

Chromosome abnormalities in 1255 cleavage-stage human embryos



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Abstract

The relationship was examined between chromosome abnormalities in cleavage stage human embryos and maternal age, embryo morphology and development rate. Embryos that were classified as suboptimal for transfer from patients undergoing IVF treatment were disaggregated, and all or most of their cells were fixed for analysis by fluorescence in-situ hybridization. Chromosomes X, Y, 13, 18 and 21, and in some instances 16 were examined. A total of 731 non-viable embryos was analysed. An increase in chromosome abnormalities with decreasing embryo competence and increasing maternal age was shown. Compared with an earlier study, the major difference was that polyploidy ($P < 0.001$) and aneuploidy were previously more common. After pooling results, it was found that aneuploidy increased with maternal age, from 3.1% in embryos from 20-34 years old patients to 17% in patients 40 years or older. Also, aneuploidy occurred more frequently in embryos with good morphology and development rate than in embryos developing poorly. In contrast, dysmorphic and slowly developing or arrested embryos had significantly more polyploidy and mosaicism than normally developing embryos. Clear associations between maternal age and aneuploidy, and between cleavage anomalies and mosaicism have been established in non-viable embryos. Arrested embryos were mostly polyploid. Moreover, polyploidy was found more frequently in embryos analysed on day 4, suggesting that developmentally compromised embryos became arrested in extended culture. A slightly higher aneuploidy rate in the earlier study may be attributed to differences in hormonal stimulation, which also resulted in different numbers of oocytes recruited and matured.

Keywords: aneuploidy, developmental arrest, mosaicism, polyploidy, PGD, preimplantation genetic diagnosis

Introduction

In a study of 524 cleavage stage embryos analysed by fluorescence in-situ hybridization (FISH), direct relationships between advanced maternal age and aneuploidy, embryonic arrest and polyploidy, and slow development and post-meiotic chromosome abnormalities were demonstrated (Munné *et al.* 1995). Some of those findings were later confirmed by Almeida and Bolton (1996) using karyotype analysis. They found that slowly developing embryos had more chromosome abnormalities, specifically mosaicism, than normally developing ones. Mosaicism has been studied by several groups using either FISH or karyotype analysis (Munné *et al.* 1994b, 1995, 1997; Almeida and Bolton 1996; Harper *et al.* 1995a; Delhanty *et al.* 1997), but its origin and impact on embryonic development are not well understood. On the other hand, the maternal age effect on aneuploidy has been studied in clinical pregnancies (Hassold and Chiu 1985, Warburton *et al.* 1986) and observed in oocytes and cleavage-stage embryos (Munné *et al.* 1995, Dailey *et al.* 1996). Aneuploidy in embryos developing in vitro was found at a frequency several fold higher than that in first trimester losses, indicating a strong in-vivo selection mechanism against aneuploid embryos. Selection against such embryos based on their morphology may improve implantation rates after IVF.

In the present study, we compare the chromosome abnormalities found in 731 embryos analysed on day 3 with those found in 524 embryos analysed on day 4 of development in a previous study (Munné *et al.* 1995). Where trends were similar, the results were pooled to establish further relationships between the incidence of chromosome anomalies and maternal age, embryo morphology and cleavage rate.

Materials and methods

Embryo source and classification

Embryos used in this study were those donated by patients undergoing IVF treatment for infertility at the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center between 1995 and 1999. The study was in accordance with guidelines set by the internal review board of the centre, and written patient consent was obtained in each case.

Embryos not suitable for either replacement or cryopreservation were used for this study. Embryos considered genetically abnormal after preimplantation genetic diagnosis (PGD) were not included.

Only non-viable monospermic embryos developing from bipronucleate zygotes were used. Study embryos were classified in three groups based on their development profile on day 3: Group A included embryos with 6 or more cells, cleavage in the previous 24 h, <20% fragmentation, and no multinucleated blastomeres. These embryos were excluded from transfer and cryopreservation because of other anomalies, most importantly, overtly uneven cell division and/or a manifestation of degenerative cytoplasm in many blastomeres. The latter includes presence of multiple vacuoles, severe cytoplasmic contraction or vesiculation, and non-uniformity of organelle distribution. Group B included embryos with <6 cells, cleavage in the previous 24 h, >20% fragmentation, and/or one or more multinucleate blastomeres. Group C included embryos that had not cleaved during a 24 h period and were considered arrested.

Embryos considered genetically abnormal after PGD were not included because they represented a population of morphologically viable but chromosomally abnormal embryos that would bias the overall population.

The results obtained in this study (study II) were compared to the results obtained previously (study I, Munné *et al.* 1995b). The embryos in study I were obtained at Cornell University Medical Center by the same group of embryologists and using the same embryology protocols as used in study II. Embryos from study I were classified into groups A, B, and C in the same fashion as for study II. The average number of oocytes obtained in studies I and II were 17.3 (37979/2196) and 10.7 (30642/2876) respectively ($P < 0.001$).

Fixation and FISH analysis

Embryos were disaggregated using 3% pronase in Ca/Mg-free M2 medium (Gibco BRL, Grand Island, NY, USA) for 5-10 min on day 3. Blastomeres were individually fixed on glass slides as described previously (Munné *et al.* 1996a). Blastomeres were analysed by FISH simultaneously using X, Y, 13, 18, 21 chromosome-specific probes following Munné and Weier (1996b) without modification, or adding to the previous mixture a chromosome 16-specific probe (Munné *et al.* 1998). The scoring criteria from Munné and Weier (1996b) and the FISH-failure criteria from Munné *et al.* (1994) were followed to distinguish FISH-failure (false positive and negatives) from mosaicism when all or most cells of a particular embryo were analysed.

Since the status of chromosome 16 was not assessed in all embryos, the incidences of trisomy and monosomy 16 were calculated based on the number of affected embryos divided by the number of embryos analysed with the 16 probe. The incidence of all other abnormalities was calculated based on the number of affected embryos divided by the total number of embryos.

An embryo was considered to be a diploid mosaic when at least one cell was normal. When all cells from a mosaic embryo were abnormal and the average number of chromosomes per cell was close to diploid, the embryo was also considered to be a diploid mosaic. A diploid mosaic embryo with 3/8 or more abnormal cells was classified as extensively diploid mosaic (Munné *et al.* 1995). Embryos with multinucleate blastomeres (MNB) were considered to be mosaics and chromosomally abnormal.

Statistical analysis

The χ^2 test was used to compare individual differences in the incidences of the various abnormalities in different age and embryo morphology groups. The Mantel-Haenszel test was used to compare heterogeneous percentages such as the proportions of abnormal embryos found using XY, 13, 18, 21 probes and those using the XY, 13, 16, 18, 21 probes.

To investigate further the associations between chromosome abnormality and age and embryo morphology group, the incidence of the abnormalities was subjected to a standard GLM (generalized linear modelling) analysis. This analysis also gave estimated mean proportions for each group and the corresponding standard errors. For any factor, these mean values represented averages over all the other factors in the study.

Results

A total of 731 non-viable monospermic embryos with varying abnormalities was analysed in this study: 425 with X, Y, 13, 18 and 21 probes and the remainder with X, Y, 13, 16, 18 and 21 probes. These embryos had 3224 blastomeres, of which 2998 (93%) produced clear signals; the rest were damaged ($n=124$), lost ($n=32$), or contained nuclei too condensed to produce a clear result ($n=70$).

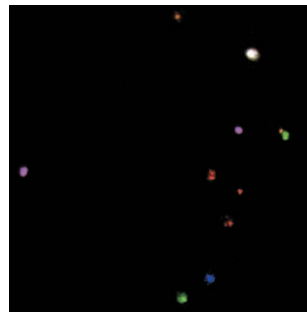


Figure 1. Blastomere analysis using probes for chromosomes X (blue), Y (white to pale yellow), 13 (orange), 16 (green), 18 (magenta) and 21 (red). This illustration shows an XY trisomy 21 blastomere

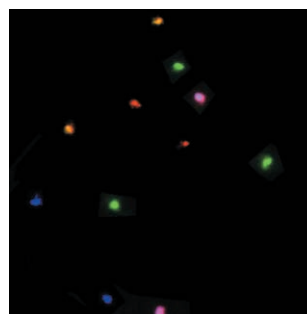


Figure 2. Probe colours as indicated in Figure 1. This illustration shows an XX trisomy 16 blastomere.

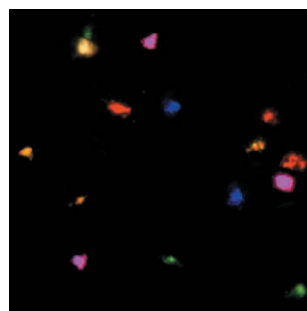


Figure 3. Probe colours as indicated in Figure 1. This illustration shows an example of polyploidy, namely an XXY triploid blastomere.

In addition, 145 cells had FISH errors: 10 with the X probe (one false positive, and nine false negatives), 47 with the 13 probe (13 false positives, and 34 false negatives), 16 with the 16 probe (10 false positives, and six false negatives), 23 with the 18 probe (five false positives, and 18 false negatives), 49 with the 21 probe (11 false positives, and 38 false negatives).

Tables 1, 2 and 3 show the chromosomal abnormalities in the three maternal age groups after analysing 254 group A, 363 group B and 114 group C embryos. Regarding maternal age groups, 255 embryos were from women of 20 to 34 years, 274 from 35 to 39 years and 202 from 40 to 47 years old.

Total aneuploidy (**Figures 1 and 2**) significantly increased with maternal age ($P<0.001$, GLM analysis) (**Table 4**) while extensive diploid mosaicism and polyploidy increased ($P<0.01$, GLM analysis) with abnormal morphology and/or abnormal development (**Table 5**). Surprisingly, aneuploidy for chromosomes 13 and 21 decreased significantly with abnormal morphology ($P<0.01$, GLM analysis) (**Table 5**).

Chromosomal abnormalities that were produced during or after fertilization (mosaicism, polyploidy (**Figure 3**) and haploidy)

significantly decreased with increasing embryonic competence, being lowest in group A and highest in C ($P<0.0001$, GLM analysis) (**Table 5**).

Comparison of studies I and II

A total of 524 monospermic embryos was analysed in study I performed at Cornell University Medical College (Munné et al. 1995b). The frequency of chromosomal abnormality was compared between study I and II for each maternal age (**Table 4**) and embryo morphology group (**Table 5**). The two studies showed similar trends. Diploid mosaicism, aneuploidy XY, 18 and aneuploidy 13, 21 occurred with similar frequencies in the two studies. The two studies differed in the following: (i) the maternal age effect on extensive diploid mosaicism detected in study I was absent in study II, (ii) extensive diploid mosaicism was found to be prevalent among embryos with abnormal morphology only in study II, (iii) polyploid embryos were found more frequently in study I in all maternal age groups, and (iv) in maternal age group 20-34 years, the rate of haploidy was lower in study I ($P<0.05$).

Table 1. Numbers of embryos with chromosome abnormalities in series II group A

	Maternal age groups (years)			
	20 to 34	35 to 39	40 to 47	Total
<i>Embryos analysed with X,Y,13,18,21 probes</i>	32	48	64	144
<i>Embryos analysed with X,Y,13,16,18,21 probes</i>	40	52	18	110
Total analysed	72	100	82	254
<i>Chromosomally abnormal embryos</i>				
Monosomy X	0	1	0	1
Trisomy XYY	0	0	1	1
Monosomy 13	0	0	1	1
Trisomy 13	0	0	1	1
Monosomy 18	0	2	2	4
Trisomy 18	0	1	0	1
Monosomy 21	0	1	8	9
Trisomy 21	0	3	5	8
Monosomy 13 and monosomy 16	0	0	1	1
Trisomy 13 and monosomy 21	0	0	1	1
Monosomy 13, diploid mosaic (16% abnormal)	0	0	1	1
Monosomy 13, diploid mosaic (60% abnormal)	0	1	0	1
Monosomy 13, diploid mosaic (63% abnormal)	0	1	0	1
Monosomy 21, diploid mosaic (6% abnormal)	0	0	1	1
Monosomy 21, diploid mosaic (25% abnormal)	1	0	0	1
Trisomy 21, diploid mosaic (10% abnormal)	0	1	0	1
Trisomy 21, mosaic (100% abnormal)	0	0	1	1
Polyploid	4	8	4	16
Haploid	5	3	3	11
Extensive diploid mosaicism	12	12	13	37
Limited diploid mosaicism	6	5	6	17
<i>Subtotals</i>				
Total aneuploidy embryos for XY,13,18,21 (%)	1.4	11.0	26.8	13.8
Total aneuploidy embryos for 16 (%)	0.0	0.0	5.6	0.9
Total aneuploid embryos (%)	1.4	11.0	32.4	14.7
Total embryos with other anomalies ^a (%)	29.2	25.0	25.6	26.4
Total abnormal embryos^b (%)	30.6	34.0	52.4	39.0

^a Total embryos with anomalies produced during or after fertilization (extensive diploid mosaicism + polyploids + haploids)

^b Embryos that presented aneuploidy and extensive diploid mosaicism were counted once

Combined results of studies I and II

Although there were differences between studies I and II, the two studies were pooled mainly to analyse aneuploidy in the combined 1255 embryos (731 from study II and 524 from study I) because the same trends were found for aneuploidy in both series.

The overall frequency of aneuploidy for chromosomes 13 and 21 increased significantly with maternal age, from 2.5% in embryos from women 20 to 34 years to 17.9% in women >40 years ($P<0.001$) (Table 4). Also, aneuploidy was found to occur at statistically higher rates in good embryos (14.7%) than in those with poor morphology (2.4%) for these chromosomes ($P<0.01$) (Table 5). Individual chromosomes involved in non-disjunction were also analysed using pooled results from the two studies (Table 6). Chromosomes 16, 18 and 21 demonstrated significantly higher frequencies of aneuploidy with increasing maternal age ($P<0.05$, $P<0.05$ and $P<0.01$ respectively).

Because of the differences in ploidy between studies I and II, as well as some inconsistencies in trends previously mentioned, the pooling of the results is less meaningful. Nevertheless, polyploidy ($P<0.001$) and extensive diploid mosaicism ($P<0.01$) significantly decreased with improved embryo quality but were not affected by maternal age.

Estimate of chromosome abnormalities in the overall IVF population

The overall rate of chromosome abnormalities in our IVF population could be estimated based on the morphological and age groups of embryos here studied. Because the frequency of each morphological and age group was available only for series II, for this estimate only series II data were used.

Series II embryos were obtained from a population of 6380 IVF cycles producing 48 765 embryos. Table 7 shows the frequency of each type of maternal age group and morphology group in

Table 2. Numbers of embryos with chromosome abnormalities in series II group B

	Maternal age groups (years)			
	20 to 34	35 to 39	40 to 47	Total
<i>Embryos analysed with X,Y,13,18,21 probes</i>	95	58	72	225
<i>Embryos analysed with X,Y,13,16,18,21 probes</i>	46	74	18	138
Total analysed	141	132	90	363
<i>Chromosomally abnormal embryos</i>				
Monosomy X	1	0	0	1
Trisomy XXY	1	0	0	1
Monosomy 13	3	0	2	5
Trisomy 13	1	3	0	4
Monosomy 16	0	2	1	3
Trisomy 16	0	1	1	2
Monosomy 18	3	0	1	4
Trisomy 18	1	0	1	2
Monosomy 21	2	3	3	8
Trisomy 21	2	3	3	8
Monosomy X and monosomy 13	0	1	0	1
Monosomy 13 and monosomy 21	0	1	0	1
Trisomy XXX and monosomy 18	1	0	0	1
Trisomy 18 and monosomy 21	0	0	1	1
Monosomy X, diploid mosaic (16% abnormal)	0	1	0	1
Monosomy 21, diploid mosaic (56% abnormal)	0	0	1	1
Trisomy XXY, diploid mosaic (33% abnormal)	1	0	0	1
Trisomy 13, diploid mosaic (20% abnormal)	0	1	0	1
Trisomy 16, diploid mosaic (50% abnormal)	1	0	0	1
Trisomy 18, diploid mosaic (30% abnormal)	0	0	1	1
Polyploidy	11	8	9	28
Haploidy	7	3	6	16
Extensive diploid mosaicism	26	32	18	76
Limited diploid mosaicism	15	12	6	33
<i>Subtotals</i>				
Total aneuploid embryos for XY,13,16,18,21 (%)	11.3	9.8	14.4	11.6
Total aneuploid embryos for 16 (%)	2.2	4.1	11.1	4.3
Total aneuploid embryos (%)	13.5	13.9	25.5	15.9
Total embryos with other anomalies ^a (%)	32.6	32.6	37.3	33.9
Total abnormal embryos^b (%)	43.3	44.7	53.3	46.3

^a Total embryos with anomalies produced during or after fertilization (extensive diploid mosaicism + polyploids + haploids)

^b Embryos that presented aneuploidy and extensive mosaicism were counted once

this population. Multiplying these frequencies by the frequency of chromosome abnormalities in each of these groups (Tables 1 to 3), we estimated that the overall rate of chromosome abnormalities for this population was 43.3%.

Discussion

Chromosomal abnormalities in 731 abnormal human embryos were studied. The findings were compared with those obtained in 524 embryos from study I (Munné *et al.* 1995). The incidence of polyploidy was different in the two studies: arrested embryos and embryos in all three maternal age groups showed significantly higher polyploidy rates in study I. The most probable reason for this difference is that embryos in study II were fixed on day 3 of development while embryos in study I were fixed on day 4. Although cellular division stops in arrested embryos, DNA synthesis has been observed to continue (Artley *et al.* 1992). Arrested embryos in study I had an extra day to replicate their DNA without cleaving thus becoming polyploid at a higher rate than arrested embryos in study II. As these embryos do not normally reach the blastocyst stage, prolonged culture may effectively eliminate them from transfer.

The overall rate of abnormalities in series II was estimated as being 43.3%. However, in this study the embryos with the best morphology and development were either transferred or frozen and not studied. Group A is therefore inferior to those best embryos, and assuming that the trend between increasing dysmorphism and chromosome abnormalities is also maintained for the best embryos, these should have fewer abnormalities than group A. Therefore, the above overall rate of chromosome abnormalities has been slightly overestimated.

Apart from polyploidy, and because other differences between study I and study II were minor, it was considered appropriate to pool the two studies to analyse the relationship between chromosome abnormalities, maternal age and dysmorphism. Two clear trends were observed: (i) aneuploidy increased with maternal age but not with morphological anomalies in embryos and (ii) embryo morphology was related to mosaicism and polyploidy, while maternal age was not.

Aneuploidy, maternal age and embryo morphology

Pooled results clearly demonstrated a highly significant relationship between maternal age and aneuploidy. This effect was mostly due to chromosome 21, but the frequency of chromosomes 16 and 18 aneuploidy was also significantly higher in women 40 or older compared to the youngest group (Table 6). Results in study II confirm those in study I, and together confirm the relationship between increasing maternal age and aneuploidy, which is well known from prenatal and postnatal data (Hassold *et al.* 1980; Hassold and Chiu 1985; Warburton *et al.* 1986). However, the large difference in the number of aneuploid embryos detected on day 3 or 4 of development compared with prenatal data, indicates a strong selection against aneuploid embryos before or shortly after implantation. For instance, while autosomal monosomies are rarely detected in first trimester pregnancies (only 1/1000 are monosomy 21), day 3 and 4 embryos have at least as much monosomy as trisomy. Monosomic embryos may be eliminated during or prior to blastocyst formation as has been demonstrated in the mouse (Magnuon *et al.* 1985) and recently in humans using embryos discarded not because of morphological abnormalities but because they were found to be abnormal after

Table 3. Numbers of embryos with chromosome abnormalities in series II group C

	Maternal age groups (years)			
	20 to 34	35 to 39	40 to 47	Total
<i>Embryos analysed with X,Y,13,18,21 probes</i>	18	20	18	56
<i>Embryos analysed with X,Y,13,16,18,21 probes</i>	24	22	12	58
Total analysed	42	42	30	114
<i>Chromosomally abnormal embryos</i>				
Monosomy 13	0	1	1	2
Trisomy 13	0	1	0	1
Monosomy 16	1	0	1	2
Trisomy 18	1	0	1	2
Tetrasomy 18	0	0	1	1
Polyploidy	10	10	3	23
Haploid	1	3	2	6
Extensive diploid mosaicism	10	13	9	32
Limited diploid mosaicism	2	2	5	9
<i>Subtotals</i>				
Total aneuploid embryos for XY,13,18,21 (%)	2.4	4.8	10.0	5.3
Total aneuploid embryos for 16 (%)	4.2	0.0	8.3	3.4
Total aneuploid embryos (%)	6.6	4.8	18.3	8.7
Total embryos with other anomalies ^a (%)	50.0	61.9	46.7	53.5
Total abnormal embryos^b (%)	54.8	66.7	60.0	60.5

^a Total embryos with anomalies produced during or after fertilization (extensive diploid mosaicism + polyploids + haploids)

^b Embryos that presented aneuploidy and extensive mosaicism were counted once

PGD (Sandalinas *et al.* 2000). Similarly, trisomies 18, 13 and 21 were found in about 8% of day 3 and 4 embryos but only in 4% of chorionic villous samples (CVS) from women 40-44 years old (Hassold and Chiu 1985; Warburton *et al.* 1986). Again, this indicates that even for those trisomies compatible with development, there is a strong negative selection at preimplantation stage. More studies are currently being performed on blastocyst stage embryos to define more closely the period when selection against aneuploid embryos occurs.

Monosomy ($n=78$) was more frequent than trisomy ($n=50$) for all chromosomes studied. Since non-disjunction theoretically produces disomic and nullisomic gametes with the same frequency, either technical error or an alternative mechanism for aneuploidy, e.g. mitotic anaphase lag during the first mitotic division (Ford *et al.* 1988) or loss of a chromosome during meiosis, could explain the latter observation.

In study I (Munné *et al.* 1995) we reported the occurrence of loss of chromosomes through nuclear and cytoplasmic fragmentation probably at the first cleavage division. Technical errors could involve polymorphisms that would render one of the targets of the probe too small to be visualized. Those polymorphisms have been described for satellite regions and affect probes X, Y and 18 (Weier and Gray 1992) but they should be uncommon for coding regions such as the ones for probes 13, 16 and 21. Since the monosomic fraction of chromosome 18 is similar to the monosomic fraction of chromosomes 13, 16 and 21, target polymorphism could not be the major cause of the difference between monosomy and trisomy rates. Thus, up to 20% of aneuploidies may be due to loss of chromosomes before or during the first mitotic division.

As discussed previously (Munné *et al.* 1995), aneuploidy does not necessarily lead to developmental arrest in the first 3 days in

Table 4. Chromosome abnormalities comparison between the three maternal age groups for the series II, I and the pooled series

		Maternal age groups (years)		
		20 to 34	35 to 39	40 to 47
<i>Extensive diploid mosaicism</i>	Series II	19.6	21.5	20.8
	Series I	13.2 ^a	25.9 ^a	14.4 ^a
	Total	16.9	23.3	18.1
<i>Polyploidy</i>	Series II ^b	9.8	9.5	7.9
	Series I ^b	20.5	21.8	17.8
	Total	14.3	14.6	12.0
<i>Haploidy</i>	Series II	5.1 ^c	3.3	5.4
	Series I	0.7 ^c	4.1	3.3
	Total	3.3	3.6	4.5
<i>Aneuploidy XY, 18</i>	Series II	3.9	2.2	4.5
	Series I	4.0	4.7	7.9
	Total	3.9	3.4	6.7
<i>Aneuploidy 13, 21</i>	Series II	3.5 ^d	80.0 ^d	15.3 ^d
	Series I	1.4 ^e	5.6 ^e	20.5 ^e
	Total	2.5^h	6.8^h	17.9^h
<i>Aneuploidy 16</i>	Series II	1.8 ^f	20.0 ^f	8.3 ^f
<i>Total aneuploidy</i>	Series II ^k	7.4^h	10.2^h	19.8^h
	Series II ^j	9.2^g	12.2^g	28.1^g
	Series I	5.4^h	7.8^h	28.4^h
	Total^l	8.2^h	12.2^h	32.9^h
Total abnormal during/after fertilization^l	Series II	34.5	34.3ⁱ	34.2
	Series I	34.4	51.8ⁱ	35.6

The mean values were averaged over the other factor 'Morphological group'. Values are percentages.

^a Significant differences between maternal age groups ($P < 0.01$, GLM analysis)

^b Significant differences between both series in all maternal age groups ($P < 0.01$, χ^2 test)

^c Significant differences between both series in all maternal age groups ($P < 0.05$, χ^2 test)

^{d,e} Significant differences between maternal age groups ($P < 0.001$, GLM analysis)

^f Not significant

^g Significant differences between maternal age groups ($P < 0.0003$, GLM analysis)

^h Significant differences between maternal age groups ($P < 0.0001$, GLM analysis)

ⁱ Significant differences between both series ($P < 0.002$, χ^2 test)

^j Total aneuploidy including chromosome 16

^k Total aneuploidy no including chromosome 16

^l Extensive diploid mosaicism + polyploidy + haploidy

culture since the embryonic genome is not fully active until day 2 or 3 of development (Braude *et al.* 1988; Tesarik *et al.* 1988). In fact, aneuploidy seems to be more common in group A embryos than in group B and C. As we argued before, this is probably an underestimation of aneuploidy in those embryos that are in addition chaotic or polyploid mosaics, where detecting aneuploidy is difficult because of the other chromosome abnormalities (Munné *et al.* 1995).

Post meiotic chromosome abnormalities, dysmorphism and maternal age

Mosaicism, haploidy and polyploidy occur post-meiotically, and mostly after fertilization (Munné *et al.* 1995). Therefore, post-meiotic chromosome abnormalities account for more than half the abnormalities detected in cleavage stage embryos. These

abnormalities significantly decreased with embryonic competence (polyploidy $P<0.001$, extensive diploid mosaicism $P<0.01$) (Table 5) but they were not affected by maternal age (Table 4).

Excluding the >40 years age group, extensive diploid mosaicism is the major chromosome abnormality in IVF-generated human embryos. For instance for the group 35-39 mosaicism was found in 23.3% of the embryos (Table 4), followed by polyploidy (21.8%), aneuploidy (10.2%), and haploidy (3.6%). Even in group A embryos, which are closer in quality to the embryos being replaced, aneuploidy (19.3%, Table 5) contributes to less than half of the chromosome abnormalities detected, with extensive diploid mosaicism (14.7%), polyploidy (4.5%) and haploidy (4%) together contributing more than aneuploidy.

Table 5. Comparison of percentages of chromosome abnormalities between the three morphological groups for the series II, I and the pooled series

		Morphological group		
		A	B	C
<i>Embryos analysed (n)</i>	Series II	254	363	114
	Series I	188	154	182
	Total	442	517	296
<i>Extensive diploid mosaicism</i>	Series II	15.7 ^a	21.8 ^a	28.1 ^a
	Series I	13.3	21.4	20.9
	Total	14.7^a	21.5^a	25.1^a
<i>Polyploidy</i>	Series II	6.3 ^{a,b}	7.7 ^a	20.2 ^{a,c}
	Series I	2.1 ^{b,d}	13.0 ^d	44.5 ^d
	Total	4.5^e	9.9^e	31.5^e
<i>Haploidy</i>	Series II	4.3	4.4	5.3
	Series I	3.7	1.9	2.7
	Total	4.0	3.4	4.2
<i>Aneuploidy XY, 18</i>	Series II	2.8	4.1	2.6
	Series I	6.4	5.8	5.5
	Total	4.6	5.0	4.1
<i>Aneuploidy 13, 21</i>	Series II	11.0 ^e	8.5 ^e	2.6 ^e
	Series I	18.4 ^d	11.2 ^d	2.2 ^d
	Total	14.7^d	9.9^d	2.4^d
<i>Aneuploidy 16</i>	Series II	0.9	4.3	3.4
<i>Total aneuploidy</i>	Series II^f	14.7	16.9	8.6
	Series II^g	13.8	12.6	5.2
	Series I	24.8	17.1	7.7
	Total^g	19.3^h	14.9^h	6.5^h
Total abnormal during/after fertilizationⁱ	Series II	26.4^j	33.9^j	53.5^j
	Series I	19.1^j	36.4^j	68.1^j

The mean values were averaged over the other factor 'Maternal age group'. Values are percentages.

^a Significant differences between all three morphological groups ($P<0.01$, GLM analysis)

^b Significant differences between both series for this morphologic group ($P<0.05$, χ^2 test)

^c Significant differences between both series in all maternal age groups ($P<0.05$, χ^2 test)

^d Significant differences between all three morphological groups ($P<0.001$, χ^2 test)

^e Significant differences between all three morphological groups ($P<0.0001$, GLM analysis)

^f Total aneuploidy including chromosome 16

^g Total aneuploidy not including chromosome 16

^h Significant differences between all three morphological groups ($P<0.03$, GLM analysis)

ⁱ Extensive diploid mosaicism + polyploidy + haploidy

^j Significant differences between all three morphological groups ($P<0.00001$, GLM analysis)

Because the embryonic genome is not fully active until day 3 of development (Braude *et al.* 1988), mosaicism, polyploidy and haploidy cannot produce dysmorphism originating in the first and second meiotic divisions. However, cytoplasmic impairment could produce both mosaicism and polyploidy, through cytoskeletal and spindle malfunction, cellular division block, or other mechanisms. For instance, abnormalities of the centriole in a fertilizing spermatozoon may produce mosaicism or other chromosome abnormalities in the resulting zygote (Palermo *et al.* 1994; Hewitson *et al.* 1997). Most chromosome studies on human embryos have focused on meiotic irregularities as the principal source of chromosome abnormalities. However, the present data indicate that other sources of chromosome abnormalities are equally or more important, and further investigation of other factors such as culture conditions, hormonal stimulation, centriole abnormalities, and cytoplasmic factors is justified.

Chromosome abnormalities, embryo selection and PGD

Selection of viable embryos for transfer is currently one of the most powerful tools in IVF to help ensure a high pregnancy rate. The frequency of chromosomal abnormalities in the embryos

transferred, and the chances of those embryos for implantation and development to term, will vary considerably depending on the type of embryo selection performed in a given laboratory. For instance, centres transferring embryos on day 2 of development cannot differentiate between slowly developing and arrested embryos. Embryo evaluation performed under powerful inverted microscopes will detect more dysmorphisms (such as multinucleation, fragmentation types, etc.) than evaluation performed on stereoscopes. Dysmorphic and arrested embryos, 50% or more of which are chromosomally abnormal, should not be transferred if better embryos are available. However, even this selection is not enough to screen for the 30% of chromosome abnormalities found in embryos with apparently normal morphology in the 35-39 years age group. For women aged >40 years, this rate increases to about 60%. For these patients, PGD for numerical chromosome abnormalities may be indicated. So far, more than 1000 PGD cases have been performed in women aged 35 years either by embryo biopsy at day 3 of development (Munné *et al.* 1993, 1999; Gianaroli *et al.* 1999) or by polar body biopsy (Verlinsky *et al.* 1995, 1996), and these studies have demonstrated an increase in implantation (Gianaroli *et al.* 1999) and a decrease in spontaneous abortions (Munné *et al.* 1999).

Table 6. Numbers of embryos showing chromosomal aneuploidy in series I, II and the pooled series

	Maternal age groups (years)			
	20 to 34	35 to 39	40 to 47	Total
<i>Embryos analysed with X,Y,18 probes</i>	406	467	382	1255
<i>Embryos analysed with 13, 21 probes</i>	255	274	202	731
<i>Embryos analysed with 16 probe</i>	110	148	48	306
Monosomy X	3	4	3	10
Monosomy Y	1	2	2	4
Trisomy XXX	1	0	1	2
Trisomy XXY	2	0	1	3
Trisomy XYY	0	0	1	1
Total gonosomes (%)	7(1.7)	5(1.1)	8(2.1)	20(1.6)
Monosomy 13	3	4	6	13
Trisomy 13	1	5	2	8
Total chromosome 13 (%)	4(1.6)	9(3.3)	8(40.0)	21(2.9)
Monosomy 16	1	2	3	6
Trisomy 16	0	1	1	2
Total chromosome 16 (%)	1(0.9)^a	3(20.0)	4(8.3)^b	8(2.6)
Monosomy 18	7	6	10	23
Trisomy 18	3	4	9 ^h	16
Total chromosome 18 (%)	10(2.5)	10(2.1)^c	19(50.0)^d	39(3.1)
Monosomy 21	3	4	15	22
Trisomy 21	2	7	9	18
Total chromosome 21 (%)	5(20.0)^e	13(4.7)^f	24(11.9)^g	40(5.5)

b>a ($P < 0.05$)

d>c ($P < 0.05$)

g>e,f ($P < 0.01$)

^h An embryo from series II classified as tetrasomy 18 was included

Aneuploidies detected with alpha-satellite 13/21 probe from series I were not included

Table 7. Estimate of the overall percentage of chromosome abnormalities in the general population of embryos in series II

	Maternal age (years)			Total
	< 35	35-39	> 39	
<i>No. embryos of each morphological group in the general population of series II</i>				
A	9309	8197	4277	21783
B	8916	7776	3949	20641
C	2792	2365	1184	6341
Total	21017	18338	9410	48765
<i>Percentage of abnormal embryos per group in series II</i>				
% A	30.6	34.0	52.3	
% B	43.3	44.7	53.3	
% C	54.8	66.7	60.0	
<i>Estimated no. of abnormal embryos in general population of series II</i>				
A	2849	2787	2237	7872
B	3861	3476	2105	9441
C	1530	1577	710	3818
Total	8239	7840	5052	21132
%	39.2	42.8	53.7	43.3

Acknowledgements

The authors would like to acknowledge the embryological skills of Adrienne Reign, Toni Ferrara, Elena Kissin, Renee Wamsley, Sasha Sadowy and Marlena Blake. Thanks are also due to Dr. Eurof Walters for providing statistical council, and Giles Tomkin for editorial assistance.

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