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Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos

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STUDY QUESTION: What are the aneuploidy rates and incidence of mosaicism in good-quality human preimplantation embryos.

SUMMARY ANSWER: High-level mosaicism and structural aberrations are not restricted to arrested or poorly developing embryos but are also common in good-quality IVF embryos.

WHAT IS KNOWN ALREADY: Humans, compared with other mammals, have a poor fertility rate, and even IVF treatments have a relatively low success rate. It is known that human gametes and early preimplantation embryos carry chromosomal abnormalities that are thought to lower their developmental potential.

STUDY DESIGN, SIZE AND DURATION: The embryos studied came from nine young (age <35 years old) IVF patients and were part of a cohort of embryos that all resulted in healthy births. These 14 embryos inseminated by ICSI and cryopreserved on Day 2 of development were thawed, cultured overnight and allowed to succumb by being left at room temperature for 24 h. Following removal of the zona pellucida, blastomeres were disaggregated and collected.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: There were 91 single blastomeres collected and amplified by multiple displacement amplification. Array-comparative genomic hybridization was performed on the amplified DNA. Array-data were normalized and aneuploidy was detected by the circular binary segmentation method.

MAIN RESULTS AND THE ROLE OF CHANCE: The good-quality embryos exhibited high rates of aneuploidy, 10 of 14 (71.4%) of the embryos being mosaic. While none of the embryos had the same aneuploidy pattern in all cells, 4 of 14 (28.6%) were uniformly diploid. Of the 70 analysed blastomeres, 55.7% were diploid and 44.3% had chromosomal abnormalities, while 29% of the abnormal cells carried structural aberrations.

WIDER IMPLICATIONS OF THE FINDINGS: Finding such a high rate of aneuploidy and mosaicism in excellent quality embryos from cycles with a high implantation rate warrants further research on the origin and significance of chromosomal abnormalities in human preimplantation embryos.

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Key words: aneuploidy / array-CGH / mosaicism / preimplantation embryo / chromosomal abnormalities

† The authors consider that the first three authors should be regarded as joint First Authors.

Introduction

Preimplantation genetic screening (PGS) has been used over the last 15 years to test the chromosome content of human IVF embryos and select for uterine transfer of those that may have a better potential to lead to a healthy birth (Munne *et al*., 1995). Typically, PGS is done by performing fluorescence *in situ* hybridization (FISH) for 5–10 chromosomes on one or two cells of a Day-3 IVF embryo. The large number of patients who have been treated worldwide with this technology has generated solid data sets on the ploidy status of human preimplantation embryos. Even if FISH only gives limited information on the chromosomal constitution of a cell, it is obvious from these data that human embryos often carry numerical chromosome errors and that cells within one embryo can have different karyotypes.

Studies using high-resolution methods for the complete karyotyping of a cell highlight the inherent limitations of FISH in detecting aneuploidy and the actual incidence of mosaicism (Fragouli *et al*., 2006, 2011; Voullaire *et al*., 2007; Fiorentino *et al*., 2011). In the studies that use FISH as a method of analysis, the cytogenetic assessment involves a limited number of chromosomes, while array-comparative genomic hybridization (array-CGH) enables analysis of the whole genome of the cell and more abnormalities can be detected than with FISH. Moreover, the size and type of aberrations that can be detected by FISH are restricted. Recently, Vanneste *et al*. (2009) used an array-based approach on the remaining blastomeres from embryos after preimplantation genetic diagnosis and showed that not only aneuploidy but also chromosome breakage is common in cleavage-stage embryos and often leads to segmental aberrations. The presence of segmental imbalances in good-quality cleavage-stage embryos was confirmed via single-nucleotide polymorphism (SNP)-based parental origin analyses (Voet *et al*., 2011a) during the clinical implementation of array-CGH (Vanneste *et al*., 2011). Johnson *et al*. (2010) found similar results using SNP-arrays on multiple cells of cleavage-stage embryos from patients of advanced maternal age. Aetiological models underlying this structural instability as well as the link to genomic profiles of healthy and diseased newborns mechanisms were reviewed by Voet *et al*. (2011b).

Until now, most of the data on full chromosomal content of human preimplantation embryos using either CGH or array-CGH were extrapolated from the information obtained from only one or two blastomeres (Voullaire *et al*., 2002; Alfarawati *et al*., 2011; Fiorentino *et al*., 2011). Although these studies give a good impression of the overall aneuploidy rates in embryos, their main limitation is that they do not provide information on the embryo as a whole. Two studies provide data from all blastomeres from good-quality embryos, using metaphase CGH, which has a lower resolution than array-based CGH (Voullaire *et al*., 2000; Wells and Delhanty, 2000). One study used high-resolution SNP arrays to analyse multiple cells from good-quality embryos from older women (Johnson *et al*., 2010) and two other studies performed array-CGH on the remaining blastomeres from preimplantation genetic diagnosis and screening embryos, giving a nearly complete image of the embryos (Le Caignec *et al*., 2006; Vanneste *et al*., 2009).

In this study, using array-CGH, we analysed all the blastomeres of normally developing cleavage-stage embryos that were part of a cohort of embryos that resulted in healthy births, to provide a high-resolution full chromosomal analysis of entire human embryos.

Materials and Methods

Embryos

Embryos used in this study were surplus to Melbourne IVF patients' needs and had been declared excess by the couple according to Australian Federal and Victorian State legislation and National Health and Medical Research Council requirements. Embryos were from six couples who had achieved a live birth from the same IVF cycle. The mean age of the women was 31.3 (range 29–35) years and a maximum of four embryos were used from any one couple. All the embryos had been inseminated by intracytoplasmic sperm injection and cryopreserved on Day 2 of development (Jericho, 2003). Fourteen embryos were thawed, cultured overnight and then allowed to succumb by being left at room temperature for 24 h. We have Institutional Review Board approval (Human Research Ethics Committee, Epworth and Freemason's Hospital, Melbourne, study number S/05/11/2) to use excess, succumbed embryos for research aimed at improving and developing novel technologies for PGD and IVF. The zona pellucida was removed from embryos by brief exposure to acidified (pH 2.4) media. Blastomeres were disaggregated by gentle pipetting.

Whole-genome amplification and array-CGH of single blastomeres

Multiple displacement amplification was performed as described in Spits *et al*. (2006).

Array-CGH was carried out using bacterial artificial chromosome/P1-derived artificial chromosome (BAC/PAC) arrays of 1 Mb resolution. All the BAC and PAC sequences are printed in duplicate and their number varies between the used batches. In particular, the array design contains in total 7744 features printed in a layout of 4 meta rows, 4 meta columns, 22 rows per block and 22 columns per block. This grid is printed twice on the glass slides allowing two microarray assays per slide. Array-CGH was carried out as described previously (Le Caignec *et al*., 2006), with minor modifications.

Equal amounts (1750 ng) of the Cy3- and Cy5-labeled probes were combined with 150 μg of Cot-1 DNA (human Cot-1 DNA; Invitrogen) followed by ethanol precipitation. Resuspension of the pellet was done in 20 μl of hybridization buffer containing 200 μg of yeast tRNA (Invitrogen) to hybridize a spotting area of 24 × 24 mm. The slide was blocked with 50 μg of Cot-1 DNA and 300 μg of salmon testes DNA (Sigma-Aldrich, St. Louis, USA) dissolved in 25 μl of hybridization buffer. The slides were then washed and scanned using an Agilent dual laser DNA microarray scanner G2566AA (Agilent technologies, Palo Alto, CA, USA). The scan images were processed with Agilent Feature Extraction Software v9.5 and data were further analysed. Arrays with autosomal standard deviation larger than 0.75 were excluded from the analysis. Array-data were sent blinded to be pre-processed using the channel clone normalization method (Cheng *et al*., 2011). In brief, array data were normalized by the channel standardization, genome composition artefact correction and recurrent genome artefact correction steps. Subsequently, aneuploidy was detected by the circular binary segmentation (CBS) method and copy number state was called using CGH call (Olshen *et al*., 2004; Van de Wiel *et al*., 2007). As described before (Vanneste *et al*., 2009), at least 18 consecutive BAC clones (half the clones of the smallest chromosome) with signal intensities 2-fold above the autosomal median background intensity were set as the threshold to consider the minimum length of an aberration.

Results

Figure 1 shows the chromosomal complements of each blastomere and embryo. A flow-chart of the experimental design can be found

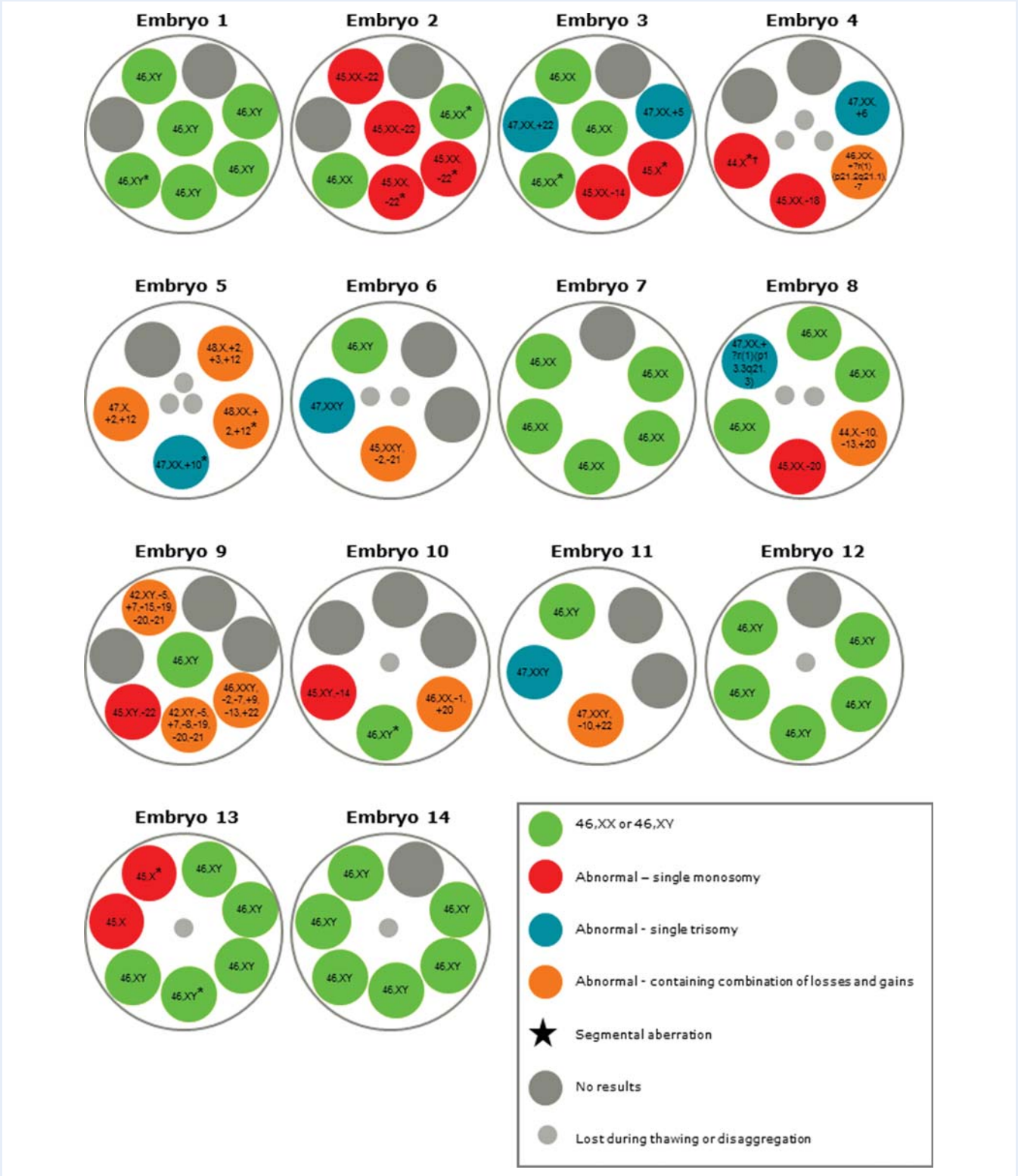

Figure 1 Chromosome complements of the blastomeres analysed by array-CGH. †Chromosome 16 has a structural aberration spanning the centromere. The acentric fragments may be translocated to other chromosomes resulting in a loss of chromosome 16.

in Fig. 2. Table I shows the detailed chromosomal complements per cell. The CBS plots for all chromosomes of all blastomeres analysed can be found in Supplementary data, Fig. The GEO accession number for the BAC-array data reported in this paper is GSE34290. According to our results, good-quality cleavage-stage embryos exhibit high rates of aneuploidy. Only 4 of 14 embryos (28.6%)

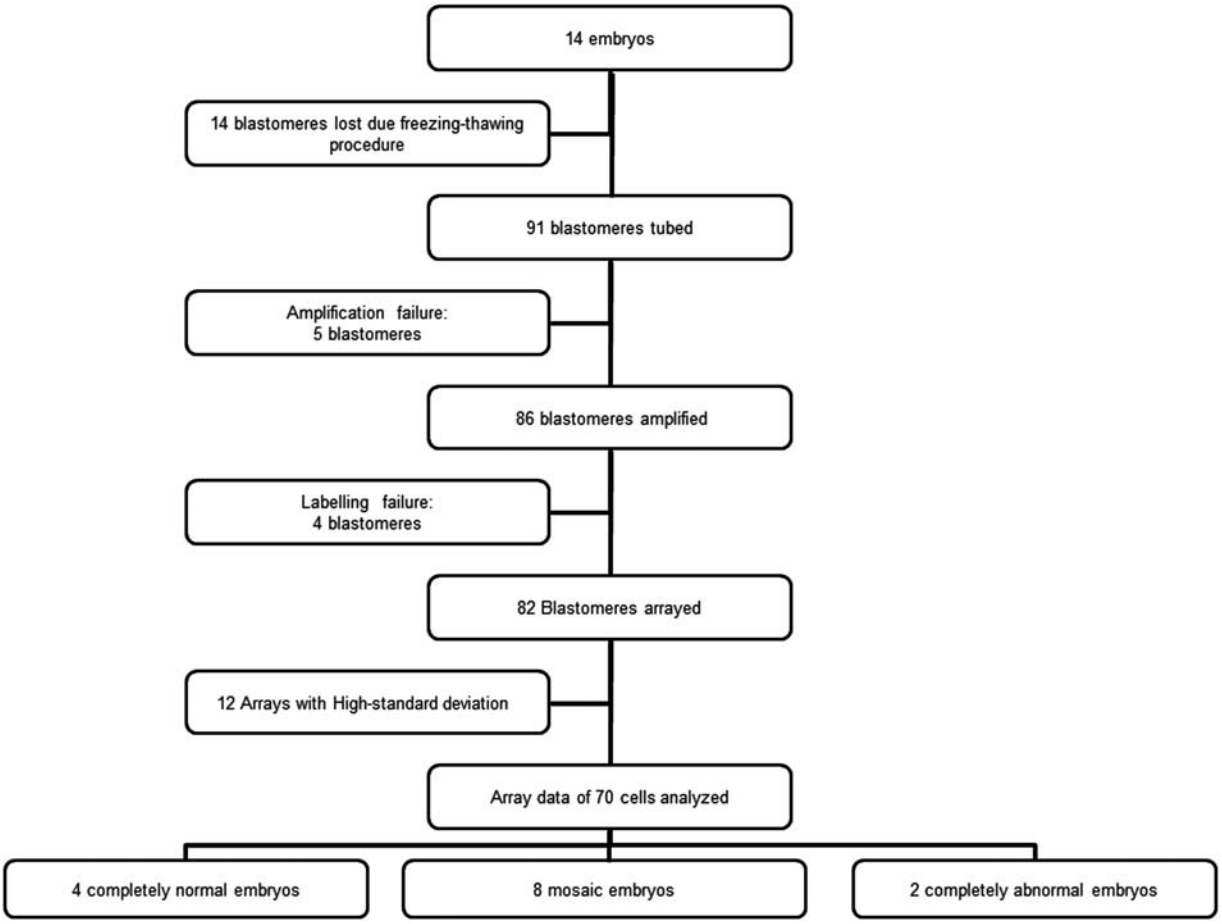

Figure 2 Flow-chart of the experimental design.

were completely normal, while 10 embryos (71.4%) were mosaic and structural aberrations were found in 12 cells. No embryo contained the same aneuploidy in all of its cells.

Of the analysed blastomeres, 39/70 (55.7%) were diploid, 5 of which contained structural aberrations and 31/70 (44.3%) had numerical abnormalities. More specifically, 18.6% (13/70) of the blastomeres had a single monosomy. Additionally, cell 4.6 had a monosomy and a structural aberration that might be a ring chromosome. There were six blastomeres (8.6%) with a single trisomy. Additionally, cell 8.4 had a trisomy and also contained a structural aberration that might be a ring chromosome. Furthermore, 2.9% (2/70) of the blastomeres had two aneuploidies and 11.4% (8/70) of the blastomeres had a complex aneuploidy. Whole chromosome abnormalities were found in almost all chromosomes except for 4, 6, 11, 16 and 17. The chromosomes 2, 19, 22 and X were most frequently involved in aneuploidy.

There were five blastomeres that had only structural aberrations (7.1%), while structural aberrations co-existed with numerical abnormalities in seven other blastomeres. The breakage events involved chromosomes 1, 2, 3, 5, 8, 9, 14, 16, 18, 22 and X. Out of a total of 16 unbalanced segments detected, 9 were interstitial, 6 were likely to be terminal and one was likely to involve the whole arm of the chromosome. Of the structural aberrations, eight were deletions and seven were duplications. Two blastomeres (4.6 and 8.4) had interstitial duplications of chromosome 1 that included the centromeric region, possibly being ring chromosomes. The smallest aberration detected was a 14 Mb deletion of the short arm of chromosome 18 at bands p11.32 through p11.21 (cell 2.4).

Discussion

In our results, half of the cells were normal, which is in line with older studies that used CGH on cells of good-quality embryos. Voullaire *et al*. (2000) carried out CGH on 63 succumbed blastomeres and found that 41% of the cells were normal. From the remaining ones, 27% had a single monosomy and 3% a single trisomy, while ~5% had two aneuploidies and 16% of the blastomeres carried complex abnormalities. Additionally, 5% of the cells had partial chromosome losses and gains. The findings of Wells and Delhanty (2000) on 64 single blastomeres from fresh embryos were comparable. They reported 56% of normal cells, 8% with single monosomy and 17% with single trisomy, while 14% of the cells had two or more aneuploid chromosomes and 6.3% of the cells had structural aberrations. Comparable data were obtained after SNP array analysis of single blastomeres. Johnson *et al*. (2010) found that 48% of cells were euploid, 32% had aneuploidy of one or two chromosomes, 20% had

Table I Array results of the analysed blastomeres.

Cell	Chromosomal status	Unbalanced regions		Euploid (bp)	Start (bp)	End (bp)	Euploid (bp)	Size (Mbp)
		Single copy loss	Single copy gain					
1.1	Euploid, male	–	–	–	–	–	–	–
1.2	Euploid, male	–	–	–	–	–	–	–
1.3	Euploid, male	–	–	–	–	–	–	–
1.4	Euploid, male	–	–	–	–	–	–	–
1.5	Unbalanced male	–	Xp22.33p21.1		480 558	31 439 987	31 992 097	31.0
1.6	Euploid, male	–	–	–	–	–	–	–
2.1	Unbalanced, female	22q11.1q13.33	–	–	15 615 802	49 464 725	–	33.8
2.2	Unbalanced, female	–	3p24.1p21.31	27 531 253	28 645 912	50 006 856	51 558 739	21.4
		22q11.1q13.33	–	–	15 615 802	49 464 725	–	33.8
2.3	Unbalanced, female	22q11.1q13.33	–	–	15 615 802	49 464 725	–	33.8
2.4	Unbalanced, female	18p11.32p11.21	–	–	130 336	14 143 291	14 843 504	14.0
2.5	Unbalanced, female	Xp21.3p22.31	–	5 415 752	6 807 524	28 277 294	28 778 243	21.5
		22q11.1q13.33	–	–	15 615 802	49 464 725	–	33.8
2.6	Euploid, female	–	–	–	–	–	–	–
3.1	Unbalanced, female	5q12.1q35.3	–	60 897 700	61 659 616	180 626 608	–	119.0
3.2	Unbalanced, female	Xp22.33q28	–	–	480 558	154 392 840	–	153.9
		2q32.2q33.3	–	189 965 350	190 397 880	206 359 675	206 942 051	16.0
		–	5q31.3q35.3	140 124 588	141 041 469	180 626 608	–	39.6
		14q12q24.3	–	31 601 773	32 378 912	74 478 914	74 588 657	42.1
3.3	Unbalanced, female	–	22q11.1q13.33	–	15 615 802	49 464 725	–	33.8
3.4	Unbalanced, female	–	5p15.33q35.3	–	2 570 762	180 626 608	–	178.1
3.5	Euploid, female	–	–	–	–	–	–	–
3.6	Euploid, female	–	–	–	–	–	–	–
3.7	Unbalanced, female	14q11.2q32.33	–	–	19 570 817	105 437 117	–	85.9
4.1	Unbalanced, female	18p11.32q23	–	–	130 336	75 940 259	–	75.8
4.4	Unbalanced, female	Xp22.33q28	–	–	480 558	154 392 840	–	153.9
		16p12.2q13	–	21 473 433	21 936 647	57 139 964	56 952 043	35.2
4.5	Unbalanced, female	–	6p25.3q27	–	90 997	170 803 707	–	170.7
4.6	Unbalanced, female	–	1p21.2q21.1	99 775 192	101 014 818	142 928 310	144 192 914	41.9
		7p22.3q36.3	–	–	188 219	158 455 135	–	158.3
5.2	Unbalanced, female	Xp22.33q28		–	480 558	154 392 840	–	153.9
		–	2p25.3q37.3	–	224 263	242 618 229	–	242.4
		–	12p13.33q24.33	–	152 582	132 136 749	–	132.0
5.3	Unbalanced, female	8q21.3q24.3	–	89 400 932	90 077 559	146 167 102	–	56.1
		–	10p15.3q26.3	–	259 607	135 286 722	–	135.0
5.4	Unbalanced, female	–	2p25.3q37.3	–	224 263	242 618 229	–	242.4
		–	3q13.33q26.33	118 326 520	119 242 817	182 470 715	183 174 957	63.2
		–	12p13.33q24.33	–	152 582	132 136 749	–	132.0
5.5	Unbalanced, female	Xp22.33q28	–	–	480 558	154 392 840	–	153.9
		–	2p26.3q37.3	–	224 263	242 618 229	–	242.4
		–	3p25.3q29	–	186 817	199 163 913	–	199.0
		–	12p13.33q24.33	–	152 582	132 136 749	–	132.0
6.1	Unbalanced, male	–	Xp22.33q28	–	480 558	154 392 840	–	153.9
		2p25.3q37.3	–	–	224 263	242 618 229	–	242.4
		21q11.2q22.3	–	–	13 462 479	46 925 923	–	33.5
6.2	Unbalanced, male	–	Xp22.33q28	–	480 558	154 392 840	–	153.9

Continued

Table I *Continued*

Cell	Chromosomal status	Unbalanced regions		Euploid (bp)	Start (bp)	End (bp)	Euploid (bp)	Size (Mbp)
		Single copy loss	Single copy gain					
6.3	Euploid, male	–	–	–	–	–	–	–
7.1	Euploid, female	–	–	–	–	–	–	–
7.2	Euploid, female	–	–	–	–	–	–	–
7.3	Euploid, female	–	–	–	–	–	–	–
7.4	Euploid, female	–	–	–	–	–	–	–
7.6	Euploid, female	–	–	–	–	–	–	–
8.1	Unbalanced, female	Xp22.33q28	–	–	480 558	154 392 840	–	153.9
		10p15.3q26.3	–	–	259 607	135 286 722	–	135.0
		13q12.11q34	–	–	19 136 604	113 927 980	–	94.8
		–	20p13q13.33	–	180 000	62 393 015	–	62.2
8.2	Euploid, female	–	–	–	–	–	–	–
8.3	Unbalanced, female	20p13q13.33	–	–	180 000	62 393 015	–	62.2
8.4	Unbalanced, female	–	1p13.3q21.3	106 794 965	107 296 837	150 651 279	150 879 988	43.4
8.5	Euploid, female	–	–	–	–	–	–	–
8.6	Euploid, female	–	–	–	–	–	–	–
9.1	Euploid, male	–	–	–	–	–	–	–
9.2	Unbalanced, male	22q11.1q13.33	–	–	15 615 802	49 464 725	–	33.8
9.3	Unbalanced, male	5p15.33q35.3	–	–	2 570 762	180 626 608	–	178.1
		–	7p22.3q36.3	–	188 219	158 455 135	–	158.3
		15q11.2q26.3	–	–	20 363 717	100 022 043	–	79.7
		19p13.3q13.43	–	–	210 823	63 771 717	–	63.6
		20p13q13.33	–	–	180 000	62 393 015	–	62.2
		21q11.2q22.3	–	–	13 462 479	46 925 923	–	33.5
9.5	Unbalanced, male	5p15.33q35.3	–	–	2 570 762	180 626 608	–	178.1
		–	7p22.3q36.3	–	188 219	158 455 135	–	158.3
		8p23.3q24.3	–	–	477 653	146 167 102	–	145.7
		19p13.3q13.43	–	–	210 823	63 771 717	–	63.6
		20p13q13.33	–	–	180 000	62 393 015	–	62.2
		21q11.2q22.3	–	–	13 462 479	46 925 923	–	33.5
9.6	Unbalanced, male	–	Xp22.33q28	–	480 558	154 392 840	–	153.9
		2p25.3q37.3	–	–	224 263	242 618 229	–	242.4
		7p22.3q36.3	–	–	188 219	158 455 135	–	158.3
		–	9p24.3q34.2	–	190	136 362 829	–	136.4
		13q12.11q34	–	–	19 136 604	113 927 980	–	94.8
		–	22q11.21q13.33	–	15 615 802	49 464 725	–	33.8
10.3	Unbalanced, male	14q11.2q32.33	–	–	19 570 817	105 437 117	–	85.9
10.5	Unbalanced	Yp11.32q12	–	–	585 968	57 501 691	–	56.9
		–	Xp22.33q28	–	480 558	154 392 840	–	153.9
		1p36.33q44	–	–	968 368	245 407 169	–	244.4
		–	20p13q13.33	–	180 000	62 393 015	–	62.2
10.6	Unbalanced, male	9q21.11q34.3	–	69 676 572	70 528 528	138 274 031	–	67.7
11.2	Unbalanced, male		Xp22.33q28	–	480 558	154 392 840	–	153.9
		10p15.3q26.3		–	259 607	135 286 722	–	135.0
			22q11.1q13.33	–	15 615 802	49 464 725	–	33.8
11.3	Euploid, male	–	–	–	–	–	–	–
11.5	Unbalanced, male	–	Xp22.33q28	–	480 558	154 392 840	–	153.9
12.1	Euploid, male	–	–	–	–	–	–	–
12.2	Euploid, male	–	–	–	–	–	–	–
12.4	Euploid, male	–	–	–	–	–	–	–
12.5	Euploid, male	–	–	–	–	–	–	–

Continued

Table I *Continued*

Cell	Chromosomal status	Unbalanced regions		Euploid (bp)	Start (bp)	End (bp)	Euploid (bp)	Size (Mbp)
		Single copy loss	Single copy gain					
12.6	Euploid, male	–	–	–	–	–	–	–
13.1	Unbalanced, male	8q21.3q24.13	–	88 302 638	89 218 072	122 875 986	123 525 975	33.7
13.2	Euploid, male	–	–	–	–	–	–	–
13.3	Unbalanced	Yp11.32q12	–	–	585 968	57 501 691	–	56.9
		–	22q11.21q12.3	–	15 615 802	34 948 762	35 473 472	19.3
13.4	Euploid, male	–	–	–	–	–	–	–
13.5	Unbalanced	Yp11.32q12	–	–	585 968	57 501 691	–	56.9
13.6	Euploid, male	–	–	–	–	–	–	–
13.7	Euploid, male	–	–	–	–	–	–	–
14.1	Euploid, male	–	–	–	–	–	–	–
14.2	Euploid, male	–	–	–	–	–	–	–
14.3	Euploid, male	–	–	–	–	–	–	–
14.4	Euploid, male	–	–	–	–	–	–	–
14.6	Euploid, male	–	–	–	–	–	–	–
14.7	Euploid, male	–	–	–	–	–	–	–

complex aneuploidy of three or more chromosomes and 12% had structural abnormalities.

A large number of chromosomal abnormalities in single blastomeres were also reported when array-CGH was performed on single blastomeres from non-PGS embryos not suitable for transfer and from PGS embryos (Gutiérrez-Mateo *et al*., 2011). In this study, 65% of the tested cells were found to be abnormal and 19.4% of the abnormal cells had a single monosomy and 14.7% a single trisomy. The same study reported that chromosomes 15, 16, 21 and 22 were most frequently involved in aneuploidy. Similarly, in a study performing array-CGH and FISH on blastocysts (Fragouli *et al*., 2011), chromosomes 15, 16, 21, 22 and X were found to be most frequently abnormal. In our hands, chromosomes X and 22 were also frequently abnormal along with chromosomes 2 and 19.

With regard to mosaicism, a recent systematic review on the chromosomal constitution of human preimplantation embryos (van Echten-Arends *et al*., 2011) found that 73% of the embryos were mosaic, 22% diploid and the remaining 5% contained other abnormalities. Almost all of the studies included in this meta-analysis used FISH to detect aneuploidy and so a limited number of chromosomes were analysed. Moreover, it has recently been reported that FISH may well overestimate the true frequency of mosaicism in early embryos (Treff *et al*., 2010). Despite these limitations, our results are in agreement showing 71.4% mosaic embryos and 28.6% diploid embryos.

The introduction of higher resolution techniques such as the single-cell metaphase- and array-CGH has enabled the detection of *de novo* partial chromosome losses and gains. Chromosome breakage leading to chromosomal imbalance is reported in several studies using CGH (Wells *et al*., 1999; Voullaire *et al*., 2000, 2002; Wells and Delhanty, 2000). Daphnis *et al*. (2008) reported that in a group of 17 embryos where CGH revealed at least one cell with abnormal chromosomal complement, 28% of the events leading to mosaicism were due to partial chromosome breakage. Using an array-based approach, Vanneste *et al*. (2009, 2011) reported that 31–70% of the embryos carried structural deletions, duplications or amplifications. In our hands, structural aberrations were found in 29% of the abnormal cells. Although it is now clear that structural aberrations are a common occurrence in preimplantation embryos, their true frequency, underlying mechanisms and biological significance are yet to be elucidated (Voet *et al*., 2011a,b).

The embryos chosen for this study came from young women whose assisted reproduction treatment (ART) cycles were successful. Sibling embryos had a clinical pregnancy rate of 100% (9/9), as evidenced by the presence of a fetal heartbeat at 6 weeks gestation, and all the nine pregnancies progressed to a live birth. The implantation rate (number of fetal hearts/embryos transferred) was 11/15 (73%). There was no difference in embryo cell number or quality between the embryos that were transferred or analysed by array-CGH. The frequency of abnormalities detected in these embryos is thus difficult to reconcile with the outcomes of the sibling embryos that were transferred in the same ART cycles, if it is assumed that only embryos with a completely normal chromosome complement could have implanted. The thorough and previous validation of the technology used in this study (Le Caignec *et al*., 2006; Vanneste *et al*., 2009; Cheng *et al*., 2011) rules out that the results were technical artefacts. This brings up the question of the minimum required number of karyotypically normal cells in an embryo to be viable. Given the high implantation rate in the transferred sibling embryos, it could be assumed that some of the mosaic embryos also have reasonable implantation potential. For instance, 70% of the cells of embryo 13 were normal, while embryo 3 had two normal cells out of the seven analysed. There is evidence that embryos that are diploid-aneuploid mosaic at the cleavage stage but are still developing with a normal cleavage rate and pattern can reach the blastocyst stage (Gonzalez-Merino *et al*.,

2003; Baart *et al*., 2006; Vanneste *et al*., 2011), although this does not imply that they all would implant. It has been suggested that embryos might 'self-correct' their chromosome complement as they develop towards the blastocyst stage (Rubio *et al*., 2007; Barbash-Hazan *et al*., 2009; Vanneste *et al*., 2009; Robberecht *et al*., 2010). Several studies show that the proportion of aneuploid cells in embryos diminish as the embryos go through the cleavage, morula and blastocyst stage (Bielanska *et al*., 2002; Gonzalez-Merino *et al*., 2003). Several mechanisms have been suggested to explain the 'self-correction', such as preferential allocation of diploid cells to the inner cell mass, loss of aneuploid cells due to apoptosis or trisomic rescue by anaphase lagging or non-disjunction (Kalousek, 2000; Los *et al*., 2004; Robberecht *et al*., 2010). Although our data do not shed light on this question, they represent a reference set for further studies.

In conclusion, in this study we present evidence that around 70% of good-quality embryos carry chromosomal abnormalities, including structural aberrations. The main strength of this work compared with other published data is that: (i) we analysed the majority of the blastomeres of (ii) top-quality embryos from a cohort of embryos with high implantation and developmental potential and for (iii) all chromosomes. This makes this data set unique and a touchstone for future experiments. These experiments should elucidate the true frequency and biological significance of chromosomal instability and the natural course of aneuploid cells in a normally developing embryo.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

A.M., L.W. and J.C. equally contributed to this work. A.M. participated in study design, execution, data analysis, manuscript drafting and critical discussion. L.W. participated in study design, execution, manuscript drafting and critical discussion. J.C. participated in study execution, data analysis, manuscript drafting and critical discussion. C.S. participated in study design, data analysis and critical discussion. E.V. participated in data analysis and critical discussion. Y.M. participated in data analysis and critical discussion. J.R.V. participated in data analysis and critical discussion. K.S. participated in study design and critical discussion.

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Conflict of interest

Y.M. holds stock in Cartagenia, which develops and distributes software for analysis of copy number variation.

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