

Chromosome abnormalities in human embryos

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The presence of numerical chromosome abnormalities in human embryos was studied using fluorescence in-situ hybridization with four or more chromosome-specific probes. When most cells of an embryo are analysed, this technique allows differentiation to be made between aneuploidy, mosaicism, haploidy and polyploidy. Abnormal types of fertilization, such as unipronucleated, tripronucleated zygotes and zygotes with uneven pronuclei, were studied using this technique. We have found a strong correlation between some types of dysmorphism with chromosomal abnormalities. In addition, the more impaired the development of an embryo, the more chromosomal abnormalities were detected in those embryos. Maternal age and other factors were linked to an increase in chromosome abnormalities (hormonal regimes, temperature changes), but not to intracytoplasmic sperm injection.

Key words: aneuploidy/FSH/mosaicism/multinucleation fragmentation

Introduction

After all the struggles that an in-vitro fertilization (IVF) clinic faces to attain a successful, high implantation rate, the final problem may well be that of excessive multiple pregnancies. Therefore, oocyte and embryo preference become of central importance, and here we review the latest methods using numerical chromosome assessment as one of the main

tools for identifying selective criteria. Certain steps are needed to study properly the numerical chromosome abnormalities in human preimplantation embryos. First, individual chromosomes need to be assessed to determine specific aneuploidy rates. Second, all or most blastomeres from an embryo should be analysed to differentiate mosaicism from other abnormalities, and finally, developmentally arrested embryos should also be analysed.

All classical cytogenetic techniques have greater or lesser limitations, starting with the need for metaphase stage, when only one-third of all embryos analysed show good quality metaphases. Of these, only one-quarter will have all their cells analysed, or <8% overall (Pellestor *et al.*, 1994; Santaló *et al.*, 1995). This means that arrested embryos cannot be analysed at all, and implies that mosaicism is severely underestimated. Fluorescence in-situ hybridization (FISH) has been used with much higher efficiencies (85–95%) to study the chromosome constitution of cleavage-stage human embryos, arrested or not (Griffin *et al.*, 1992; Benkhalifa *et al.*, 1993; Munné *et al.*, 1993, 1994a, 1995a; Harper *et al.*, 1994a; Munné and Weier, 1996; Laverge *et al.*, 1998). FISH with multiple probes can differentiate polyploidy from aneuploidy and also haploidy from monosomy, and when most or all cells of an embryo are analysed, mosaicism can be differentiated from FISH or fixation failure, as well from aneuploidy (Munné *et al.*, 1994b,c). However, FISH only supplies information on a limited number of chromosomes for which the probes are specific. Another technique, primed in-situ (PRINS) labelling, shows efficiencies similar to FISH when used on blastomeres (see review by Pellestor, 1996). PRINS has the same limitations as FISH because cells need to be fixed, and the fluorochromes can rarely visualize more than five or six chromosomes simultaneously; however, it also has the limitation of any polymerase chain reaction (PCR) -based technique, because contaminant DNA could be accidentally amplified. Other approaches such as comparative genome hybridization (CGH) (Kallioniemi *et al.*, 1992) and quantitative PCR (Von Eggeling *et al.*, 1993), cannot as yet perform single cell analysis, but show potential for the future. Finally, spectral imaging (SKY) based on FISH technology, has

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been able to karyotype poor-quality metaphases of polar bodies (PB), oocytes and blastomeres, but still requires those cells to be at metaphase stage (Márquez *et al.*, 1998).

Chromosome abnormalities in embryos derived from zygotes with abnormal pronuclear number or morphology

The centrosome inheritance in human embryos needs to be understood to comprehend the different patterns of chromosome abnormalities produced by different numbers and the parental origin of pronuclei. Centrosome inheritance, unipronuclear, triprounuclear, apronuclear zygotes and zygotes with uneven pronuclei will be discussed here. Chromosome abnormalities produced by an abnormal number of haploid chromosome sets and the importance of the male centrosome will also be covered.

Centrosome inheritance in human zygotes

The centrosome is a centre of organization involved in forming the mitotic spindle. It reproduces and doubles during interphase in anticipation of cell division. In most cells, it consists of two morphologically distinct objects called centrioles, a pair of cylinders enclosed in a complex and asymmetrical arrangement, and the pericentriolar material from which spindle microtubules are generated. These organelles reproduce by forming daughter copies which segregate from their mother. In the development of fertilized animal eggs, specific mechanisms must exist at the gamete or zygote level to control centrosome inheritance. If centrosomes from both gametes were retained and remained functional, the zygote would enter mitosis with two doubled sets of centrosomes and four centrioles, resulting first in multipolar or extra spindles, and then in mosaicism (Sluder *et al.*, 1989). Three types of centrosomal inheritance have been described, that is under paternal or maternal control, or controlled by both gametes. Wilson and Matthews (1895) discovered over a century ago that in sea urchins, it is paternally inherited. Similar mechanisms exist for all mammals studied, with the exception of the mouse, which apparently displays no distinct centrosomal complex (Schatten *et al.*, 1986).

The presence of sperm centrioles in fertilized monospermic and dispermic human embryos was demonstrated by Sathananthan *et al.* (1991) using transmission electron microscopy. Tripolar spindles were found in triprounuclear zygotes with two centrioles. Analysis of the incidence and onset of mosaicism in human digynic, dispermic and enucleated embryos provides evidence that the sperm centrosome controls the first mitotic division after fertilization, although the use of maternal centrosomal material cannot

be excluded. Results prove that dispermic zygotes usually do not have bipolar spindles and become mosaic. This situation is determined by an extranuclear factor, since removal of a single pronucleus does not correct the abnormal division pattern (Cohen *et al.*, 1995). In contrast, monospermic digynic embryos become triploid, indicating that the three pronuclei are organized in a single spindle at syngamy. Removal of one pronucleus from such zygotes restores the embryo to a normal diploid state. Both the incidence of mosaicism and the onset of the anomaly in digynic embryos are similar to those found in normally developing monospermic embryos (Palermo *et al.*, 1994).

Single pronucleate zygotes

Single pronucleate (1PN) zygotes can be obtained following IVF and intracytoplasmic sperm injection (ICSI) at frequencies ranging from 2% to 5%. Single pronucleate embryos occasionally (3%) develop following insemination in our laboratory, but they occur more frequently (9%) following ICSI.

Chromosome studies in 1PN after IVF

Karyotype studies indicate that between 46–80% of 1PN embryos are in fact diploid (Plachot, 1991; Staessen *et al.*, 1993). However, those studies could not differentiate between diploidy produced by pronuclear fusion or fertilization by parthenogenetic activation and subsequent diploidization by asynchronous pronuclei inflation. To differentiate between parthenogenetic activation and true fertilization (resulting in asynchronous pronuclear inflation or fusion), sexing of these embryos is necessary since the presence of a Y chromosome will indicate the occurrence of fertilization. FISH studies, summarized in Table I, have shown that when embryos are diploid, 45–48% of them are fertilized (Sultan *et al.*, 1995; Staessen and Van Steirteghem, 1997). However, true parthenogenesis may also be present since haploid embryos were also detected. These FISH studies suggest that most diploid 1PN embryos develop from fertilized oocytes. However, two mechanisms could be responsible, namely asynchronous appearance of the two pronuclei, or formation of a single fertilization or zygote pronucleus (fused pronuclei). Levron *et al.* (1995) demonstrated that single pronucleate zygotes from IVF are usually formed after fusion of the male and female pronuclei. They partitioned the pronucleus (karyoplast) from the rest of the egg (cytoplast), and analysed them by FISH. Of the diploid pronuclei, 66% contained XY chromosomes and 33% contained XX chromosomes. The corresponding cytoplasts were DNA-free, indicating that asynchronous inflation of pronuclei is rare in such zygotes, or does not occur at all.

Table I. Fluorescence in-situ hybridization (FISH) studies of 1PN embryos from in-vitro fertilization (IVF)

Reference	n	Mosaic (%)	3n (%)	2n (%Y)	n (%)
Sultan <i>et al.</i> (1995)	21	19	0	66 (48)	14
Staessen and Van Steirteghem (1997)	115	37	1	49 (45)	13

Table II. Fluorescence in-situ hybridization (FISH) studies of 1PN embryos from intracytoplasmic sperm injection (ICSI)

Reference	n	Mosaic (%)	3n (%)	2n (%)	n (%)	%Y
Sultan <i>et al.</i> (1995)	21	39	0	14	47	19
Staessen and Van Steirteghem (1997)	61	41	0	28	31	31

Table III. Fluorescence in-situ hybridization (FISH) studies of 3PN embryos from in-vitro fertilization (IVF), with and without enucleation

Reference	n	Mosaic (%)	Triploid (%)	Diploid (%)	Haploid (%)
Intact					
Cohen <i>et al.</i> (1995)	36	89	3	3	0
Staessen and Van Steirteghem (1997)	71	63	13	21	3
Enucleated					
Cohen <i>et al.</i> (1995)	15	100	0	0	0

Table IV. Fluorescence in-situ hybridization (FISH) studies of 3PN embryos from intracytoplasmic sperm injection (ICSI)

Reference	n	Mosaic (%)	Triploid (%)	Diploid (%)	Haploid (%)
Palermo <i>et al.</i> (1994)	7	14	86	0	0
Staessen and Van Steirteghem (1997)	71	28	56	13	0

Chromosome analysis of 1PN after ICSI

Single pronucleate zygotes from ICSI have also been analysed by FISH in two studies (Sultan *et al.*, 1995; Staessen and Van Steirteghem, 1997). The results, summarized in Table II, indicate that single pronucleate ICSI zygotes are usually activated, not fertilized, but able to cleave. Such embryos are not being replaced into the patient.

Trippronuclear (3PN) zygotes

Dispermy (3PN after IVF)

Dispermy is the most common fertilization anomaly in the human (Kola *et al.*, 1987). The majority of these embryos will cleave, but arrest prior to differentiation. Cleavage patterns are highly irregular; most dispermic zygotes immediately divide into three or four cells following the first division, and only a minority divides into two cells (Kola *et al.*, 1987). Although several karyotype studies have been performed on 3PN (Pellestor, 1995), they suffer from the same problems of most karyotype studies and also do not distinguish between 3PN–2PB (dispermic) from

3PN–1PB zygotes (digynic). Multi-probe FISH studies (Cohen *et al.*, 1995; Staessen and Van Steirteghem, 1997) have found that while most of the embryos are mosaics, very few are pure triploids (Table III). The different number of diploids between centres, ranging from 3% to 21%, could be due to differences in recording vacuoles and pronuclei. Staessen and Van Steirteghem (1997) found a ratio of XXX:XXY:XYX similar to 25%:50%:25% which would be expected from a dispermic origin. Some of these embryos could still be digynic, since polar bodies are occasionally fragmented and it is difficult to distinguish if there is one fragmented PB or two PBs. They also observed that 3PN zygotes after IVF cleaved more rapidly than 2PN oocytes, with more 3-cell embryos resulting from 3PN (20%) than 2PN (12%) embryos. Zygotes dividing into 3-cell embryos had less uniformly triploid cells than those dividing into two or four cells.

In human zygotes, size appears to be variable and sperm tail remnants can almost never be identified by light microscopic observation (Malter and Cohen, 1989). To ascertain

the presence of the male pronuclei, the X/Y ratio needs to be determined. The X/Y ratio is determined by the fact that the female pronucleus always contains an X-chromosome, whereas male pronuclei can have X- as well as Y-chromosomes. The X/Y ratio in dispermic zygotes and their daughter blastomeres should be 1:3. Removal of a male pronucleus should render a normal X/Y distribution of 1:1 if the distal pronucleus (the one furthest from the polar body) is indeed of paternal origin. A total of 51 dispermic embryos were biopsied between the 2- to 11-cell stages, of which 15 were previously enucleated. The proportion of dispermic and enucleated embryos with X- as well as Y-chromosomes was similar to that of the theoretical value; 72% and 53% respectively (Tang *et al.*, 1994). However, even though the sex ratio was corrected, the resulting embryos continue to be mosaics (Cohen *et al.*, 1995) (Table III). Analyses of the types of mosaicism and the frequency of abnormal cells have indicated that the anomaly occurs at syngamy (Munné *et al.*, 1994b). Nevertheless, mosaicism in monospermic embryos usually occurs after the embryo has divided into two cells (Munné *et al.*, 1994b). The mosaicism probably occurs as a consequence of abnormal tripolar division in some embryos (Plachot, 1991).

Digynic zygotes (3PN after ICSI)

Some 4% of eggs injected with single sperm cells have three pronuclei and a single polar body. We consider these embryos to be monospermic digynic, and their genetic status can be analysed by performing FISH in blastomeres (Palermo *et al.*, 1994; Staessen and Van Steirteghem, 1997). Most of these embryos were found to be perfect triploids (Table IV), and the differences in the percentage of diploid embryos could be caused by the presence of a misleading vacuole. None of the studies found XYY embryos, and equal numbers of XXX and XXY were found, again indicating that the mechanism was digyny. In addition, significantly less mosaics were found in 3PN after ICSI than 3PN after IVF, and mosaicism occurred mostly at the second division after ICSI compared with the first division after IVF. This, and the fact that many perfectly triploid embryos were found, indicates that the chromosomes of the three pronuclei had organized in a single bipolar spindle at syngamy, and suggests that only one centrosome is active in such zygotes.

Three of the four monospermic and digynic embryos from which the pronucleus next to the first polar body was removed cleaved properly and became normally diploid, also indicating that a single centrosome is active in such embryos. The fourth one was a diploid mosaic embryo.

Apronuclear (0PN) zygotes

About 1% of zygotes with two polar bodies do not show pronuclei. Using FISH with XY,18 and 13/21 probes, Manor *et al.* (1996) have found that 57% of them are normal diploid, 30% polyploid and or mosaic, and 13% aneuploid. They recommend the transfer of these embryos when insufficient dipronucleated embryos are available. It is likely that pronuclei are hidden due to granularity, or missed because of an abnormal development speed.

Uneven pronuclei

Preimplantation development and chromosomal contents were evaluated in individual embryos derived from monospermic zygotes with uneven pronuclei. The average pronuclear sizes were 12.5 μm and 22.3 μm . Less than 2% of the studied 2PN zygotes ($n = 4527$) had this dysmorphism, but they were found in 14% of patients. Chromosomal status was assessed in 15 of these zygotes and 87% were considered abnormal, mostly mosaics (Sadowy *et al.*, 1998). More studies are needed to identify the origin of each pronucleus. In the meantime, transfer of such embryos is not recommended, especially since the data were derived by analysing a restricted number of chromosome pairs.

Analysis of cleavage-stage human embryos

Karyotype analysis of morphologically normal embryos

Some reports have been published giving a rate of chromosome abnormalities between 20% and 40%. The disparity of these results may in fact be caused by the small number of embryos and the low number of cells analysed. Observations are also complicated, since the follicular stimulation protocols were different as well as the aetiology of patients and culture systems. From recent data collected in several programmes, it is becoming apparent that many factors determine chromosomal aneuploidy rates (Munné *et al.*, 1997). For instance, when only an embryo for which at least two cells were analysed is included, lower rates of aneuploidy and higher rates of mosaicism were reported (Almeida and Bolton, 1996) than when only one or more cells were analysed (Pellestor, 1995). The results are summarized in Table V. The difference between these studies is remarkable and indicates that a sizeable part of aneuploidy detected before the data of Almeida and Bolton (1996) were published may not have been true aneuploidy, but mosaicism. These results underline the need for different and/or improved techniques, such as FISH and SKY, to study chromosomal abnormalities in cleavage-stage embryos.

Table V. Difference in chromosome abnormalities found by karyotyping when one or more cells were analysed

	Good quality 2PN		Poor quality 2PN	
	(a)	(b)	(a)	(b)
No. processed	1574	163	686	178
No. analysed (%)	32	72	39	49
Diploidy (%)	63	78	13	38
Aneuploidy (%)	15	3	30	10
Mosaicism (%)	10	15	13	40
Other abnormal (%)	12	5	49	11

(a) = one or more cells analysed (see review by Pellestor, 1995).

(b) = two or more cells analysed (data from Almeida and Bolton, 1996).

Table VI. Summary of morphological abnormalities and relation to chromosomal abnormalities

Embryo morphology	FISH analysis	Reference
<i>Normal morphology</i>		
20–34 years old	16% abnormal	Munné <i>et al.</i> (1995)
35–39 years old	37% abnormal	Munné <i>et al.</i> (1995)
40–45 years old	53% abnormal	Munné <i>et al.</i> (1995)
<i>Dysmorphic zygotes</i>		
3PN after regular IVF	80–100% abnormal	Cohen <i>et al.</i> (1995); Staessen and Van Steirteghem (1997)
3PN after ICSI	100% abnormal	Cohen <i>et al.</i> (1995); Staessen and Van Steirteghem (1997)
Cleaving 1PN after regular IVF	34–50% abnormal	Sultan <i>et al.</i> (1995); Staessen and Van Steirteghem (1997)
Cleaving 1PN after ICSI	70–85% abnormal	Sultan <i>et al.</i> (1995); Staessen and Van Steirteghem (1997)
Uneven PN	87% abnormal	Sadowy <i>et al.</i> (1998)
Cleaving 0PN	43% abnormal	Manor <i>et al.</i> (1996)
<i>Dysmorphic embryos</i>		
Giant embryos (>220 µm)	triploid	Munné <i>et al.</i> (1994d)
Dominant single blastomere	polyploid	Munné <i>et al.</i> (1994d)
>20% fragments, normal development	56% abnormal	S.Munné (unpublished)
Multinucleated embryos	74% abnormal	Kligman <i>et al.</i> (1996)

ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilization.

Morphological traits and chromosome abnormalities

Certain types of dysmorphism have been studied in correlation to chromosome abnormalities: fragmentation, multinucleation, giant eggs and dominant blastomere embryos. As summarized in Table VI, some of these morphological abnormalities are very well correlated with chromosomal abnormalities, but others are not. A review of each dysmorphism follows and its association (or lack

of) with chromosomal abnormalities.

Fragmentation

Fragmentation percentage has been associated with chromosome abnormalities (Plachot *et al.*, 1987; Pellestor and Sele, 1988), in particular mosaicism. We have found that the percentage of fragmentation is correlated with mosaicism (S.Munné, unpublished results), whereas aneuploidy does not appear to predict fragmentation (Table VII).

Table VII. Chromosomal abnormalities detected by fluorescence in-situ hybridization (FISH) and fragmentation rate

Fragments (%)	Aneuploid (%)	Mosaic and other (%)
0–15	11	29
20–40	8	47
45–100	11	89
	Not significant	$P < 0.001$

In addition, different patterns of fragmentation have been observed, some of them associated with programmed cell death (Warner *et al.*, in press; M.Alikani *et al.*, unpublished results). The mechanical removal of fragments is only recommended in those embryos with <40% fragmentation because they can still be chromosomally normal. Removal of fragments from embryos with such limited fragmentation may improve implantation, probably by allowing the establishment of proper cell–cell interaction, especially with the microsurgical removal of fragments associated with adjacent blastomeres (M.Alikani *et al.*, unpublished results).

Multinucleation

The occurrence of multinucleated blastomeres (MNB) has been studied by Balakier and Cadesky (1997) in 1885 embryos. About 44% of patients possessed embryos with at least one MNB, and 15% of embryos contained MNB, but there was no correlation with maternal age. MNB occurred at any time between the first cleavage division and blastocyst stage, but were found more often in 2-cell than 4-cell or later-stage embryos. This last observation may be less precise since nuclear observations are hindered once embryos contain more cells and fragments.

MNB are normally associated with abnormal embryo development or dysmorphism. For instance, we found that there are more dysmorphic embryos with MNB (47%) than normally developing embryos (34.4%) (Munné *et al.*, 1995). Hardy *et al.* (1993) suggested that multinucleation indicates a reduced developmental competence of the embryo, since binucleated cells are usually arrested. Similarly, Balakier and Cadesky (1997) found that 57% of embryos containing MNB arrested at 2–15 cells, and only 14% reached expanded blastocyst stages and contained a normal-looking inner-cell mass.

Chromosome abnormalities: FISH studies on MNB showed that the chromosomal content of each MNB nucleus was not always the same as the chromosomal content of the nuclei of the sibling blastomere MNB (Munné and Cohen, 1993; Munné *et al.*, 1994a). A more recent study has found that the presence of multinucleated cells in non-arrested day 2 or day 3 human embryos is indicative in 74%

of the cases of extensive mosaicism and/or polyploidy (Kligman *et al.*, 1996). Similar results were obtained by Laverge *et al.* (1997). This pattern differs from previous analyses in which MNB were detected on day 4 after biopsy. In those studies, morphologically normal embryos with a single or few MNB were mostly chromosomally normal (Munné and Cohen, 1993; Munné *et al.*, 1994a). This suggests two patterns of multinucleation. One pattern occurs at the 2-cell stage, which normally involves multinucleation and produces chromosomally abnormal embryos. Van Blerkom *et al.* (1997) have recently found a correlation between embryos with at least one MNB at the 2-cell stage and follicular under-oxygenation. These embryos normally have a disorganized M-II spindle, thus probably resulting in extensive chromosome abnormalities. The other pattern occurs at the 4- to 16-cell stage and usually involves binucleation probably produced by cytokinesis failure, with each of the two nuclei being chromosomally normal. Normally, we do not recommend the transfer of embryos with MNB at the 2-cell stage, while embryos with binucleated cells at the 8-cell stage may be transferred if no other morphologically normal embryos are available.

Giant eggs and dominant blastomere embryos

We have detected two instances in which monospermic embryos with a particular morphological abnormality were chromosomally unique (Munné *et al.*, 1994c). The first were embryos with only one large dominant cell surrounded by smaller blastomere-sized extracellular fragments (Figure 1). These embryos were polyploid and frequently polyploid mosaic, and the single cell was normally multinucleated. The second group developed from giant oocytes with diameters of $\geq 220 \mu\text{m}$, and zygotes displaying two polar bodies and two pronuclei (Figure 2). These embryos are invariably triploid or triploid mosaics, with XXX or XXY gonosome constitutions, which suggests a higher contribution of maternal genomes. We have seen also giant germinal vesicle (GV)-stage eggs containing two nuclei, indicating that giant GV-stage oocytes originate from cytokinetic failure or from fusion of two GV. These embryos should not be transferred.

Chromosomal abnormalities and embryo development

Significant differences in the total amount of chromosome abnormalities are found between arrested, slowly developing and normally developing embryos when studied by FISH (Munné *et al.*, 1995). These differences have been later confirmed by karyotyping, although only slow and



Figure 1. Two oocytes at germinal-vesical stage. One is normal size and mononucleated; the other is much larger, with two nuclei.

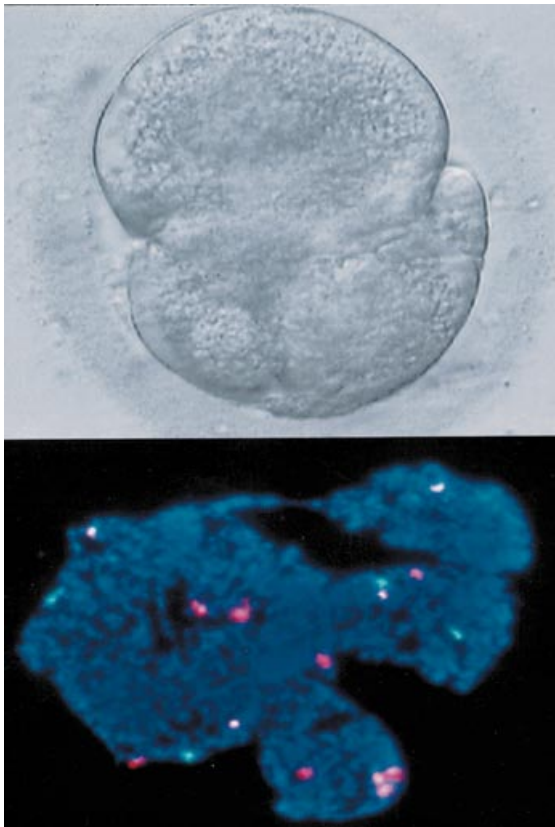


Figure 2. 'Dominant blastomere' embryo, and fluorescence in-situ hybridization (FISH) on the large blastomere, showing that it is polyploid.

normally developing embryos could be studied (55% abnormal versus 27%, $P < 0.001$; Almeida and Bolton, 1996).

Arrested embryos: The major chromosomal abnormality detected in arrested monospermic embryos is polyploidy (43%), followed by mosaicism and aneuploidy. In total, 71% of arrested embryos are chromosomally abnormal

(Munné et al., 1995). It is likely that this is an underestimation considering the relatively low number of chromosomes investigated. The origin of polyploidy in arrested embryos was discussed previously (Munné et al., 1994a), and polyspermic fertilization is considered unlikely since only two pronuclei are observed after insemination. It may be that for polyploid monospermic embryos, their DNA synthesis continued, although cellular division had stopped. In some instances karyokinesis continues, producing multinucleation in almost half their cells. That DNA synthesis is not prevented by cleavage arrest has been demonstrated by Artley et al. (1992). According to Winston et al. (1991), even if karyokinesis and gene activation do not fail, impaired cytokinesis may arrest the embryo because there are insufficient cells to produce a functional inner-cell mass. Since most polyploid embryos arrest before the onset of genome activation, which occurs around the 4- to 8-cell stage (Braude et al., 1988), oocyte quality or culture conditions may be the cause of their arrest. Interestingly, embryos that at the time of freezing were not arrested but that were not cleaved after 24 h in culture after thawing are rarely chromosomally normal (20%; Laverge et al., 1998) and show high rates of polyploidy and mosaicism. This reinforces the hypothesis that cytokinesis arrest, at any stage, results in polyploidy in all (pure polyploidy) or some cells (diploid/polyploid mosaicism).

Slow and/or fragmented embryos: These embryos are chromosomally abnormal in 57% of cases. The major chromosome aneuploidy in 'fragmented or slowly' developing embryos is aneuploidy (23%), followed by extensive mosaicism (22%) and polyploidy (13%) (Munné et al., 1995). 'Arrested' and 'slow or fragmented' embryos appear to have similar rates of diploid mosaicism. This rate is double that of normally developing embryos (Table III). Since many arrested polyploid embryos are also mosaics, it can be argued that arrested embryos show more mosaicism than do slowly developing ones. Almeida and Bolton (1996) found that slow embryos at the 2- to 4-cell stage had 55% abnormalities compared with 28% of embryos at the 5- to 8-cell stage ($P < 0.001$). Similar results were also obtained using FISH with X, Y and 1 chromosome specific probes (Laverge et al., 1997).

Normally developing embryos: These are rarely polyploid or mosaic, but frequently aneuploid. Polyploidy is very rare in normally developing embryos, and mosaicism and aneuploidy are also lower than in slow embryos. In total, 29% of morphologically normal embryos are chromosomally abnormal (Munné et al., 1995). Obviously, it should be borne in mind that the actual values are underestimated, since only a restricted number of chromosomes were investigated simultaneously.

Table VIII. Cleavage origin of mosaicism

Cleavage stage onset of mosaicism	Monospermic			Dispermic
	Diploid	Polyploid	Haploid	
1st day	1	9	3	17
2 days	17	15	1	11
≥3 days	17	3	1	0
Total	35	27	5	28

Table IX. Types of mosaics according to the number of abnormal cells per embryo

Type	<3/8 abnormal	>3/8 abnormal	Total
Diploid mosaics			
Chaotic	9 (12)	105 (44)	114 (36)
2n/4n	42 (54)	35 (15)	77 (24)
Non-disjunction	8 (10)	38 (16)	46 (15)
With MNB	11 (14)	6 (3)	17 (5)
Anaphase lag	2 (3)	12 (4)	14 (4)
Endoreduplication	1 (1)	0 (0)	1 (>1)
Haploid mosaics	1 (1)	16 (8)	17 (5)
Polyploid mosaics	4 (5)	26 (11)	30 (9)
Total mosaics	78	238	316

Database from Saint Barnabas Medical Centre (up to June 1997, unpublished).

Values in parentheses are percentages.

Accelerated cleavage: Harper et al. (1994b) have described the occurrence of embryos with accelerated cleavage. These embryos had already cleaved by day 1, being at the 2- to 4-cell stage. After FISH analysis, most of them appeared mosaic. Harper and co-workers suggested that the embryos could have been polypenetrated, which would explain both the mosaicism and the accelerated cleavage. Chromosome abnormalities impair embryo development: As demonstrated before, cleavage-stage embryos with normal development can be chromosomally abnormal. This is because genome activation does not start until the 4- to 8-cell stage (Braude et al., 1988) and therefore, chromosomal abnormalities cannot produce embryonic arrest until later stages, as shown in other species. In bovine species, depending on the abnormality, chromosomally abnormal embryos analysed on day 5 of development have less cells than chromosomally normal ones (7.9 for polyploids, 16.8 for aneuploids, 30 for normal diploids) (Kawarsky et al., 1996).

Study of specific types of chromosome abnormalities occurring in human embryos

Mosaicism in monospermic human embryos

The high frequency of mosaicism after FISH analyses of only four chromosome pairs has been criticised as a FISH artefact. Recently, high rates of mosaicism have also been reported using PRINS (Pellestor *et al.*, 1996), or by karyotyping when analysing at least two blastomeres (Almeida and Bolton, 1996). In karyotype studies, the rate of mosaicism detected has been underestimated based on the analysis of only one or two cells per embryo, while aneuploidy was overestimated (Almeida and Bolton, 1996). For mosaicism studies to be accurate, most cells from each embryo should be analysed.

Onset of mosaicism

The onset of mosaicism in cleavage-stage embryos can be determined by assessing the number of blastomeres of each type. This can only be accomplished when the majority of cells in a given embryo are analysed. All blastomeres of monospermic embryos are abnormal when the chromosome abnormality occurs during the first embryonic division. When one-half or one-quarter of the blastomeres are abnormal, mosaicism arises at the second and third divisions, respectively (Munné *et al.*, 1994b). Striking differences in the mechanisms and onset of mosaicism appear when monospermic embryos with varying ploidy are compared with dispermic embryos. Polyploid and haploid mosaic embryos are usually created at the first division, whereas monospermic diploid mosaics are usually generated at the second or later divisions (Munné *et al.*, 1994b) (Table VIII). The results suggest that in diploid embryos, pronuclear syngamy usually occurs correctly, whereas mosaicism is generated by ensuing mitotic aberrations. Triploid dispermic zygotes, on the contrary, may have problems distributing their chromosomes equally in two nuclei due to the presence of several paternal centrosomes.

Types of mosaics

The types of mosaics observed in cleavage-stage embryos are more diverse than those observed in spontaneous abortions, probably because some of them are incompatible with implantation. We have classified mosaics according to the overall ploidy (haploid, diploid, polyploid mosaics), and in addition, according to the mechanism (non-disjunction, endoreduplication, chaotic mosaics, diploid/other ploidy) (Table IX; Figure 3). Another classification has been suggested which differentiates mosaic embryos with an understandable mechanism of origin (non-disjunction, 2n/4n) from chaotic embryos, which are not classified as

mosaics (Harper and Delhanty, 1996; Harper *et al.*, 1995). However, we feel that chaotic embryos should be classified as mosaics, that is, embryos with more than one cell type.

Table IX shows recent data from our laboratory for the different types of mosaics found and their frequency according to the number of abnormal cells (Munné *et al.*, 1994b, 1995, and unpublished data). The types of mosaics involving a few cells tend to be diploid/tetraploid mosaics, while mosaics affecting most cells of an embryo tend to be chaotic mosaics. The types of mosaics also change with embryonic development, as shown in Table X, with diploid/tetraploid embryos being most common in normally developing embryos and chaotic and polyploid embryos being more common in arrested embryos. While aneuploidy is found to increase significantly with maternal age, both at the oocyte and embryo level (Munné *et al.*, 1995; Dailey *et al.*, 1996), mosaicism does not appear to be correlated.

Table X. Types of mosaics in different developmental groups of embryos

	Arrest	Slow	Good
n	182	154	188
Mosaicism	85 (47%)	68 (44%)	56 (30%)
Diploid mosaics			
2N/4N, 2N/3N, 2N/N, etc.	10%	40%	36%
By chaotic division	24%	12%	23%
By mitotic non-disjunction	14%	19%	21%
Others	0%	1%	2%
Polyploid mosaics	46%	24%	5%
Haploid mosaics	6%	4%	13%

Data from Munné *et al.* (1995), excluding binucleated cells.

Fate of mosaic cells

It has been suggested repeatedly that abnormal cells within mosaic embryos are self-correcting, that they have less developmental potential than normal cells, and/or locate ultimately in the trophoectoderm (Kola *et al.*, 1987; Plachot, 1991; James and West, 1994). Nevertheless, studies in several laboratories show a different outcome for abnormal cells, depending on the type of mosaicism.

Diploid/tetraploid mosaics: These apparently represent a normal developmental process leading to trophoblast formation. Polyploid nuclei have been described in most mammals studied, including human blastocysts (Benkhali-fa *et al.*, 1993). Experimental production of tetraploid/diploid mouse embryos suggests that a mechanism exists to exclude chromosomally abnormal cells from the primitive ectoderm lineage, since at 12 days no tetraploid cells can be

detected in the fetus (James and West, 1994). The cells derived from the tetraploid part appear mostly on trophoectoderm derivative tissues or to amnion, yolk sac mesoderm, or primitive endoderm (tissues derived from the inner cell mass, other than the fetus). This means that although some tetraploid cells can become incorporated in the inner cell mass, they do not contribute, at least in the mouse, to form the fetus. On the other hand, the fact that they appear as early as the cleavage stage has been suggested to indicate cytokinesis breakdown due to unsuitable culture conditions (Angell *et al.*, 1987). In that case, their fate is probably cellular arrest.

Multinucleated blastomeres: The fate of MNB found in cleavage-stage embryos has been determined by measuring blastomere size. During cleavage, blastomeres become increasingly smaller, as there is no cellular growth, and consequently by measuring their size it is possible to determine the stage of every individual blastomere. Using this approach, Hardy *et al.* (1993) concluded that MNB are mostly arrested. It is unlikely that mural-trophoectoderm giant cells, which are polyploid, could originate from binucleated cells because these giant cells are formed at the blastocyst stage while most binucleated cells originate and arrest at the third cleavage division, before trophoectoderm differentiation (Hardy *et al.*, 1993).

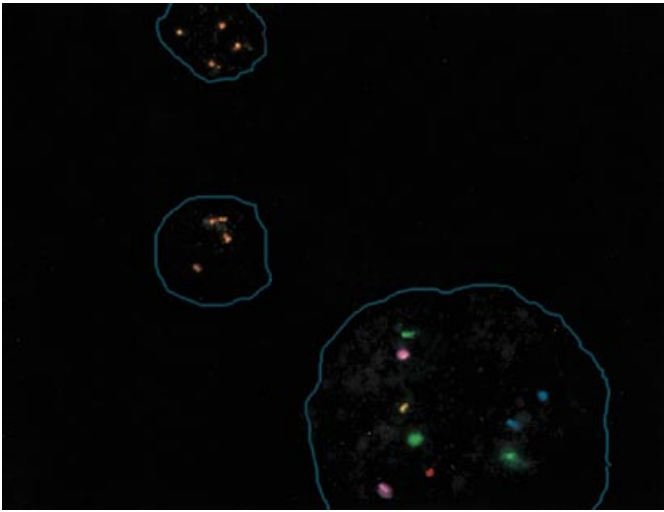
Aneuploid cells: Unlike polyploid and multinucleated cells, aneuploid cells experimentally combined with normal diploid cells to form mosaic mammalian embryos are demonstrably able to participate in embryogenesis and contribute to post-implantation stages and viable offspring (Epstein *et al.*, 1984; Dyban and Baranov, 1987).

Chaotic embryos: Most or all cells of these embryos are chromosomally abnormal, and in most cases, each cell has a different chromosome complement. As indicated by Delhanty *et al.* (1997), who first described this type of mosaicism, they probably do not progress beyond implantation.

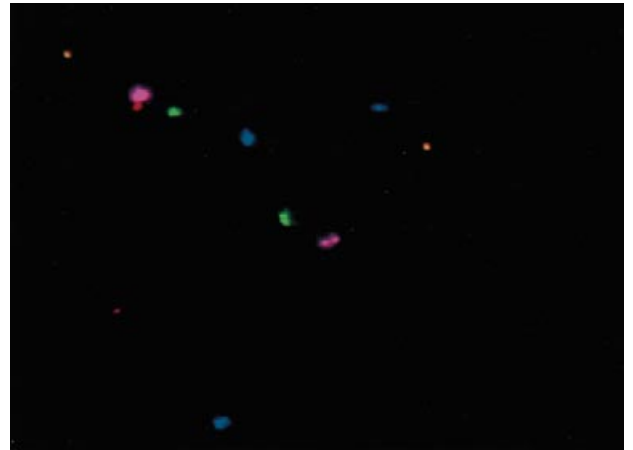
Factors inducing mosaicism

The suspected factors contributing to the formation of mosaicism are diverse and are by no means fully investigated. Chromosomal abnormalities may be caused by an abnormal number of centrioles (Palermo *et al.*, 1994). Otherwise, a defective sperm centriole/centrosome may also impair the first embryonic division resulting in mosaicism (Sathananthan *et al.*, 1996). Another factor inducing mosaicism has been identified by Van Blerkom *et al.* (1997), who found a statistically significant correlation between embryos with at least one MNB at the 2-cell stage and follicular under-oxygenation. These embryos also normally have a disorganized M-II spindle. Therefore, these

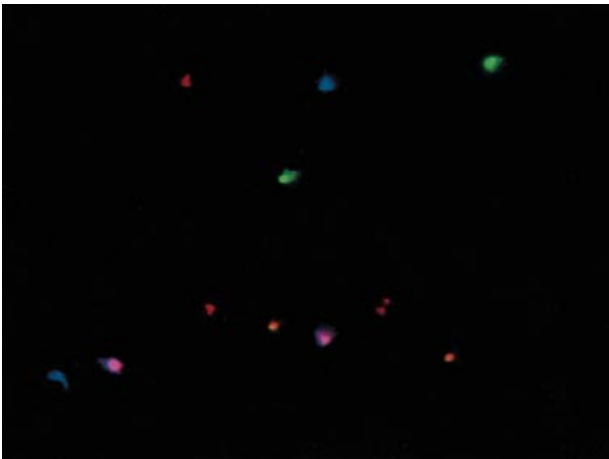
3



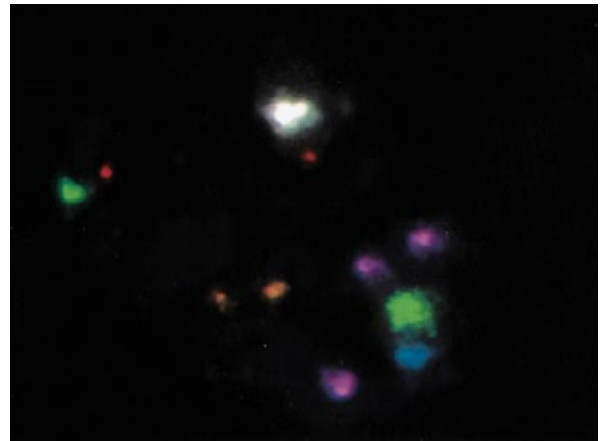
6



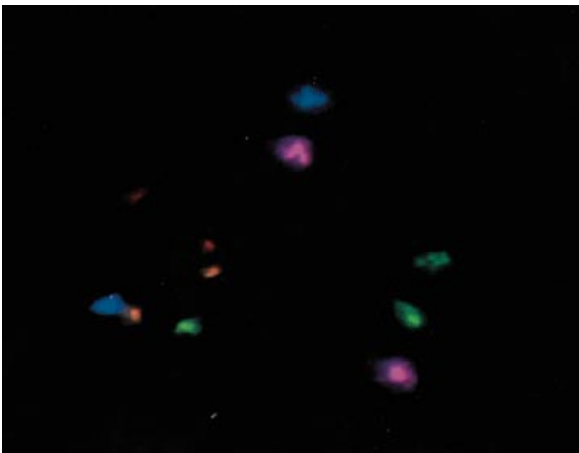
4



7



5



Figures 3–7. Fluorescence in-situ hybridization (FISH) with X (blue), Y (white), 13 (yellow), 16 (green), 18 (pink) and 21 (red) specific probes on blastomeres from trisomic embryos. Figure 3 shows a blastomere which belonged to an embryo with all its cells trisomic 16, but endoreduplication of chromosome 13 has also occurred (nine copies). Figure 4 shows a blastomere from a trisomy 21 embryo; Figure 5 shows a blastomere from a trisomy 16 embryo; Figure 6 shows a blastomere from a trisomy X embryo and Figure 7 shows a blastomere from a trisomy 18 embryo.

mechanisms will probably cause mosaicism such that all the cells become abnormal, though with different ploidies. However, this type of mosaic is rare. Embryos with only a fraction of their cells abnormal may be produced by culture conditions more often than by abnormal gametes. For instance, a drop in temperature may affect cytokinesis, resulting in diploid/polyploid embryos or embryos with MNB. Recently, we have demonstrated that embryos from different laboratories cultured under different conditions and stimulation protocols have very diverse rates of mosaicism (Munné *et al.*, 1997).

Aneuploidy in oocytes and cleavage-stage embryos

Genetic analysis of abortuses and live offspring has shown that older women are at a higher risk of delivering trisomic fetuses. Most of this aneuploidy is the result of non-disjunction in maternal meiosis-I (Hassold and Chiu, 1985; Warburton *et al.*, 1986; Antonorakis *et al.*, 1991), and even trisomy 18, which occurs in similarly in maternal meiosis-I and -II (Fisher *et al.*, 1995) may also originate mostly in meiosis-I but be classified by molecular techniques as originating at meiosis-II (Lamb *et al.*, 1996). Most karyotype studies of oocytes and embryos have failed to show any correlation between maternal age and aneuploidy, largely because of problems inherent in both unrepresentative patient populations and karyotyping itself. While FISH reviews only a few chromosomes, it can detect trisomy with accuracy, and in patients with a large maternal age spectrum, a very significant ($P < 0.001$) increase in aneuploidy with maternal age was found both in embryos (Munné *et al.*, 1995) (Table III) and oocytes (Dailey *et al.*, 1996). This corroborates the hypothesis that oocytes of older women are more prone to non-disjunction caused by meiotic errors at the gamete level.

The risk of conceiving a trisomic fetus has been estimated to increase from 1.9% of clinically recognized pregnancies in women 25–29 years old, to 19.1% in women over 39 years of age (Hassold and Chiu, 1985). For chromosomes 13, 18 and 21, aneuploidy in clinically recognized pregnancies increases from 1.3% in the 35- to 39-year-old group to 4.3% in the 40- to 45-year-old group (Snijders *et al.*, 1994). However, in morphologically and developmentally normal cleavage-stage embryos, we found that aneuploidy rates for chromosomes XY, 13, 18 and 21 increased from 4% in the 20- to 34-year-old group, to 37% in the 40- to 45-year group.

The differences between aneuploidy in cleavage-stage embryos and clinically recognized pregnancies appear to be that many aneuploid embryos are being eliminated before a pregnancy is clinically recognized. Monosomies, for instance, seldom implant. The data from our embryo study (Munné *et al.*, 1995) shows that the rate of monosomy is

similar to that for trisomy, while with the exception of monosomy 21 (1/1000 karyotyped abortions), the other autosomal monosomies are normally undetected in clinically recognized pregnancies. Therefore, monosomic embryos must be the ones eliminated during the first days or weeks of pregnancy. Similarly, some trisomies are commonly detected in cleavage-stage embryos and seldom or never detected in spontaneous abortions, as is the case of trisomy 1 (Watt *et al.*, 1987; Laverge *et al.*, 1997). Figures 4–7 show FISH results from trisomic embryos.

Because aneuploidy is only linked to maternal age, negative selection of aneuploid embryos can only be done through preimplantation genetic diagnosis either by polar body analysis or by embryo analysis. So far XY, 13, 16, 18 and 21 chromosomes can be analysed simultaneously in single blastomeres or polar bodies with an efficiency of 90% (Munné *et al.*, 1993; Verlinsky *et al.*, 1995; Munné and Weier, 1996). More than 500 cycles have been performed using this technique, resulting in more than 100 chromosomally normal babies. However, an increase in implantation rate or a decrease in abnormal offspring has not yet been demonstrated because of insufficient data.

Factors influencing chromosome abnormalities

Many factors have a role in the high rate of chromosome abnormalities in cleavage embryos, especially with regard to differences between laboratories, but few have been studied in detail. Here, we review some effects of hormonal stimulation, of light, temperature, water, the ICSI procedure, high basal FSH levels and patient selection.

Hormonal stimulation

The frequency of premature chromosome condensation in human oocytes after karyotyping has been observed to depend on the type of hormonal stimulation (Ederisinghe *et al.*, 1992; Benkhalifa *et al.*, 1996). Using FISH analysis, chromosome abnormalities—particularly mosaicism—have been alluded to increase in human embryos obtained through clomiphene citrate or gonadotrophin stimulation rather than through downregulation ($P < 0.05$; Munné *et al.*, 1997).

Light, temperature and water

Temperature: Spindle microtubules are highly thermo-sensitive and even a small change in temperature can disturb the spindle structure of the oocytes. Pickering *et al.* (1990) found that human oocytes have a disassembled spindle in 50% of cases after 10 min at room temperature, and in 100% after 30 min at room temperature, accompanied by chromosome anomalies.

Table XI. Chromosome abnormalities found in intracytoplasmic sperm injection (ICSI) and in-vitro fertilization (IVF) embryos developing from bipronucleated zygotes

Chromosome abnormalities	Age ≤ 39 years		Age >39 years		All ages	
	IVF	ICSI	IVF	ICSI	IVF	ICSI
No. of embryos analysed	135	102	110	34	245	136
% Normal	39	48	28	24	34	42
% Aneuploid ^a	10	6	12	18	11	9
% Gonosomal					2	1
% Haploid (mosaic or not)	2	5	2	6	2	5
% Polyploid (mosaic or not)	10	4	13	12	11	6
% Extensive 2N mosaicism	28	20	38	38	33	24
% Low 2N mosaicism	10	18	7	3	9	14

^aFour monosomies 13; two nullisomy 13; six monosomies 21; two monosomies 18; six monosomy 16; two trisomy 13; three trisomy 16; seven trisomy 21; one trisomy 18; three monosomy X; one disomy Y; one trisomy XXY; one trisomy XYY.

Water and air quality: Unsuitable water and air quality has been recently linked to sudden decreases in implantation rate (Cohen et al., 1997). However, no chromosomal analysis has been performed on those embryos. Furthermore, while some chemicals have been suggested as the culprits for lower than expected implantation rates, the damage that they might produce is at the gene level, and not the chromosome level.

The ICSI procedure

After controlling for morphological and maternal age differences, a study involving 380 bipronucleated cleavage-stage embryos obtained by conventional IVF or ICSI showed that the rates of aneuploidy (autosomal and gonosomal), mosaicism and other abnormalities were similar (Table XI). These results indicate that ICSI is not a teratogenic method (Munné *et al.*, 1998). However, chromosomal abnormality rates may still be higher in a fraction of ICSI patients, these being carriers of chromosomal abnormalities, and patients with oligoasthenoteratozoospermia, who have more gonosomal disomic spermatozoa (Vidal *et al.*, personal communication). For these cases, preimplantation genetic diagnosis (PGD) could be offered once blood or sperm analysis from a certain patient demonstrates a real risk of transmitting chromosome abnormalities.

High basal follicle stimulating hormone (FSH) concentrations

Scott *et al.* (1997) have compared chromosomal abnormalities in relation to FSH concentrations to evaluate the relationship between age, ovarian reserve and genetic errors during meiosis and embryonic cleavage. There is a significant rise in abnormality rates with increasing FSH

concentrations in women below the age of 40 years ($P < 0.02$). The prevalence of genetic abnormalities is uniformly high in embryos from women over the age of 40 years, independent of their basal FSH status. Abnormality rates in women under 40 with elevated FSH concentrations (>10 mIU/ml) are equivalent to those over age 40. The finding of uniformly high chromosome abnormality rates in women over the age of 40 years, independent of their FSH status, demonstrates that an elevated FSH concentration is a marker which becomes abnormal late in the process of depleting ovarian reserve and is consistent with lower pregnancy rates seen in this population.

Final remarks

Successful embryo selection is one of the best tools used in embryology for achieving high implantation rates. The selection can be: (i) active, by selecting against morphologically, developmentally or genetically (by PGD) abnormal embryos; or (ii) indirect, by culturing the embryos as long as possible, in which process a sizeable number of unsuitable embryos will arrest. Now that most morphological, developmental and chromosomally abnormal types of embryos have been identified, the next step should be to ascertain—and if possible prevent—the action of agents causing chromosomal abnormalities as well as embryonic dysmorphism and arrest. This may imply changes in hormonal stimulation to produce better-matured oocytes, prevention of molecular organic and inorganic contamination using better air and water filters (as well as purer culture ingredients and products), and better selection of spermatozoa to avoid damaged centrioles producing mosaicism. Although most of these agents are not known, or the anomalies cannot be identified without destroying the em-

bryo, new methods are being proposed to prevent these processes in the gamete or embryo, such as cytoplasm donation (Cohen *et al.*, 1998).

References

- Almeida, P.A. and Bolton, V.N. (1996) The relationship between chromosomal abnormality in the human preimplantation embryo and development in-vitro. *Reprod. Fertil. Dev.*, **8**, 235–241.
- Angell, R.R., Sumner, A.T., West, J.D. *et al.* (1987) Post-fertilization polyploidy in human preimplantation embryos fertilized *in vitro*. *Hum. Reprod.*, **2**, 721–727.
- Antonarakis, S.E., Lewis, J.G., Adelsberg, P.A. *et al.* (1991) Parental origin of the extra chromosome in trisomy 21 revisited: DNA polymorphism analysis suggests maternal origin in 95% of cases. *N. Engl. J. Med.*, **324**, 872–876.
- Artley, J.K., Braude, P.R. and Johnson, M.H. (1992) Gene activity and cleavage arrest in human pre-embryos. *Hum. Reprod.*, **7**, 1014–1021.
- Balakier, H. and Cadesky, K. (1997) The frequency and developmental capability of human embryos containing multinucleated blastomeres. *Hum. Reprod.*, **12**, 800–804.
- Benkhalifa, M., Janny, L., Vye, P. *et al.* (1993) Assessment of polyploidy in human morulae and blastocysts using co-culture and fluorescent in-situ hybridization. *Hum. Reprod.*, **8**, 895–902.
- Benkhalifa, M., Menezo, Y., Janny, L. *et al.* (1996) Cytogenetics of uncleaved oocytes and arrested zygotes in IVF programs. *J. Assist. Reprod. Genet.*, **13**, 140–148.
- Braude, P., Bolton, V. and Moore, S. (1988) Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature*, **333**, 459–461.
- Cohen, J., Levron, J., Palermo, G. *et al.* (1995) Atypical activation and fertilization patterns in humans. *Theriogenology*, **43**, 129–140.
- Cohen, J., Gilligan, A., Esposito, W. *et al.* (1997) Ambient air and its potential effects on conception. *Hum. Reprod.*, **12**, 1742–1749.
- Cohen, J., Scott, R., Alikani, M. *et al.* (1998) Ooplasmic transfer in mature human oocytes. *Mol. Hum. Reprod.*, **4**, 269–280.
- Dailey, T., Dale, B., Cohen, J. and Munné, S. (1996) Association between non-disjunction and maternal age in meiosis-II human oocytes detected by FISH analysis. *Am. J. Hum. Genet.*, **59**, 176–184.
- Delhanty, J.D.A., Harper, J.C., Ao, A. *et al.* (1997) Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. *Hum. Genet.*, **99**, 755–760.
- Dyban, A.P. and Baranov, V.S. (1987) Functional activity of chromosomes and control mechanisms of early embryonic development. In *Cytogenetics of Mammalian Embryonic Development*. Clarendon Press, Oxford, pp. 267–294.
- Ederisinghe, W.R., Murch, A.R. and Yovich, J.L. (1992) Cytogenetic analysis of human oocytes and embryos in an in-vitro fertilization program. *Hum. Reprod.*, **7**, 230–236.
- Epstein, C.J., Smith, S. and Cox, D.R. (1984) Production and properties of mouse trisomy 15 diploid chimeras. *Dev. Genet.*, **4**, 159–165.
- Fisher, J.M., Harvey, J.F., Morton, N.E. and Jacobs, P.A. (1995) Trisomy 18: studies of the parent and cell division of origin and the effect of aberrant recombination on nondisjunction. *Am. J. Hum. Genet.*, **56**, 669–675.
- Griffin, D.K., Wilton, L.J., Handyside, A.H. *et al.* (1992) Dual fluorescent in-situ hybridization for simultaneous detection of X and Y chromosome-specific probes for the sexing of human preimplantation embryonic nuclei. *Hum. Genet.*, **89**, 18–22.
- Hardy, K., Winston, R.M.L. and Handyside, A.H. (1993) Binucleate blastomeres in preimplantation human embryos in-vitro: failure of cytokinesis during early cleavage. *J. Reprod. Fertil.*, **98**, 549–558.
- Harper, J.C. and Delhanty, J.D.A. (1996) Detection of chromosomal abnormalities in human preimplantation embryos using FISH. *J. Assist. Reprod. Genet.*, **13**, 137–139.
- Harper, J.C., Coonen, E., Ramaekers, F.C.S. *et al.* (1994a). Identification of the sex of human preimplantation embryos in two hours using an improved spreading method and fluorescent in-situ hybridization (FISH) using directly labeled probes. *Hum. Reprod.*, **9**, 721–724.
- Harper, J.C., Robinson, F., Duffy, S. *et al.* (1994b) Detection of fertilization in embryos with accelerated cleavage by fluorescence in-situ hybridization (FISH). *Hum. Reprod.*, **9**, 1733–1737.
- Harper, J.C., Coonen, E., Handyside, A.H. *et al.* (1995) Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic preimplantation human embryos. *Prenatal. Diagn.*, **15**, 41–49.
- Hassold, T. and Chiu, D. (1985) Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum. Genet.*, **70**, 11–17.
- James, R.M. and West, J.D. (1994) A chimeric animal model for confined placental mosaicism. *Hum. Genet.*, **93**, 603–604.
- Kallioniemi, A., Kallioniemi, O.P., Sudar, D. *et al.* (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*, **258**, 818–821.
- Kawarsky, S.J., Busurur, P.K., Stubbings, R.B. *et al.* (1996) Chromosomal abnormalities in bovine embryos and their influence on development. *Biol. Reprod.*, **54**, 53–59.
- Kligman, I., Benadiva, C., Alikani, M. and Munné, S. (1996) The presence of multinucleated blastomeres in human embryos correlates with chromosomal abnormalities. *Hum. Reprod.*, **11**, 1492–1498.
- Kola, I., Trounson, A., Dawson, G. and Rogers, P. (1987) Triprenuclear human oocytes: altered cleavage patterns and subsequent karyotypic analysis of embryos. *Biol. Reprod.*, **37**, 395–401.
- Lamb, N., Freeman, S., Savage-Austin, A. *et al.* (1996) Susceptible chiasmate configurations of chromosome 21 predispose to nondisjunction in both maternal meiosis I and meiosis II. *Nature Genet.*, **14**, 400–405.
- Laverge, H., De Sutter, P., Verschraegen-Spae, M.R. *et al.* (1997) Triple colour fluorescent in-situ hybridization for chromosomes X, Y and 1 on spare human embryos. *Hum. Reprod.*, **12**, 809–814.
- Laverge, H., Van der Elst, J., De Sutter, P. *et al.* (1998) Fluorescent in-situ hybridization on human embryos showing cleavage arrest after freezing and thawing. *Hum. Reprod.*, **13**, 425–429.
- Levron, J., Munné, S., Willadsen, S. *et al.* (1995) Male and female genomes associated in a single pronucleus in human zygotes. *Biol. Reprod.*, **52**, 653–657.
- Malter, H. and Cohen, J. (1989) Embryonic development following microsurgical repair of polyspermic human zygotes. *Fertil. Steril.*, **52**, 373–380.
- Manor, D., Kol, S., Lewit, N. *et al.* (1996) Undocumented embryos: do not trash them, FISH them. *Hum. Reprod.*, **11**, 2502–2506.
- Márquez, C., Cohen, J. and Munné, S. (1998) Chromosome identification in human oocytes and polar bodies by spectral karyotyping. *Cytogenet. Cell Genet.*, **81**, 254–258.
- Munné, S. and Cohen, J. (1993) Unsuitability of multinucleated human blastomeres for preimplantation genetic diagnosis. *Hum. Reprod.*, **8**, 1120–1125.
- Munné, S. and Weier, U. (1996) Simultaneous enumeration of chromosomes 13, 18, 21, X and Y in interphase cells for preimplantation genetic diagnosis of aneuploidy. *Cytogenet. Cell Genet.*, **75**, 263–270.
- Munné, S., Lee, A., Rosenwaks, Z. *et al.* (1993) Diagnosis of major chromosome aneuploidies in human preimplantation embryos. *Hum. Reprod.*, **8**, 2185–2191.
- Munné, S., Grifo, J., Cohen, J. and Weier, H.U.G. (1994a) Chromosome abnormalities in arrested human preimplantation embryos: a multiple probe fluorescence in-situ hybridization (FISH) study. *Am. J. Hum. Genet.*, **55**, 150–159.
- Munné, S., Weier, H.U.G., Grifo, J. and Cohen, J. (1994b) Chromosome mosaicism in human embryos. *Biol. Reprod.*, **51**, 373–379.
- Munné, S., Alikani, M., Grifo, J. and Cohen, J. (1994c) Monospermic polyploidy and atypical embryo morphology. *Hum. Reprod.*, **9**, 506–510.
- Munné, S., Alikani, M., Tomkin, G. *et al.* (1995) Embryo morphology, developmental rates and maternal age are correlated with chromosome abnormalities. *Fertil. Steril.*, **64**, 382–391.

- Munné, S., Magli, C., Adler, A. *et al.* (1997) Treatment-related chromosome abnormalities in human embryos. *Hum. Reprod.*, **12**, 780–784.
- Munné, S., Márquez, C., Reing, A. *et al.* (1998) Chromosome abnormalities in embryos obtained following conventional IVF and ICSI. *Fertil. Steril.* **69**, 904–908.
- Palermo, G., Munné, S. and Cohen, J. (1994) The human zygote inherits its mitotic potential from the male gamete. *Hum. Reprod.*, **9**, 1220–1225.
- Pellestor, F. (1995) The cytogenetic analysis of human zygotes and preimplantation embryos. *Hum. Reprod. Update*, **1**, 581–585.
- Pellestor, F. (1996) The primed *in situ* technique and preimplantation chromosome analysis. *Assist. Reprod. Rev.*, **6**, 62–66.
- Pellestor, F. and Sele, B. (1988) Assessment of aneuploidy in the human female by using cytogenetics of IVF failure. *Am. J. Hum. Genet.*, **42**, 274–283.
- Pellestor, F., Dufour, M.C., Arnal, F. and Humeau, C. (1994) Direct assessment of the rate of chromosomal abnormalities in grade IV human embryos produced by in-vitro fertilization procedure. *Hum. Reprod.*, **9**, 293–302.
- Pickering, S.J., Braude, P.R., Johnson, M.H. *et al.* (1990) Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. *Fertil. Steril.*, **54**, 102–108.
- Plachot, M. (1991) Chromosome analysis of oocytes and embryos. In Verlinsky, Y. and Kuliev, A. (eds), *Preimplantation Genetics*. Plenum Press, New York, pp. 103–112.
- Plachot, M., Junca, A.M., Mandelbaum, J. *et al.* (1987) Chromosome investigations in early life. II. Human preimplantation embryos. *Hum. Reprod.*, **1**, 29–35.
- Sadowy, S., Tomkin, G., Munné, S. *et al.* (1998) Impaired development of zygotes with uneven pronuclear size. *Zygote*, **63**, 137–141.
- Santaló, J., Veiga, A., Calafell, J.M. *et al.* (1995) Evaluation of cytogenetic analysis for clinical preimplantation diagnosis. *Fertil. Steril.*, **64**, 44–50.
- Sathananthan, H., Kola, I., Osborn, J. *et al.* (1991) Centrioles in the beginning of human development. *Proc. Natl Acad. Sci. USA*, **88**, 4806–4810.
- Sathananthan, H., Ratman, S.S., Ng, S.C. *et al.* (1996) The sperm centriole: its inheritance, replication and perpetuation in early human embryos. *Hum. Reprod.*, **11**, 345–356.
- Schatten, H., Schatten, G., Mazia, D. *et al.* (1986) Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. *Proc. Natl Acad. Sci. USA*, **83**, 105–109.
- Scott, R.T., Márquez-Guevara, C., Tomkin, G. *et al.* (1997) The incidence of chromosomal errors during human meiosis and cleavage correlates with basal FSH levels. *Fertil. Steril.* (in press).
- Sluder, G., Miller, F.J., Lewis, K. *et al.* (1989) Centrosome inheritance in starfish zygotes: selective loss of the maternal centrosome after fertilization. *Dev. Biol.*, **131**, 567–579.
- Snijders, R.J.M., Holzgreve, W., Cuckle, H. and Nicolaides, K.H. (1994) Maternal age-specific risks for trisomies at 9–14 weeks' gestation. *Prenat. Diagn.*, **14**, 543–552.
- Staessen, C. and Van Steirteghem, A.C. (1997) The chromosomal constitution of embryos developing from abnormally fertilized oocytes after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Hum. Reprod.*, **12**, 321–327.
- Staessen, C., Janssenwillen, C., Devroey, P. and Van Steirteghem, A.C. (1993) Cytogenetic and morphological observations of single pronucleated human oocytes after in-vitro fertilization. *Hum. Reprod.*, **8**, 221–223.
- Sultan, K.M., Munné, S., Palermo, G.D. *et al.* (1995) Ploidy assessment of embryos derived from single-pronucleated human zygotes obtained by regular IVF and intra-cytoplasmic sperm injection (ICSI). *Hum. Reprod.*, **10**, 132–136.
- Tang, Y.X., Munné, S., Reing, A. *et al.* (1994) The parental origin of the distal pronucleus in dispermic human zygotes. *Zygote*, **2**, 79–85.
- Van Blerkom, J., Antezak, J. and Schrader, R. (1997) The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Hum. Reprod.*, **12**, 1047–1055.
- Verlinsky, Y., Cieslak, J., Friedline, M. *et al.* (1995) Pregnancies following pre-conception diagnosis of common aneuploidies by fluorescence in-situ hybridization. *Hum. Reprod.*, **10**, 1923–1927.
- Von Eggeling, F., Freytag, M., Fashold, R. *et al.* (1993) Rapid detection of trisomy 21 by quantitative PCR. *Hum. Genet.*, **1**, 567–570.
- Warburton, D., Kline, J., Stein, Z. and Strobino, B. (1986) Cytogenetic abnormalities in spontaneous abortions of recognized conceptions. In Porter, I.H. and Willey, A. (eds), *Perinatal Genetics: Diagnosis and Treatment*. Academic Press, New York, pp. 133–148.
- Warner, C.M., Cao, W., Exley, G.E. *et al.* (1998) Genetic regulation of egg and embryo survival. *Hum. Reprod.*, **13**, (Suppl. 3) 178–190.
- Watt, J.L., Templeton, A.A., Messinis, I. *et al.* (1987) Trisomy 1 in an eight-cell human pre-embryo. *J. Med. Genet.*, **24**, 60–64.
- Wilson, E.B. and Mathews, A. (1895) Maturation, fertilization and polarity in the ectoderm egg. *J. Morphol.*, **10**, 319–342.
- Winston, N.J., Braude, P.R., Pickering, S.J. *et al.* (1991) The incidence of abnormal morphology and nucleocytoplasmic ratios in 2-, 3- and 5-day human pre-embryos. *Hum. Reprod.*, **6**, 17–24.