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Tripronuclear Human Oocytes: Altered Cleavage Patterns and Subsequent Karyotypic Analysis of Embryos

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ABSTRACT

Between 1 and 4% of human oocytes fertilized in vitro are tripronuclear. It has been reported that these tripronuclear oocytes can develop to grossly normal-appearing morulae and that chromosomally, these embryos could be triploid, diploid, or severely depleted. The etiology and proportion of apparently diploid and aneuploid embryos deriving from tripronuclear human oocytes is unknown. This study provides evidence for the first time that most (18 of 29) tripronuclear human oocytes cleave directly to 3-cells at the first cleavage division. These embryos have a severely abnormal (but not triploid) chromosomal complement. Furthermore, some (4 of 29) tripronuclear human oocytes cleave to 2-cells plus an extrusion, and these embryos are diploids, whereas some (7 of 29) cleave to 2-cells, and these embryos are triploid after the first cleavage division. These findings demonstrate that most tripronuclear human oocytes have an altered cleavage pattern at the first cleavage division, that most tripronuclear human oocytes (76% in this study) do not develop into triploid embryos, and that a correlation exists between the pattern of the first cleavage division and the subsequent karyotype of these embryos. Insight into the mechanisms by which these oocytes fail to develop into triploid embryos is also provided.

INTRODUCTION

Although 1–4% (Mahadevan and Trounson, 1984) of human oocytes fertilized in vitro are tripronuclear (contain 3 pronuclei), there is little information about the subsequent development and chromosomal composition of preimplantation embryos that derive from these oocytes, and the data that does exist suggest a complex picture. We (Trounson, 1982) previously found that three of five cleaving embryos from tripronuclear human oocytes were triploid, and two were near diploid. Van Blerkom et al. (1984) showed that two such embryos may develop to at least the 8-cell stage and demonstrated, by serial-sectioning a 12-cell embryo and fluorescent staining with 4,6-diamidino-2-phenylindole (DAPI), that one blastomere contained 46 chromosomes. In addition, Rudak et al. (1984) reported that in an embryo fixed 50.5 h after insemination, one blastomere had a severely abnormal complement of 33 chromosomes. Recently, Angell et al. (1986) evaluated the chromosomal constitution of 5 embryos derived from tripronuclear oocytes and found 1 diploid, two near triploid embryos and two embryos with 38 and 39 chromosomes per blastomere, respectively. Two other embryos, evaluated by DNA microfluorimetry, were near diploid. Most of the embryos in the Angell et al. (1986) study were evaluated after the second cleavage division.

We were interested in the derivation of the apparent diploidy and aneuploidies in tripronuclear oocytes, especially as electron microscopic observations of tripronuclear oocytes had confirmed the presence of three pronuclei arising through dispermy (Sathananthan and Trounson, 1985). Furthermore, Zenzes et al. (1985) have shown that a tripronuclear oocyte was hypotriploid (had 64 chromosomes) before the first cleavage division. Thus, it appears that tripronuclear oocytes have a triploid or near triploid chromosomal complement before the first cleavage division and that if deviation from triploidy occurs, it occurs at later stages of embryogenesis. Theoretically,

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for a tripronuclear oocyte (consisting of 69 chromosomes before the first cleavage division) to develop to a diploid embryo, one of two mechanisms have to operate. Either a haploid set of chromosomes is ejected, or the oocyte cleaves to 3 cells at the first cleavage division. Against this background, we investigated the pattern of the first cleavage division of tripronuclear human oocytes and the chromosomal constitution of these embryos before the second cleavage division to determine both the proportion of tripronuclear human oocytes that develop into triploid embryos and to gain insight into the mechanism by which these tripronuclear human oocytes fail to develop into triploid embryos.

MATERIALS AND METHODS

Oocytes obtained from superovulated patients undergoing treatment of infertility by in vitro fertilization (IVF) were collected and fertilized as previously described (Trounson and Mohr, 1983). The number of pronuclei were scored under dissecting microscopy 12–20 h after insemination. Each tripronuclear oocyte was reexamined under phase and/or interference contrast microscopy and confirmed to be tripronuclear. In addition, all oocytes had two polar bodies, which indicates that the tripronuclear state of these human oocytes is almost certainly due to dispermy.

In an initial investigation, 4 oocytes that were evaluated as being tripronuclear (Fig. 1a shows a human oocyte with three clearly visible pronuclei) were incubated with colcemid (0.1 μg/ml) just before the first cleavage division (24 h after insemination). The oocytes were assessed for chromosomal constitution by using a modification of the technique previously described (Kola et al., 1986). Briefly, embryos were incubated in hypotonic solution (1% trisodium citrate) for 3–5 min at room temperature. The embryos were then transferred onto clean glass slides in a minimal amount of hypotonic solution. One drop of fixative (methanol/glacial acetic acid, 3:1) was dropped onto the embryo from behind it. Immediately after adherence to the slide, a second drop of fixative was dropped onto the embryo. Finally, 3 more drops of fixative were added 1 at a time to the side of the embryo. Chromosomes were stained with 10% Giemsa (BDH, Poole, England) in buffer, pH 6.8, for 10 min.

In subsequent experiments, tripronuclear oocytes were monitored constantly for dissolution of pronuclei and cleavage. Three tripronuclear oocytes were filmed for pronuclear dissolution and cleavage by using time-lapse cinematography. The embryos were incubated with colcemid beginning 6–8 h after the first cleavage division. The first 8 embryos were assessed for chromosomal constitutions 12 h after the first cleavage division. The remaining embryos were fixed 14 h after the first cleavage division because a number of embryos did not have any cells in mitosis when fixed earlier. Chromosome scoring was conducted independently by at least two investigators.

RESULTS

The 4 oocytes that were fixed before the first cleavage division each had 69 chromosomes.

Of 29 tripronuclear oocytes allowed to cleave, 18 (62%) cleaved directly to 3-cells (Table 1, Fig. 1b). We found that the dissolution of the pronuclei and cleavage to 3-cells was synchronous (as determined by both microscopic evaluation and time lapse cinematography). The regularity of the blastomeres varied; 6 embryos had 3 equally sized blastomeres, whereas the remaining 12 embryos had 1 large and 2 smaller blastomeres. Figure 1(b) shows a 3-cell human embryo, with one large and two smaller blastomeres that had resulted from the direct and synchronous cleavage of a tripronuclear oocyte. The variation of blastomere size was not related to synchrony of cleavage, since those embryos that had 1 large and 2 smaller blastomeres had also cleaved synchronously to 3-cells. Chromosomal analysis of 10 of these 3-cell embryos just before the second cleavage division revealed that all of these embryos had a severely abnormal but near-diploid chromosomal constitution (Table 1). Furthermore, in all cases the 3-cells within an embryo had different numbers of chromosomes. It also appeared that the number of chromosomes in individual cells was random. This is the first study demonstrating that tripronuclear human oocytes cleave directly to 3-cells. Angell et al. (1986) observed a 3-cell embryo, but were unable to differentiate between direct cleavage to 3-cells or cleavage to 2-cells and a rapid second division.

We found that 14% (4 of 29) of tripronuclear human oocytes cleaved to 2-cells plus an extrusion (a mass that is extruded from the embryo simultaneously with cleavage of the embryo) (Table 1). Figure 2a shows a 2-cell-plus-extrusion embryo, with the extrusion indicated by an arrow. Three of these 4 embryos were successfully karyotyped, and both the cells of these embryos were found to be diploid. Figure 2b shows a chromosomal spread of 1-cell of a

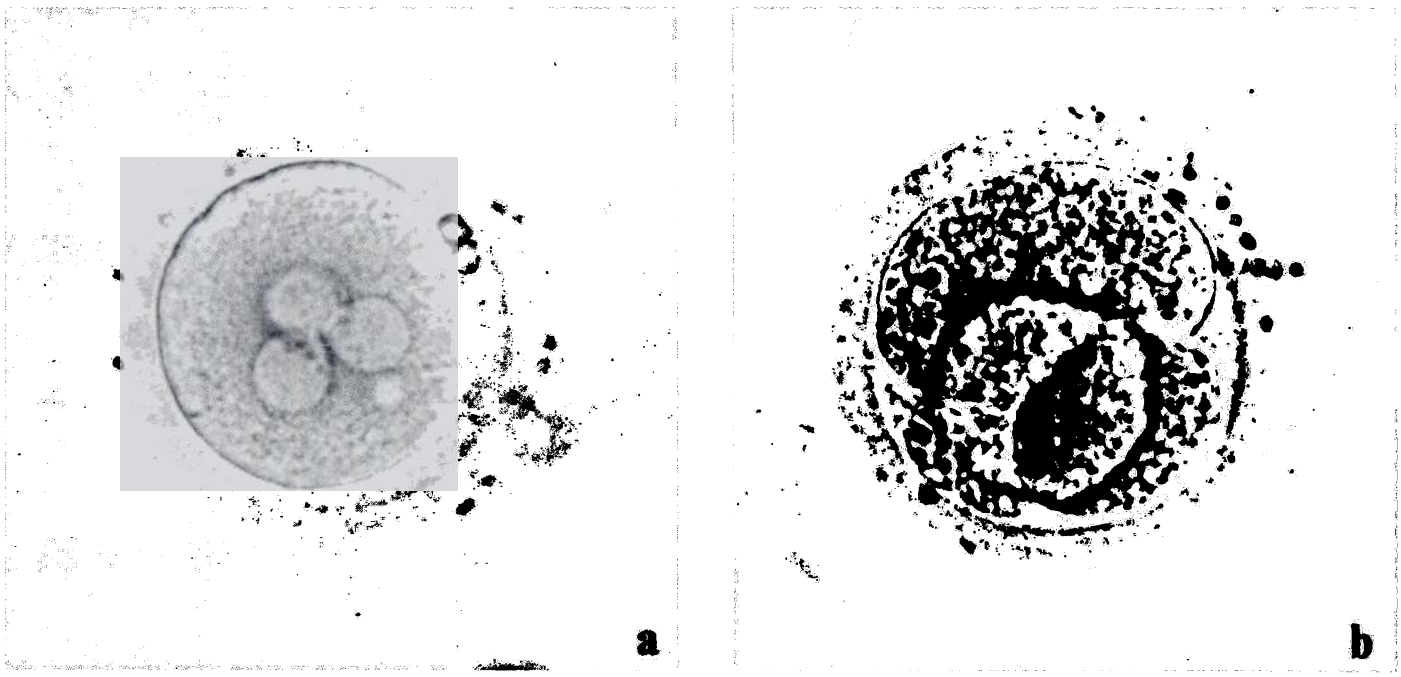

FIG. 1. (*a*) Photomicrograph of a tripronuclear human oocyte (X200). The photomicrograph clearly shows three pronuclei that have migrated to the center of the oocyte. The first and second polar bodies are also seen, demonstrating that the tripronuclear state of the oocyte is due to dispermy. (*b*) Photomicrograph of a 3-cell embryo (1 large, 2 smaller blastomeres) derived from the direct cleavage of a tripronuclear oocyte (X200).

2-cell-plus-extrusion embryo, and Figure 2c shows the diploid karyotype (46XY) of the metaphase spread shown in Figure 2b. The remaining embryo in this group that had not been successfully karyotyped, had two interphase nuclei, and one haploid set of chromosomes, possibly derived from the extrusion.

Twenty-four percent (7 of 29) of tripronuclear oocytes in this study cleaved to morphologically normal 2-cell embryos (Fig. 3a, Table 1). Figure 3a is a photomicrograph of a morphologically normal 2-cell embryo. Chromosomal analysis of 4 of these embryos at the second cleavage division revealed triploid or near-triploid embryos (Table 1). Figure 3b shows a metaphase spread of a 2-cell embryo, and Figure 3c shows the triploid karyotype (69XXX) of the spread shown in Figure 3b.

DISCUSSION

The findings in this study have demonstrated that a large majority of tripronuclear human oocytes do not develop into triploid embryos, and that the first cleavage division is a critical stage that influences the subsequent chromosomal constitution of tripronuclear human oocytes. We have shown that three types of events occur at the first cleavage division of tripronuclear human oocytes: 1) they cleave to three cells, 2) they cleave to 2-cells-plus-extrusion, and 3) they cleave to regular 2-cell triploid embryos. Our findings also demonstrate that a correlation exists between the pattern of cleavage at the first division and the subsequent chromosomal composition of these embryos—all the embryos that cleaved to 3-cells had a severely abnormal chromosomal composition; the 2-cell-plus-extrusion embryos were diploid whereas the morphologically regular-looking embryos were triploid or near-triploid.

For an embryo to cleave from 1- to 3-cells, the spindle formed at the first cleavage division should be tripolar. The data in this study indicate that the movement of chromosomes on such a tripolar spindle is disorganized, resulting in the cells in an embryo having different numbers of chromosomes in each cell, and the cells thus having an abnormal chromosomal constitution. Tri- and/or quadripolar spindles have been observed in the sea urchin (Wilson, 1925) and in rat oocytes (Austin and Walton, 1960).

The finding that some embryos cleave to 2-cells-plus-extrusion, which are karyotypically diploid, suggest that in this situation one haploid set of

TABLE 1. Cleavage details and chromosome findings of tripronuclear human oocytes.

Number of cells after first cleavage division	Oocyte code	Time of first cleavage division (h after insemination)	Number of metaphase cells	Chromosome findings
3-cell	MCK	29	2	57,51
	MUL	32	0	–
	DEM7	28	1	57
	DEM1	27	0	–
	MIT	30	0	–
	JAN	28	1	54
	CHR	33	ND*	ND
	SHA4	26	3	42,44,52
	TS1	28	1	34
	BAL	26	ND	ND
	GAN	33	ND	ND
	SUM	28	ND	ND
	COP	28	1	51
	FRA3	33	2	52,39
	HOL	33	1	38
	COA	27	ND	ND
	OTT	29	–	34
+ 3 fragments	MAR	30	3	34,42,32,9,12,6**
2-cells + extrusion	COC	30	2	46,46
	THE	30	2	46,46
	DOM	27	2	46,46
	COR	27	2 nuclei + 23 chromosomes	
2-cells	DEM4	34	2	69,69
	SM1	24	0	–
	BRO	33	0	–
	HOO	31	ND	ND
	SHA1	36	1	69
	ATT	27	1	72
	FRA4	29	1	69

*ND = Not done.

**The derivation of these 6 groups of chromosomes is unclear.

chromosomes is excluded from the metaphase plate at the first cleavage division, and that this division only involves 46 chromosomes and a normal bipolar spindle. It may be that the chromosomes of one pronucleus has a different degree of condensation from the other two; thus, a haploid set of chromosomes do not gather on the metaphase plate. However, we have not yet elucidated whether the subsequent development of these embryos is normal nor determined the fate of the haploid set of chromosomes. Van Blerkom et al. (1984) found that one tripronuclear oocyte that cleaved to 2-cells was diploid at the 8-cell stage, but it is possible that a nucleated extrusion was overlooked.

The data in this study suggest that in the third situation (tripronuclear oocytes cleaving to morphologically normal 2-cell embryos; chromosomally, these embryos are triploid), all the chromosomes participate in mitosis. These embryos have a regular division, the production of daughter cells having a full triploid chromosomal complement. In the Angell et al. (1986) study, two of the embryos that cleaved to 2-cells and were fixed at the 2-cell stage were also found to be triploid.

This study has demonstrated that only 24% of tripronuclear human oocytes develop into triploid embryos after the first cleavage division. One percent of all in vivo clinically recognized conceptions in man are triploid (Jacobs et al., 1978). Uchida and Freeman (1985) have reported the live birth of 7 triploid infants, thus demonstrating that some triploid embryos do develop to later stages of pregnancy. There have also been reports of 5 triploid infants who have survived from 2 to 7 mo (Borgaonkar, 1984), and recently one that survived to 10½ mo (Sherard et al., 1986).

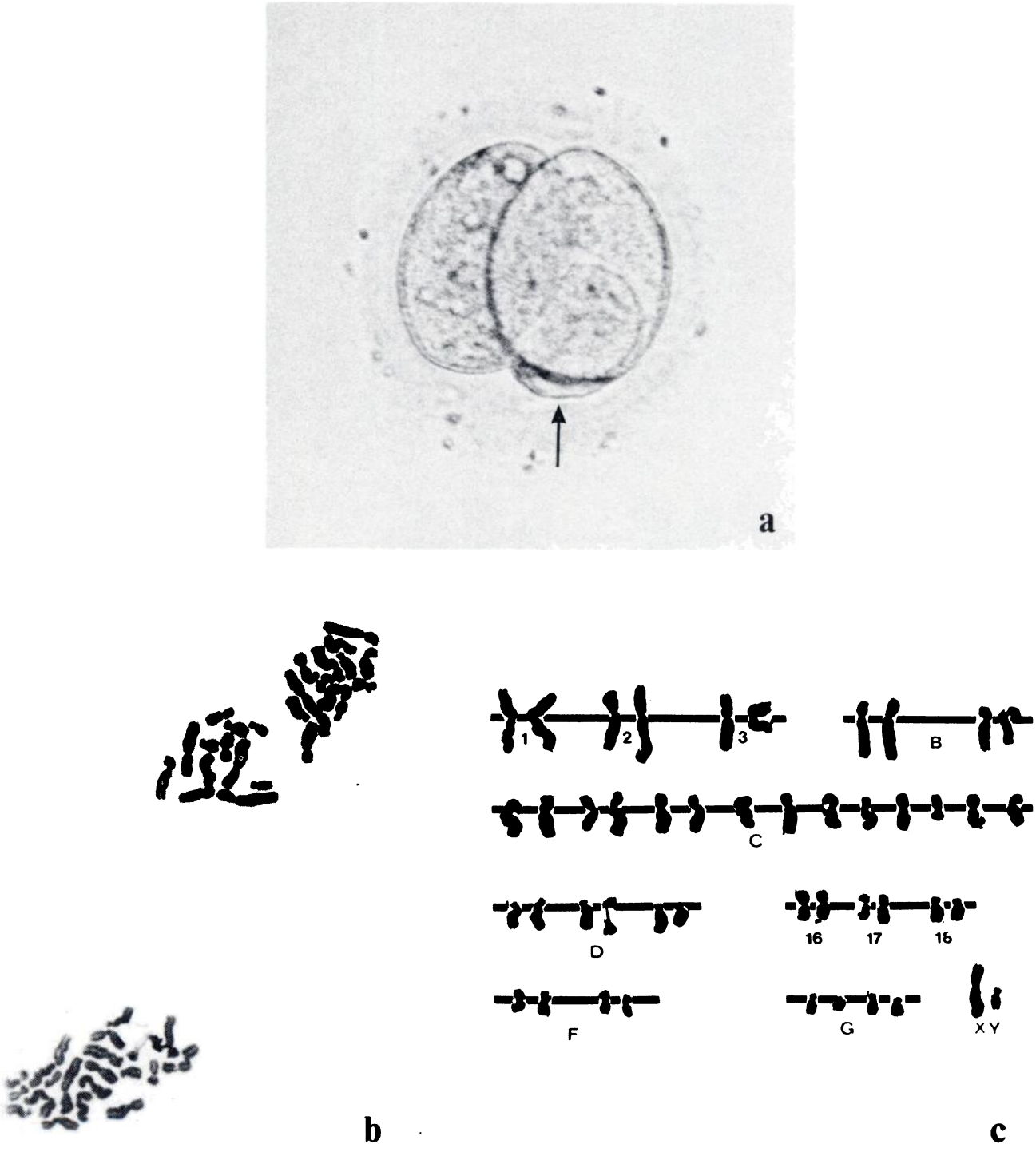

FIG. 2. (*a*) Photomicrograph of 2-cells-plus-extrusion embryo, derived from the direct cleavage of a tripronuclear oocyte (X200). The two blastomeres are clearly visible. The extrusion is indicated by an *arrow*. (*b*) Metaphase spread and (*c*) karyotype of 1-cell from a 2-cell-plus-extrusion embryo (X1000). These photomicrographs demonstrate that the cell had a diploid (46 XY) karyotype.

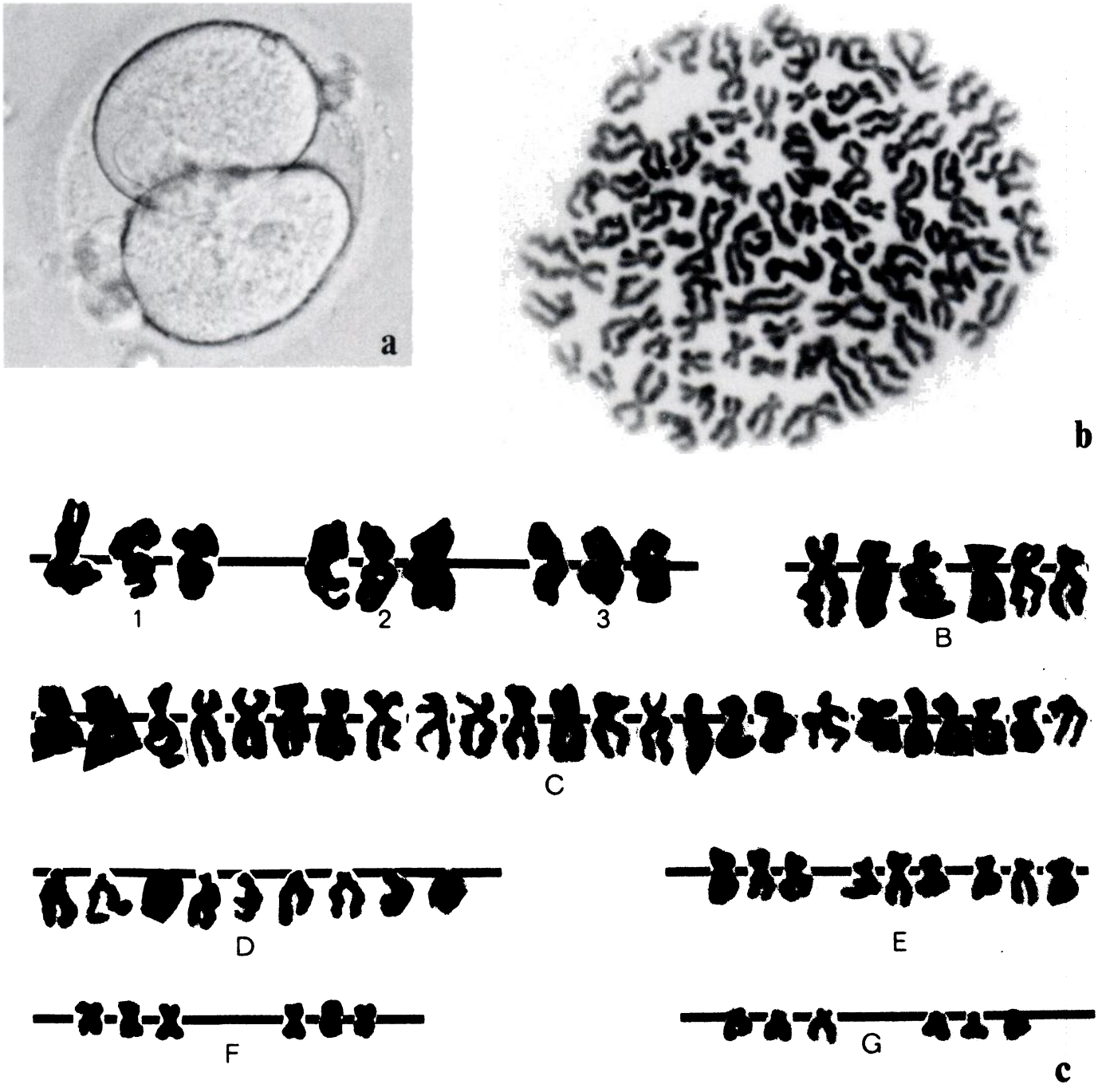

FIG. 3. (*a*) Photomicrograph of 2-cell embryo derived from the direct cleavage of a tripronuclear oocyte (×200). This embryo is a morphologically normal 2-cell embryo. (*b*) Metaphase spread and (*c*) karyotype of 1-cell from a 2-cell embryo (×1000). These photomicrographs demonstrate that the cell was triploid (69 XXX karyotype).

The findings in this study have demonstrated that the vast majority of tripronuclear human oocytes do not develop into triploid embryos and that a correlation exists between the pattern of the first cleavage division and the subsequent karyotype of these embryos. This study has also provided insight into the mechanisms by which tripronuclear oocytes fail to develop into triploid embryos in the human. However, research is being focused on staining the microtubules (Schatten et al., 1985, 1986), centro-

somes, and chromosomes to further investigate the mechanisms whereby tripronuclear human oocytes fail to develop into triploid embryos.

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