181818[4]
Aneuploid mosaic	A mosaic embryo without the presence of diploid cells	XX,181818[3]/XXX,181818[4] X,18[1]/X,181818[4]/X,1818[2]
Other abnormalities		
Haploid	All cells contain one chromosome for each chromosome pair tested	X,18[7] Y,18[7]
Polyploid	All cells contain more than two chromosomes for each chromosome pair tested	XXX,181818 [7] XXYY,18181818 [7]
Aneuploid	All cells contain the same abnormality for one chromosome pair tested	XX,18 [7] XX,181818 [7]
Complex abnormal	All cells contain the same abnormalities for multiple chromosome pairs tested	X,181818 [7] XXY,18 [7]

\* Embryo should have at least three cells. Criteria can be used for cleavage stage as well as blastocyst stage embryos.

\*\* For each category two examples are provided for illustrative purposes. Number between brackets is the number of cells.

With a chi-square test we analysed whether the distribution of the embryos over the different categories (diploid, diploid-aneuploid mosaic, aneuploid-mosaic and other abnormalities) was confounded by the collected variables. Due to the possible covariance between embryos from the same cycle, couple, center or study, an overestimation of the analysed effect could

be expected. Therefore we considered P values of  $<0,01$  to be significant. In addition we determined the mosaicism rate in developing cleavage stage embryos which were analysed for eight or more chromosomes.

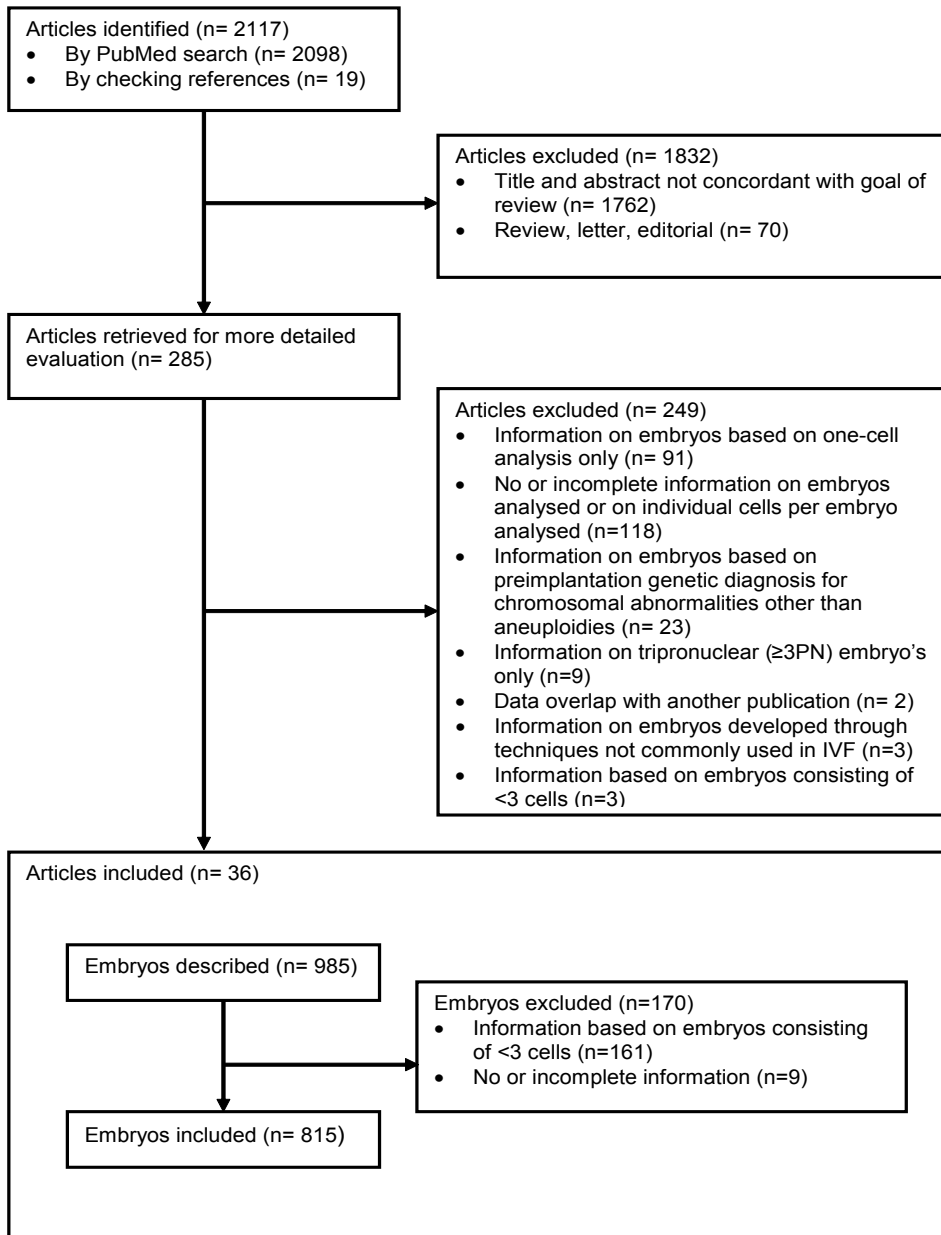


Figure 1. Flow diagram summarising inclusion of articles.

## Results

Our search identified 2117 citations. Of these, 1832 were excluded based on their title and abstract and 284 were retrieved for more detailed evaluation (Figure 1 and Supplementary Appendix). This led to the further exclusion of 249 studies (Figure 1 and Supplementary Appendix), leaving 36 studies that fulfilled the inclusion criteria and that provided the chromosomal constitution of each separate cell of each embryo analysed (Schrurs et al., 1993; Delhanty et al., 1993; Munne and Cohen, 1993; Munne et al., 1993a; Munne et al., 1993b; Harper et al., 1994; Munne et al., 1994a; Munne et al., 1994b; Harper et al., 1995; Jakobsson et al., 1995; Kligman et al., 1996; Pellestor et al., 1996a; Pellestor et al., 1996b; Iwarsson et al., 1999; Veiga et al., 1999; Bielanska et al., 2000; Magli et al., 2000; Harrison et al., 2000; Wells and Delhanty, 2000; Emiliani et al., 2000; Voullaire et al., 2000; Ruangvutilert et al., 2000b; Katz et al., 2002; Voullaire et al., 2002; Gonzalez-Merino et al., 2003; Liu and Zhu, 2003; Trussler et al., 2004; Baart et al., 2004a; Baart et al., 2004b; Daphnis et al., 2005; Chatzimeletiou et al., 2005; Baart et al., 2006; Le Caignec et al., 2006; Baart et al., 2007b; Daphnis et al., 2008; Vanneste et al., 2009b). These 36 studies reported on a total of 976 embryos of which 815 could be included and categorized according to the pre-specified criteria.

Of these 815 embryos, 177 (22%) were diploid, 599 (73%) were mosaic, and 39 (5%) contained other abnormalities (Table 2 and Supplementary Appendix). The 599 mosaic embryos could be divided into 480 embryos (59% of the total number of embryos) that were diploid-aneuploid mosaic and 119 embryos (15% of the total number of embryos) that were aneuploid mosaic (Table 2 and Supplementary Appendix). Seventy-two percent of the cells of the diploid-aneuploid mosaic embryos (10155/14116) was diploid.

**Table 2. Summary of the findings of 36 studies on the chromosomal makeup of human preimplantation embryos**

	All embryos (n=815)		Developing, cleavage stage embryos analysed for ≥8 chromosomes (n=107)	
<b>Diploid</b>	177	22%	15	14%
<b>Mosaic</b>	599	73%	77	72%
Diploid-aneuploid mosaic	480	59%	49	46%
% diploid cells	(10155/14116)	72%	(151/324)	47%
Aneuploid mosaic	119	15%	28	26%
<b>Other abnormalities</b>	39	5%	15	14%
<b>Haploid</b>	3	<1%	1	1%
<b>Polyploid</b>	5	<1%	1	1%
<b>Aneuploid</b>	18	2%	4	4%
Monosomy	13	2%	3	3%
Trisomy	5	<1%	1	1%
<b>Complex abnormal</b>	13	2%	9	8%

Subgroup analysis showed that the origin of the embryos ( $p$  0,03) and the developmental history of the embryo ( $p$  0,14) were not significantly correlated with the distribution of the embryos over the different categories of chromosomal makeup (diploid, diploid-aneuploid

mosaic, aneuploid-mosaic and other abnormalities) (Table 3). The developmental stage at which the embryo was analysed ( $p<0.001$ ), the method of analysis ( $p<0.001$ ) and the number of chromosomes analysed ( $p<0.001$ ) did correlate with the distribution of the embryos over the different categories of chromosomal makeup. The incidence of diploid embryos was lower and the incidence of diploid-aneuploid mosaic embryos was higher if more chromosomes were analysed. Similarly, the incidence of diploid embryos was lower and the incidence of diploid-aneuploid mosaic embryos was higher in blastocysts compared to cleavage stage embryos. The percentage of diploid cells in diploid-aneuploid mosaic blastocysts was also higher than in cleavage stage embryos. The incidence of diploid embryos and diploid-aneuploid mosaic embryos was lower when CGH was used in comparison to FISH.

**Table 3. Analysis of confounding factors in the distribution of embryos over different categories.**

	Origin of the embryo				Developmental history				Developmental stage					
	spare IVF		spare PGD		developing		arrested		cleavage		blastocyst			
	n=520		n=295		n=631		n=184		n=548		n=267			
Diploid	126	24%	51	17%	128	20%	49	27%	169	31%	8	3%		
Mosaic														
Diploid-aneuploid mosaic	306	59%	174	59%	383	61%	97	53%	251	46%	229	86%		
% diploid cells		73%		71%		73%		61%		62%		74%		
Aneuploid mosaic	65	13%	54	18%	88	14%	31	17%	95	17%	24	9%		
Other abnormalities	23	4%	16	5%	32	5%	7	4%	33	6%	6	2%		
p value	p=0.03				p=0.14				p<0.001					
	Method of analysis						Number of chromosomes analysed							
	FISH		CGH		other		<3		3-5		6-10		>10	
	n=719		n=70		n=26		n=191		n=236		n=214		n=174	
Diploid	160	22%	11	16%	6	23%	115	60%	21	9%	26	12%	15	9%
Mosaic														
Diploid-aneuploid mosaic	440	61%	34	49%	6	23%	61	32%	166	70%	143	67%	114	63%
% diploid cells		72%		52%		54%		62%		77%		63%		74%
Aneuploid mosaic	95	13%	14	20%	10	38%	8	4%	41	17%	38	18%	32	18%
Other abnormalities	24	3%	11	16%	4	15%	7	4%	8	3%	7	3%	17	10%
p value	p<0.001						p<0.001							

Developing cleavage stage embryos which were analysed for eight or more chromosomes ( $n=107$ ) showed a diploid-aneuploid mosaicism rate of 46% with a mean of 47% diploid cells in these diploid-aneuploid mosaic embryos (Table 2). Eighteen of these embryos were analysed with FISH (Harrison et al., 2000; Baart et al., 2007a), 70 with CGH (Voullaire et al., 2000; Wells and Delhanty, 2000; Voullaire et al., 2002; Trussler et al., 2004; Le Caignec et al., 2006), and 19 with an array-based method (Vanneste et al., 2009b).



## Discussion

Our systematic review of the literature showed diploid-aneuploid chromosomal mosaicism to be the most common chromosomal constitution in spare human preimplantation embryos after in vitro fertilisation. Out of 815 embryos, only 177 (22%) were diploid, 599 (73%) were mosaic, and 39 (5%) contained other abnormalities. Of the mosaic embryos 480 (59% of the total number of embryos) were diploid-aneuploid mosaic.

The outcomes of our review could be flawed by the use of spare embryos after IVF. Not surprisingly, the studies included in this review mainly used spare embryos rather than embryos that are transferred or cryopreserved. When evaluating the prevalence of mosaicism it is best to know the chromosomal constitution of the total cohort of embryos before selection, transfer or cryopreservation. When performing our review we encountered only one study that performed such an analysis (Ziebe et al., 2003). This study could not be included in our review since it did not provide information on all cells of each embryo analysed, but it did report that 55% of the embryos (57 out of 103) was diploid-aneuploid-mosaic and that 55% of all blastomeres from these embryos (235 out of 424) was diploid. A subset of embryos from our review that resembled the conditions in this study best, i.e. all cleavage stage, developing embryos which were analysed by FISH for five to fifteen chromosomes (Munne et al., 1993a; Kligman et al., 1996; Iwarsson et al., 1999; Harrison et al., 2000; Gonzalez-Merino et al., 2003; Baart et al., 2007a), showed 54% of the embryos (66 out of 123) to be diploid-aneuploid-mosaic with 49% of all blastomeres (521 out of 1064) being diploid. Thus, although based on just one available study, the data from spare embryos included in our review seem not to differ much from the total cohort of embryos after in vitro fertilisation.

Nearly all studies (88%) analysed embryos by FISH. It is well known that FISH analysis has an accuracy per probe of 92-99% (Ruangvutilert et al., 2000a), so when using a multi-probe panel on a single cell there is an inherent risk of misdiagnosis. Since multiple cells were analysed per embryo, suboptimal FISH accuracy could potentially have skewed the observed rates of chromosomal abnormalities and mosaicism in this review. It still needs to be confirmed whether novel promising methods of analysis, such as those based on array-technology (Wells et al., 2008), can achieve higher accuracy rates than FISH for this purpose. The first studies conducted with these novel array methods also show mosaicism to be present, thereby confirming the conclusions of our review, but again, with varying percentages (Vanneste et al., 2009a; Vanneste et al., 2009b; Treff et al., 2010). Future research using these technologies should further assess the mosaicism rates found in this review.

The high rate of chromosomal mosaicism found in our review of the literature might indicate that mitotic errors are a common feature of human preimplantation development, even though the molecular basis for this remains elusive. The human embryonic genome is not active until the eight cell stage (Braude et al., 1988), which makes the first cleavage divisions fully dependent on maternally derived gene transcripts and proteins stored in the oocyte. By studying human embryonic stem cells it was hypothesized that high levels of mitotic and cell cycle proteins from the oocytes are necessary to prevent aneuploidy formation and for

prompt activation of the cell cycle checkpoint machinery (Ambartsumyan and Clark, 2008). The quality of human oocytes and their gene transcripts and proteins could diminish over time by the accumulation of radiation or toxic agents, oxidative stress (Tarin, 1996), compromised mitochondria (Keefe et al., 1995), or telomere shortening (Keefe et al., 2006). This may result in a less functional cell cycle checkpoint mechanisms, especially in women of advanced maternal age, which may lead to chromosomal segregation errors in the first cleavages of human preimplantation embryos and thus to mosaicism (Harrison et al., 2000; Delhanty, 2005; Steuerwald, 2005). However, high rates of numerical chromosomal abnormalities were also found in embryos from young women suggesting that these abnormalities are not exclusively related to maternal age (Baart et al., 2006; Vanneste et al., 2009b). Unfortunately, in our systematic review we were not able to determine whether maternal age was a confounding factor, since only a minority of studies reported on maternal age for each embryo separately.

Chromosomal mosaicism might also be an induced phenomenon in an IVF treatment caused by the ovarian hyperstimulation and/or in vitro culture of human preimplantation embryos. Indeed, it has been shown that the intensity of ovarian hyperstimulation could influence the rate of chromosomal mosaicism (Baart et al., 2007b). It has also been shown in mouse model studies that changes in oxygen tension during embryo culture could affect chromosomal mosaicism rates (Bean et al., 2002). The question whether chromosomal mosaicism is a physiological or a pathological phenomenon in humans deserves future attention.

The varying definitions of mosaicism that have been used in the literature, most importantly the classification of embryos as 'diploid' or 'normal' and not as 'mosaic' despite the presence of a certain percentage (up to 50%) of aneuploid blastomeres in these embryos (e.g. (Munne et al., 1995; Ziebe et al., 2003; Baart et al., 2006)), explains, at least in part, the varying mosaicism rates reported. Our meta-analysis indicates that three other factors are also important: the number of chromosomes analysed (<3, 3-5, 6-10, >10), the method of analysis (FISH, CGH, other), and the developmental stage at which the embryos were analysed (cleavage stage or blastocyst stage). The incidence of diploid embryos was lower and the incidence of diploid-aneuploid mosaic embryos was higher when more chromosomes were analysed. Analysing all chromosomes by CGH showed higher aneuploidy rates compared to FISH analysis. The underlying mechanism may well be that more aneuploidies are detected when more chromosomes are tested, since the aneuploidies found in human preimplantation embryos are not limited to one specific chromosome. The lower incidence of fully diploid blastocysts as compared to cleavage-stage embryos could be caused by the fact that the chance that one or more mitotic errors have occurred is simply higher in blastocysts as these embryos have undergone more mitotic divisions. The incidence of diploid-aneuploid mosaicism was indeed found to be higher among blastocysts. Also contributory to the high rate of diploid-aneuploid mosaicism in blastocysts could be that diploid-aneuploid mosaic embryos more easily reach blastocyst stage in comparison with embryos containing only aneuploid blastomeres because of apoptosis or arrest of aneuploid cells during preimplantation development (James and West, 1994; Gonzalez-Merino et al., 2003). Indeed less completely aneuploid blastocysts were found compared to cleavage stage embryos. The higher percentage of diploid cells in diploid aneuploid blastocysts compared to cleavage-stage embryos (74% versus 62% respectively)

also supports this hypothesis.

To determine whether the observed rates of diploid-aneuploid mosaicism underlie the poor outcomes after PGS in clinical trials (Mastenbroek et al., 2011), we evaluated a subset of developing, cleavage stage embryos which were analysed for at least eight chromosomes (Table II), as this resembles clinical practice in recent years and current guidelines best (Harper et al., 2010; Harton et al., 2011a; Harton et al., 2011b). The use of FISH in this subgroup is perhaps underrepresented (18 out of 107 embryos in this subset were analysed by FISH), but this fits with the current trend to analyse all chromosomes in PGS (89 out of 107 embryos were analysed for all chromosomes). If we assume that the data in this subset is representative for the cohort of embryos in which PGS is applied, then, in 22% ( $0,46 \pm 0,47$ ) of all embryos in PGS a diploid cells is biopsied, while the remaining embryo is at least in part aneuploid. These embryos are potentially transferred, but with one diploid cell missing. Furthermore, in 24% ( $0,46 \pm 0,53$ ) of all embryos in PGS an aneuploid cell is biopsied, while the remaining embryo contains one or more diploid cells. These potentially viable embryos are discarded, thereby lowering the number of viable embryos in an IVF treatment. It seems logical to assume that this results in an overall decrease in live birth rates. It is clear however, as previously suggested (Vanneste et al., 2009a), that one of the rationales behind cleavage stage PGS, i.e. that the biopsied cell is representative for the entire embryo, is incorrect.

In regard to the above reasoning, it is important to know the probability of diploid-aneuploid mosaic embryos to result in live birth. Indirect evidence supports the idea that diploid-aneuploid mosaic embryos are viable. The injection of donor ES cells of which only a small percentage were diploid (20% diploid cells combined with 80% cells with chromosomal abnormalities) in tetraploid blastocysts resulted in fully diploid normal adult mice (Eggan et al., 2002). Since tetraploid cells are excluded from the embryo proper, offspring resulting from these injected blastocysts must have originated from the injected ES cells. These results give evidence of selection against aneuploid cells during development (Eggan et al., 2002). Similar mechanisms, such as preferential allocation of diploid cells to the inner cell mass or embryo proper, preferential cell proliferation of diploid cells, loss of aneuploid cells through cell death, or aneuploidy rescue by anaphase lagging or chromosome demolition, have been suggested for human embryos once the embryonic genome has been activated (Los et al., 2004). Finally, frozen-thawed human embryos that lost nearly half of their blastomeres due to the cryopreservation procedure are still able to result in live births, implying that not all blastomeres of human preimplantation embryos are necessary for proper development into a child (Munne et al., 1995; Edgar et al., 2007).

In conclusion, diploid-aneuploid mosaicism is by far the most common chromosomal constitution of human preimplantation embryos after in vitro fertilisation. This undermines the reliable determination of the ploidy status of an in vitro embryo based on the analysis of a single cell. Further studies are needed to elaborate on the origin of chromosomal mosaicism in human preimplantation embryos, on factors that affect its incidence, as well as on the fate of diploid-aneuploid mosaic embryos.

## Supplementary data

Supplementary data are available at <http://humupd.oxfordjournals.org>.

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