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The presence of multinucleated blastomerés in human embryos is correlated with chromosomal abnormalities

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The purpose of the present study was to determine whether the presence of one or more multinucleated blastomeres during early embryonic development is associated with chromosomal abnormalities in sibling blastomeres of that embryo. Embryos with multinucleated cells (n = 47) detected on day 2 or 3 of development were compared to dividing embryos without multinucleation. Arrested embryos were excluded from this study. Chromosome abnormalities were detected using fluorescent in-situ hybridization (FISH) with X, Y, 18 and 13/21 chromosome-specific probes. Of 47 embryos included in this study, 76.6% were chromosomally abnormal, compared to 50.9% in the control group ($P < 0.001$). Excluding aneuploidy, which is originated in the gametes and not the embryo, the differences were even higher, with 74.5% of multinucleated embryos being chromosomally abnormal compared to 32.3% of non-multinucleated embryos ($P < 0.001$). Day of multinucleation appearance, number of nuclei per cell, number of multinucleated cells per embryo and developmental quality of the embryos as well as the type of fertilization (intracytoplasmic sperm injection versus standard insemination) were not found to affect the rate of chromosomal abnormalities in embryos with multinucleated cells. These results suggest that embryos with multinucleated cells may not be suitable for replacement and should be excluded unless no other embryos are available.

Key words: embryo biopsy/FISH/mosaicism/mulunucleation

Introduction

Chromosomal disorders occurring at syngamy or during the cleavage stages of human development are common and result in embryos with limited developmental ability. The presence of such abnormalities in a living embryo cultured *in vitro* can only be recognized by performing an embryo biopsy with preimplantation genetic diagnosis. Some investigators have suggested that embryonic morphology and chromosomal disorders are related. For instance, all karyotype studies on morphologically abnormal monospermic embryos have detected a higher rate of chromosome abnormalities compared to morphologically normal embryos (Plachot *et al.*, 1987; Bongso *et al.*, 1991; Zenzes and Caspar, 1992; Pellestor *et al.*, 1994; Munné *et al.*, 1994a, 1995). However, this broad relationship between embryo morphology and chromosomal status is sufficiently ambiguous not to dictate against the transfer of such embryos in in-vitro fertilization (IVF)–embryo transfer cycles. Nevertheless, some specific types of morphological abnormalities have been found to be invariably linked to chromosomal abnormalities. Polyspermy, producing tri- or multi-pronuclear zygotes, has been found to result in embryos that are chromosomal mosaics, although seldom purely polyploid (Cohen *et al.*, 1995). Rougier and Plachot (1993) demonstrated a high incidence of mosaicism when performing karyotypes of single blastomeres from abnormal embryos (uni- or tripronuclear) obtained after IVF. Other studies demonstrate that chromosomal mosaicism can be efficiently detected in apronuclear, unipronuclear and polypronuclear human embryos using the fluorescent in-situ hybridization (FISH) technique (Coonen *et al.*, 1994). In contrast, cleaving embryos developing from unipronuclear zygotes are mostly chromosomally normal and diploid when obtained by standard insemination (Munné *et al.*, 1994b; Levron *et al.*, 1995). Following intracytoplasmic sperm injection (ICSI), however, these are found to be mostly haploid (Sultan *et al.*, 1995). Cell size and distribution also seem to be a reflection of the chromosomal complement. For instance, embryos with a large cell (bigger than a 2-cell stage cell) surrounded by cellular fragments, and embryos developing from oocytes larger than normal were found to be polyploid and triploid respectively (Munné *et al.*, 1994c).

Multinucleation is another morphological abnormality described in cleaving embryos developing *in vitro* as well as *in vivo*. Its frequency ranges from 17 to 69% of human embryos (Hertig *et al.*, 1954; Lopata *et al.*, 1983; Plachot, 1985; Hardy *et al.*, 1993; Munné *et al.*, 1994a) but is significantly higher when other morphological abnormalities are also present alongside multinucleation (Munné *et al.*, 1995a). Hardy *et al.* (1993) found no correlation between morphology and the incidence of binucleate cells in a large series of human embryos, although there was a correlation between anucleate and multinucleate blastomeres and morphology. Multinucleation first occurs at the 2-cell stage (Tesarik, 1994), but desegregration and biopsy studies indicate that it occurs most frequently at the 8-cell stage (Hardy *et al.*, 1993; Munné and Cohen, 1993). Although multinucleated embryos clearly contain a chromosomal abnormality, it still remains unclear whether the chromosomal aberration is limited to the

multinucleated cell or is shared by the rest of the cells. Since multinucleated blastomeres (MNB) have arrested development (Hardy *et al.*, 1993), embryos with one or two of these may still develop normally, but embryos with many MNB will have compromised development. However, if multinucleation occurs at the first embryonic division, all the cells of the embryo may be affected, and thus, the presence of MNB may indicate that the embryo is completely chromosomally abnormal. FISH studies on 8-cell stage human embryos have been performed to assess MNB as a source of misdiagnosis during preimplantation genetic diagnosis (Munné and Cohen, 1993; Munné *et al.*, 1994a). It was found that the chromosomal content of each nucleus of a MNB was not always the same as the chromosomal content of the nuclei of sibling blastomeres. However, in most cases in which MNB showed two nuclei, and each of the two nuclei had a normal chromosomal complement, the rest of the cells were usually chromosomally normal. These studies also suggest that multinucleation at the 8-cell stage or later is a benign morphological abnormality if it is limited to one or two cells per embryo. However, no studies have been performed in embryos in which multinucleation was observed at the first embryonic division.

In the present study, embryos containing MNB were identified during routine morphology assessments performed on day 2 and 3 of in-vitro culture after conventional IVF or ICSI. Embryos containing MNB were analysed by FISH using multiple probes in order to determine whether the presence of a MNB was indicative of chromosomal abnormalities in the other blastomeres, which appeared normal and mononucleated

Materials and methods

Embryos for this study were obtained from patients undergoing IVF treatment for infertility or preimplantation genetic diagnosis at The Center for Reproductive Medicine and Infertility of The New York Hospital–Cornell Medical Center, USA. These embryos were investigated under protocols #0890–701 and #0893–107, approved by the institute's Committee on Human Rights in Research and after obtaining written, signed consent from the patients. Embryos were cultured in droplets of human tubal fluid (made on site with water base from a Millipore water system) covered with equilibrated mineral oil (Squibb Pharmaceuticals, Princeton, NJ, USA). The presence of multinucleation was recorded on a daily basis by an embryologist, and in most cases, the day MNB appeared, the number of MNB per embryo and number of nuclei in each blastomere were recorded. For that purpose, all embryos were observed with a ×40 objective on an inverted Nikon Diaphot microscope using Hoffman interference optics The degree of embryonic fragmentation and number of cells on days 2 and 3 were also recorded, as well as the method of fertilization (i.e conventional insemination or ICSI) Embryos with MNB appearing 2 or 3 days after insemination were then biopsied and the blastomeres were fixed on the fourth day. Monospermic embryos were classified in three main groups 'arrested', 'slow and/or fragmented' and 'good' Arrested embryos were those that had not cleaved during the previous 24 h and had not gone beyond the 4-cell stage 3 days after insemination. Slow and/or fragmented embryos were those that had cleaved in the previous 24 h and had >15% fragmentation and/or had not reached the 8-cell stage 3 days after insemination Good embryos were those that had reached the 8-cell stage within 3 days of insemination and had <15% fragmentation Only non-arrested, monospermic embryos developing from bipronucleated zygotes were used for this study.

Embryos were biopsied on day 4 of development. A hole was drilled through the zona pellucida with acidified Tyrode's solution and all blastomeres were removed from each embryo by micromanipulation as described previously (Grifo *et al.*, 1992). All biopsied blastomeres were placed in a culture dish containing hypotonic solution (1% sodium citrate in water, 6 mg bovine serum albumin/ml, Sigma, St. Louis, MO, USA) for 2–5 min and fixed on glass slides (Munné *et al.*, 1993a). Slides were dehydrated (70, 85, 95% ethanol, 2 min each) and either analysed after incubation in a dry oven at 65°C for 1 h or stored at –20°C until further analysis. All or most blastomeres fixed from each embryo were analysed by FISH, using simultaneously X, Y, 18 chromosome-specific probes combined or not with 13/21 or 16 chromosome-specific probes as previously described (Munné *et al.*, 1993b, 1994a, 1995b) without modification (Figure 1) The FISH analysis and failure criteria used have been previously described by Munné *et al.* (1994a).

It has been observed from many hundreds of procedures of both embryo biopsy (Hardy *et al.*, 1990) and freeze–thaw cryopreservation, that 8-cell stage embryos can still develop normally after the loss of two or three cells, but viability diminishes considerably when 3/8 to half of the embryo is destroyed. Accordingly, we have considered an 8-cell embryo as abnormal if >3/8 blastomeres were either chromosomally abnormal or multinucleated. Binucleated cells were also considered abnormal, even when each nucleus had a normal number of chromosomes.

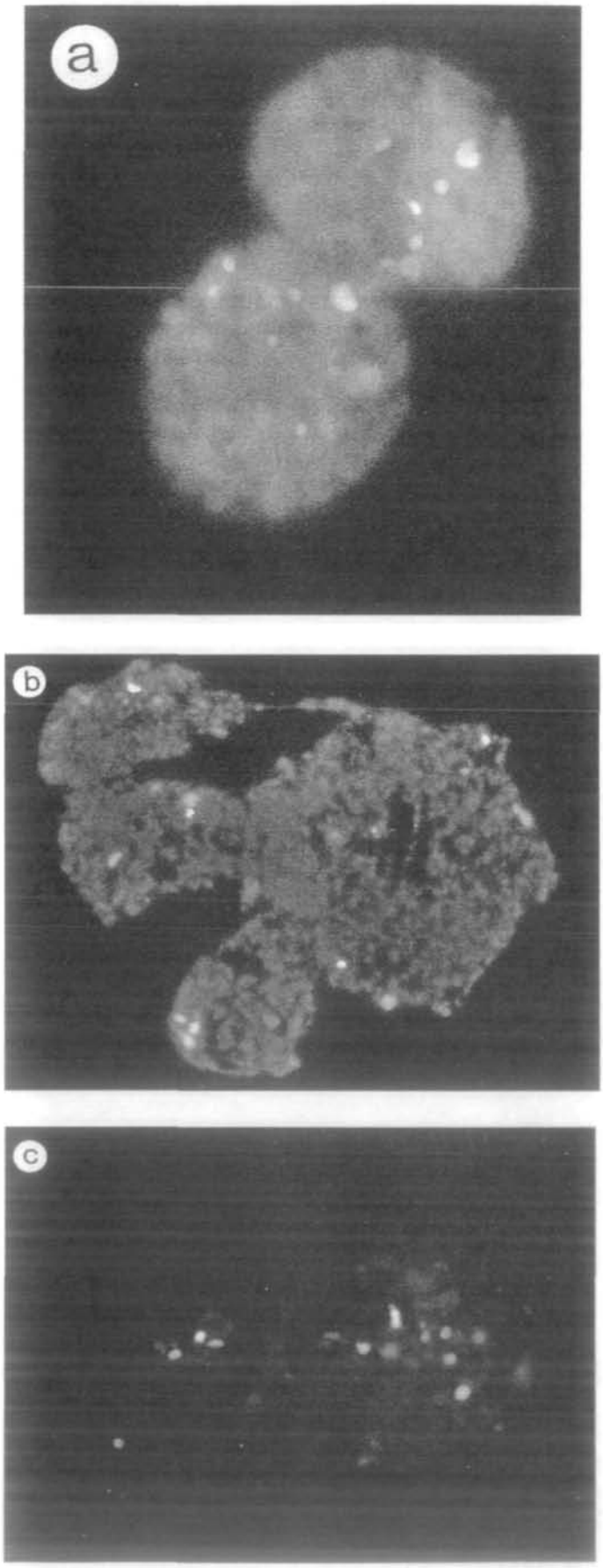
As a control group, 403 non-arrested embryos without multinucleation on day 2 or day 3 of development were used. Of those, 201 had good development and 198 were slow and/or fragmented Average maternal age was 36.4 years Most of these embryos have been described in a previous report (Munné *et al*, 1995a) The χ^2 test was used to compare morphological groups for abnormalities detected by the same probe.

We have previously classified MNB into four groups according to the chromosomal content of each nucleus (Munné and Cohen, 1993) In this study those groups have been simplified to just two: group A, consisting of MNB in which each nucleus has a normal diploid number of chromosomes, and group B, in which they have any other combination of numbers of chromosomes.

Results

In all, 47 embryos containing MNB were included in this study (Table I): 18 with normal and 29 with slow development. These embryos contained a total of 318 cells, 23 of which were lost during biopsy or fixation. In 45 others, no nucleus was detected even though the cell was not lost during fixation; these were considered to be anucleate cells. The remaining 250 blastomeres were analysed by FISH, and results were obtained in 90.8% (227/250) of these. The rest were either damaged or were FISH failures as defined in the Methods section.

After biopsy and subsequent fixation, only 32 embryos contained MNB, 15 of which had one MNB, eight had two, and nine had three or more MNB In total, 61 blastomeres were multinucleated, with 36 of them having two nuclei, 17 with three nuclei or more, and eight with an unrecorded number of nuclei The 15 remaining embryos did not show multinucleation after fixation, even though in nine of them all the cells were correctly biopsied and fixed. In the other six



embryos, one cell was lost in five of them, and four cells were lost in the sixth embryo. One of the lost cells could have been the multinucleated. The 61 MNB were classified into groups A and B according to their chromosomal content per nucleus: 18 (29.5%) belonged to group A (only diploid nuclei), and the rest to group B. This proportion of group A MNB was significantly lower ($P < 0.01$) than that previously described in MNB of embryos studied on day 4 (42/72, 58.3%; Munné and Cohen, 1993).

From the FISH analysis, chromosome abnormalities were found in 76.6% (36/47) of the embryos. Of those, 26 were diploid mosaics with extensive mosaicism (38% or more abnormal and/or multinucleated cells), two were aneuploid with extensive mosaicism, six were polyploid or polyploid mosaics, one was polyploid and aneuploid, one was aneuploid without mosaicism (embryo no. 9 in Table I) and one was haploid. Embryos presenting mosaicism are shown in Table II. Interestingly, the five detected aneuploidies were all monosomies: three for chromosomes 13 or 21 and two for chromosome 18. These embryos came from women of average maternal age that was apparently slightly higher (36.4 years) than that of women producing the rest of the multinucleated embryos (35.1 years). No significant differences in maternal age (35.3 and 36.4 years) or aneuploidy rates (10.6 and 20.3%) were found between the groups of multinucleated and non-multinucleated embryos respectively. However, the proportion of total chromosome abnormalities (76.6%) was significantly higher ($P < 0.001$) in multinucleated embryos than the 50.9% found in non-arrested embryos without multinucleation. Similarly, the percentage of chromosome abnormalities when aneuploidy was excluded was significantly higher ($P < 0.001$) in multinucleated embryos (74.5%) than in non-multinucleated embryos (32.3%).

Multinucleated embryos were sorted and compared according to different characteristics. One was the persistence of multinucleation until day four (biopsy day). In this category, 15 (32%) of the embryos that had been previously observed to contain MNB were not multinucleated at the time of biopsy. Within this group, six (40%) either had normal chromosomal complements in all their cells ($n = 3$) or had <38% abnormal cells ($n = 3$), and the other nine embryos were either diploid mosaics with ≥38% or more abnormal cells ($n = 4$), polyploid or polyploid mosaics ($n = 4$), or were aneuploid without mosaicism ($n = 1$).

Embryos were also sorted by day of detection of multinucleation (day 2 or day 3), by developmental characteristics (slow or good development), by number of nuclei per MNB (two or

Figure 1. In-situ hybridization of fluorochrome-labelled X, Y, 18, 13/21 chromosome-specific DNA. The chromosome X-specific probe was labelled in white or yellow, chromosome 18 in green, and the probe specific for chromosomes Y, 13 and 21 was stained in red or pink. Chromosomes 13/21 and Y can be differentiated by their size, with the signal for chromosome Y being much larger. (**a**) A binucleated blastomere with each nucleus having a normal XY 1818 4[13/21] complement. (**b**) A cell with four nuclei with a total tetraploid chromosome content (4X 4[18] 8[13/21]). (**c**) A binucleated blastomere with an XO1818 1[13/21] nucleus and an XO1818 3[13/21] nucleus.

Table I. Individual morphological, developmental and chromosomal characteristics of 47 embryos with multinucleated blastomeres assessed microscopically

Embryo no	No of cells on day			No of cells lost	MNB appearance		No of nuclei/MNB	No. of MNB on day 4	Morphology	ICSI	Chromosome abnormality
	2	3	4		Day	No of MNB					
1	2	7	7	1	2	2	>2	1	Good	Yes	Aneuploid (100%)
2	2	6	7	0	2	2	>2	2	Good	No	Mosaic (100%)
3	2	7	8	0	2	2	2	2	Good	No	Mosaic (61 6%)
4		4	4	0	3	3	>2	3	Slow	No	Polyploid (100%)
5	4	6	12	2	3	1	>2	4	Good	Yes	Mosaic (82%)
6	2	3	5	0	2	2	>2	1	Slow	No	Mosaic (100%)
7	2	4	7	0	2	2	>2	1	Slow	Yes	Mosaic (66%)
8	4	8	12	1	3	2	>2	5	Good	Yes	Mosaic (100%)
9	2	6	10	0	2	2	>2	0	Good	No	Aneuploid (monosomy 18)
10	2	6	10	2	2			1	Good	No	Mosaic (38%)
11	5	5	8	1	2	1	>2	2	Slow	Yes	MNB only (50%)
12	2	5	8	0	2	1	>2	1	Good	Yes	Mosaic (50%)
13	2	8	8	0	2	2	>2	1	Good	No	Mosaic (66%)
14	2	4	11	0	2	1	>2	0	Slow	No	Mosaic (55%)
15	4	5	8	2	2	1	>2	1	Good	Yes	Mosaic (50%)
16	2	4	7	0	3	2	2	0	Slow	Yes	Normal
17	2	6	8	0	2	2	>2	2	Good	No	Mosaic (43%)
18	4	6	7	0	3	2	2	2	Slow	Yes	Monosomy 18, polyploid (100%)
19	3	4	7	0	3	1	2	0	Slow	No	Normal
20	2	4	5	0	3	1	>2	1	Slow	No	MNB only (20%)
21	3	4	6	1	3	1		2	Slow	No	MNB only (40%)
22	2	6	7	0	2	2	>2	3	Good	No	Mosaic (100%)
23	2	4	7	0	2	2	>2	3	Slow	No	MNB only (42%)
24	6	6	4	0	3			0	Slow	Yes	Mosaic (100%)
25	2	4	8	1	2	2	>2	0	Slow	No	Mosaic (17%)
26	2	6	6	1	2	2	>2	1	Slow	No	Mosaic (50%)
27	2	5	6	0	2	1	>2	1	Slow	Yes	MNB only (25%)
28	2	6	5	1	2	2	>2	0	Slow	Yes	Polyploid (100%)
29	4	6	5	1	2	1	>2	0	Slow	Yes	Polyploid (100%)
30	3	4	4	0	3	1	2	3	Slow	No	Haploid (100%)
31	3	6	8	1	2	2	>2	1	Slow	Yes	Monosomy 13/21, mosaic (100%)
32	2	4	6	1	2	1	2	0	Slow	Yes	Mosaic (60%)
33	4	6	9	0	2	1		0	Good	Yes	Mosaic (80%)
34	5	7	7	1	2	2	>2	3	Good	No	Mosaic (75%)
35	2	4	4	1	2	2	>2	0	Slow	No	Mosaic (25%)
36	2	4	10	0	2	2	>2	3	Slow	No	Mosaic (27%)
37	2	4	6	0	2	2	>2	0	Slow	Yes	Normal
38	2	4	4	0	2	1	2	1	Slow	Yes	Polyploid (100%)
39	2	4	4	0	3	1	2	1	Slow	Yes	Mosaic (100%)
40	3	5	5	0	2			1	Slow	No	MNB only (33%)
41	3	5	5	0	3	1	>2	0	Slow	No	Polyploid (100%)
42	2	8	8	0	2	2	>2	3	Good	No	Mosaic (86%)
43	2	7	8	0	2	2	>2	0	Good	No	Mosaic (13%)
44	2	8	7	0	2	2	>2	2	Good	No	MNB only (29%)
45	2	8	8	4	2	2	>2	0	Good	Yes	Polyploid (100%)
46	2	4	6	1	2	1	2	1	Slow	No	Mosaic (75%)
47	2	4	5	0	3	2	>2	2	Slow	No	Monosomy 13/21, mosaic (80%), aneuploid

MNB = multinucleated blastomere, ICSI = intracytoplasmic sperm injection

more nuclei), by number of MNB per embryo (some cells or all cells were MNB), and by method of fertilization (ICSI or standard insemination). The results are shown in Table III. None of these subclassifications showed any significant difference in the percentage of chromosome abnormalities present.

With regard to individual cells, 13 MNB had a normal chromosomal complement in each of the nuclei, two combined normal and abnormal complements in each of the nuclei, while the remainder had chromosome abnormalities in all of their nuclei.

Discussion

The purpose of this study was to uncover any association between multinucleation and chromosomal abnormalities in the MNB as well as in other blastomeres of embryos containing MNB. FISH analysis of embryos with multinucleation illustrates a highly significant association between the presence of multinucleation 2–3 days after development and chromosomal abnormalities occurring in all or most cells of these embryos. This finding supports previous observations indicating a pathological state of cleavage-stage embryos carrying MNB (Tesarik *et al.*, 1987; Tesarik, 1994)

Table II. Fluorescent in-situ hybridization (FISH) results in mosaic embryos

Embryo no	Results[a,b]						Abnormality	% cells abnormal
1	MNB = XY-18O-4[1321]	XY 818:5[1321]	XY 1818.3[1321] (2)				Mosaic 2N/mitotic non-disjunction 13/21	100
2	MNB = XX 1818-4[1321]	XO-1818 1[1321]	MNB = XX.4[18]:4[13/21]	XY-O: 2[1321]	O- 2[1321]		Mosaic chaotic	100
3	XX18183[1321]	XX1818 4[1321] (2)	XO18O:2[1321]	MNB = 4X4[18]6[1321]	O-18O-1[1321]	XO18O:3[1321] (3)	Mosaic chaotic	62
4	MNB = 3X18O 1[1321]	MNB = 3X3[18]6[13/21]	MNB = XX.1818.7[1321]				Polyploid, mosaic 3N/MNB	100
5	18O:1[1321]	XO-1818:NR	MNB = YY 4[18]:NR	XY1818 4[1321] (3)	MNB = XXYY 4[18] 8[13/21] (3)	MNB = XX5[18]4[1321]	Mosaic chaotic	82
6	3X.4[18]:6[1321]	XO:1818.3[1321]	XO-1818 4[1321]	MNB = XO1818.2[1321]			Mosaic chaotic	100
7	XY 1818.5[1321]	XY 1818.4[1321] (2)	XY.3[18] 3[1321][c]	XY 1818 3[1321]	MNB = XXYY4[18]8[13/21]		Mosaic 2N/4N/mitotic non-disjunction 13/21	66
8	YO-18O-O	MNB = YY18O-1[1321]	MNB = O-4[18]:13[1321]	MNB = 4X4Y-4[18] 4[13/21] (2)	O-O:2[1321] (2)		Mosaic chaotic	100
11	XX18181616 (2)	MNB = 4X4[18]4[16]	MNB = 18O				Mosaic 2N/MNB	50
12	XX181816O[c]	XX18181616 (2)	MNB = 4X4[18]4[16] (2)	4X4[18]NR[c]			Mosaic 2N/4N/MNB	50
13	XY1818	XYY181818	MNB = XX1818				Mosaic 2N/3N	66
14	XX18O 3[1321]	XO18O3[1321]	XO18183[1321]	XX1818 2[1321] (2)	XX 1818 4[1321] (4)		Mosaic chaotic	55
15	XX1818 (2)	MNB = XX.3[18]	4X4[18]				Mosaic 2N/4N/MNB	50
17	4X4[18]	MNB = 4X4[18] (2)	XX1818 (4)				Mosaic 2N/4N/MNB	43
18	3X3Y 3[18] 12[1321]	MNB = XXYYY.3[18]:10[1321]	XXYY 2[18] 8[1321]	MNB = 4X4Y 4[18] 16[1321]			Aneuploid (monosomy 18), mosaic 3N/4N/6N/8N	100
21	XY1818 4[1321] (3)	MNB = XY1818 4[1321]	MNB = XXYY 4[18]:8[13/21]				Mosaic 2N/MNB	40
22	MNB = 2X3Y.7[18]10[1321]	3X3Y6[18] 11[1321]	XY1818 4[13/21] (2)	XXYY 4[18] 6[1321]	4X4Y 8[18]12[1321]	MNB = 6X6Y 12[18]:23[1321]	Polyploid, mosaic 2N/4N/8N/12N/MNB	100
23	XX.1818.3[1321] (2)[c]	XX.1818 4[1321] (2)	MNB = 4X.4[18] 8[1321] (3)				Mosaic 2N/MNB	42
24	XX.1818.5[1321]	YO-18O:2[1321]	O-18O:2[1321]				Mosaic chaotic	100
26	YO-18O:2[1321] (2)	XY-1818 4[1321] (3)	MNB = 2X2Y-O-O				Mosaic 2N/N/MNB	50
28	4X.5[18] 8[1321]	8X. 8[18] 14[1321]					Polyploid, mosaic chaotic	100
29	XXYY 5[18]:5[13/21]	XXYY 4[18] 12[13/21]					Polyploid, mosaic chaotic	100
30	MNB = 18O 2[1321]	MNB = XO-O-O	MNB =O				Mosaic chaotic	100
31	XO18O-1[1321]	MNB = 4X 4[18]:6[1321]	3X3[18] NR				Aneuploid (monosomy 21), mosaic N/2N/3N	100
32	2[13/21]5[14/22]	5[13/21]2[14/22]	4[13/21]3[14/22]	2[13/21]5[14/22]	5[13/21]5[14/22]		Mosaic chaotic	60
33	4[13/21] 3[14/22]	2[13/21] 1[14/22]	4[13/21] 4[14/22]	3[13/21] 1[14/22]	2[13/21] 4[14/22]		Mosaic chaotic	80
34	MNB = 4X4[18]	XX1818 (2)	MNB = 3X3[18]	XXX1818	MNB = XX1818		Mosaic 2N/3N	75
38	XO-18O:2[1321] (2)	MNB = XO:3[18].3[1321]					Haploid, mosaic chaotic	100
39	MNB = XXY1818	XY181818	XXY5[18]	XY18O (2)			Mosaic chaotic	100
41	XXYY-4[18]	4X4Y 8[18] (2)					Polyploid, mosaic 4N/8N	100
42	XO-O-16O	MNB = XY-O-18O	MNB = XO-16O-O	YO-1818 16O (2)	MNB = XY1818181616	XY 1818:1616	Mosaic chaotic	86
45	XXYY:4[18]:8[1321]	6X4Y:8[18]- 16[1321]					Polyploid, mosaic 4N/8N	100
46	XX.1818:O	XX:1818.8[13/21]	4X.4[18] 8[13/21]	XX 1818 4[13/21]			Mosaic 2N/4N/mitotic non-disjunction 13/21	75
47	MNB = XY 1818.4[13/21] (2)	XY 1818.3[13/21]	XY 1818.2[13/21]	MNB = 2X2Y 4[18]-6[1321]			Aneuploid (monosomy 21), mosaic (mitotic non-disjunction 13/21)	80

NR = no result, MNB = multinucleated blastomere.

[a]The chromosome constitution in the table is the addition of all the chromosomes from all the nuclei in that cell

[b](2), (3), (4) = number of cells with the same karyotype.

[c]Probably a FISH error.

Table III. Relationship between chromosomal abnormalities and day of multinucleation, development characteristics and number of nuclei per cell, in multinucleated embryos

	Percentage (number) of embryos with chromosome abnormalities	*P* value
Multinucleated on day 2	76.5 (26/34)	
Multinucleated on day 3	76.9 (10/13)	NS
Good development	88.8 (16/18)	
Slowly developing	69.0 (20/29)	NS
Two nuclei	88.9 (8/9)	
Three or more nuclei	72.7 (24/33)	NS
Unrecorded	80.0 (4/5)	
All cells multinucleated	71.4 (15/21)	
Some cells multinucleated	82.6 (19/23)	NS
Unrecorded	66.7 (2/3)	
ICSI multinucleated	63.0 (17/27)	
No ICSI multinucleated	95.0 (19/20)	NS
Multinucleation persists on day 4	81.3 (26/32)	
Multinucleation does not persist on day 4	60.0 (9/15)	NS
MNB embryos	76.6 (n = 47)	
Control embryos	50.9 (n = 403)	<0.001
MNB embryos (excluding aneuploidy)	74.5 (n = 47)	
Control embryos (excluding aneuploidy)	32.3 (n = 403)	<0.001

NS = not significant, ICSI = intracytoplasmic sperm injection, MNB = multinucleated blastomere

In the present study the incidence of chromosomal abnormalities was the same among embryos with two or three or more nuclei. Furthermore, no differences were found when comparing embryos with a few MNB or where all cells were multinucleated and those where all cells were multinucleated, nor between slow or normally developing embryos with multinucleation; nor between embryos obtained by standard insemination or by ICSI; and neither between embryos with multinucleation detected on day 2 or 3 of development.

According to Van Blerkom (personal communication), the best stage to detect multinucleation is after the first embryonic division. If the embryo is observed just prior to cell division, it is possible to miss the number of nuclei contained in the dividing cell; this would explain the similar rates of abnormalities detected on days 2 and 3 in embryos without multinucleation on day 2 but with multinucleation on day 3. In fact, most of the multinucleated embryos observed on day 2 in this study were found to be at the 2- to 3-cell stage. In these embryos, half or more of the cells were chromosomally abnormal and, under our definition of detrimental chromosome mosaicism, the chances of developing normally were slim. This is in contradiction to previous analysis, in which MNB were detected on day 4, after biopsy. In those studies, most cells of morphologically normal embryos with one or more MNB were chromosomally normal (Munné and Cohen, 1993; Munné *et al.*, 1994a). The present results indicate therefore two distinct patterns of multinucleation, one occurring on day 2 or 3 of development and producing mostly chromosomally abnormal embryos, and another occurring on day 4 or later, yielding mostly chromosomally normal embryos.

It is also interesting to note that 70% of the embryos with multinucleation observed on days 2 and 3 presented three or more nuclei, while after biopsy on day 4, most MNB were binucleated. Previous studies had reported that most MNB were also binucleated (Tesarik *et al.*, 1987; Hardy *et al.*, 1993; Munné and Cohen, 1993; Winston *et al.*, 1993). While in the present study embryos were selected for nucleation observed mostly on day 2, other studies observed multinucleation mostly on day 4. Furthermore, comparing group A and B MNB, the proportion in group A in the present study was significantly lower than in previous studies of multinucleation on day 4 (Munné and Cohen, 1993). Group A blastomeres are produced by cytokinesis arrest, but each nucleus is chromosomally normal. Group B MNB, however, have chromosomally abnormal nuclei and are probably the result of random distribution of chromosomes. It is possible that the number of nuclei per MNB is not stable and that nuclear fusion occurs, explaining both a reduction in the number of multinucleated cells and the absence of day 4 multinucleation in embryos presenting it on day 2 or day 3. All these differences between multinucleation before and after day 3 again support the notion of a double pattern of nucleation: one occurring on day 2 or 3, producing multinucleated and chromosomally abnormal embryos, and another on day 4 or later generating mostly binucleated cells and chromosomally normal embryos

The type of mosaic embryos detected in this study are mostly produced through chaotic divisions. Such a pattern of mosaicism has been described previously in several FISH studies (Delhanty *et al.*, 1993; Coonen *et al.*, 1994; Munné *et al.*, 1994a,c,d, 1995a; Harper *et al.*, 1995), and it is probably related to centriole or spindle deficiencies as well as disturbance of pronuclear syngamy.

Of the embryos that were previously diagnosed as multinucleated and for which no MNB were detected after biopsy on day 4, 60% (9/15) were either mosaics, polyploid, polyploid mosaics or complex mosaics. This rate of abnormality is similar to the overall rate for multinucleated embryos, and it suggests that the nuclei of those MNB may have fused, yielding a new nucleus with an abnormal chromosomal complement. Such an hypothesis may contradict the observations of Hardy *et al.* (1993), who noted that MNB remained arrested without fusing their nuclei. In that study, however, multinucleation was detected on day 3 or 4 and not on day 2 of development. Another finding related to missing MNB is that some embryos with all their cells found to be multinucleated on day 2 continued development and became chromosomally normal by day 4. The explanation for this could be either that cytokinesis is resumed later on, or that the MNB fragments, or that the presence of multinucleation was misdiagnosed. In order to confirm nuclear fusion and/or resumption of cytokinesis, further cell lineage studies should be performed.

The present results indicate that multinucleated embryos detected on day 2 or 3 of development have a much greater incidence of chromosomal abnormalities than embryos without multinucleation. Taking all these findings into consideration, it appears advisable not to replace embryos with multinucleated blastomeres observed on day 2 or 3 unless no other embryos are available.

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