

Centrioles in the beginning of human development

(sperm centriole/fertilization/syngamy/pronucleus/ultrastructure)

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ABSTRACT We demonstrate the presence of centrioles in fertilized human oocytes at syngamy. Single or double centrioles within centrosomes were detected by transmission electron microscopy at one pole of the first cleavage spindle in normal and dispermic embryos (25–26 hr after insemination). Sperm centrioles were also closely associated with the male pronucleus (16–20 hr after insemination) in pronuclear stage embryos. A tripolar spindle derived from a tripronuclear embryo is also demonstrated with two centrioles at one pole. The data provide evidence that human centrioles, as those in most other animals, and unlike the mouse, are paternally derived, thus supporting Boveri's classical theory. Furthermore, this study provides insights to the proposed mechanisms of aberrant cleavage patterns of dispermic human embryos.

It is widely believed that mature mammalian oocytes and early cleavage stage embryos do not have centrioles (1–6). Most cells, however, do possess centrosomes, which are microtubule (MT) organizing centers at spindle poles (3). In his classical theory of fertilization, Boveri in 1900 (7) stated that unfertilized eggs derive their centrosomes from male gametes, and this has subsequently been shown to be the case in a number of animal species, including the sea urchin (2), where centrioles associated with centrosomes organize mitotic bipolar spindles (3). On the contrary, in mice, centrosomes are maternally derived (2) and this has been proposed to be true for other mammals.

Meiotic spindles of mammalian oocytes are anastral, barrel shaped, and composed of numerous MTs (1, 2, 4). The structure of the human meiotic spindle has already been described to conform to the mammalian pattern (5, 6, 8, 9). Mammalian meiotic spindles have centrosomes but no centrioles. Centrosomes and centrioles are both self-reproducing organelles and centrioles merely advertise the presence of centrosomes (3). After fertilization, the mitotic spindle of the sea urchin embryo is organized by paternally inherited centrioles and centrosomes (10, 11). In the sea urchin, each sperm carries two centrioles associated with centrosomes (12), which duplicate and separate to form a bipolar spindle during the first mitosis and are the ancestors of these organelles in all cells during subsequent development (2, 10, 11). In a fashion similar to sea urchin sperm, human sperm also have centrioles. A well-defined proximal centriole is present next to the basal plate of the sperm head (13–15), while the distal centriole (which is a remnant) gives rise to the sperm tail axoneme during spermiogenesis. The proximal centriole consists of nine triplets of MTs showing the typical 9 + 0 organization and is associated with osmiophilic centrosomal material. After gamete fusion, the sperm midpiece and tail are invariably incorporated into the ooplasm, and the centriolar region often remains attached to the decondensing sperm

nucleus and persists after male pronuclear formation (16–18). This study demonstrates the presence of centrioles associated with centrosomes in the first mitotic spindle of the human fertilized oocyte.

MATERIALS AND METHODS

Both normal two-pronuclear (2PN) embryos and dispermic tripronuclear (3PN) embryos were examined for centrioles at the pronuclear stage and at syngamy soon after fertilization. The 2PN embryos were obtained from the *in vitro* fertilization (IVF) clinic at the National University Hospital (Singapore), while the 3PN embryos were collected from the IVF unit at Epworth Hospital (Melbourne). The 2PN embryos were obtained with the patient's consent after four embryos were used for embryo replacement. One patient who donated two embryos became pregnant with twins that same cycle. Women were routinely stimulated with either follicle-stimulating hormone/human menopausal gonadotrophin (hMG) or clomiphene citrate/hMG (19, 20) followed by human chorionic gonadotropin (hCG) administered when two or three follicles reached 16–17 mm diameter when viewed by ultrasonography. Oocytes were recovered by ultrasound or laparoscopy 36 hr after hCG and inseminated 4–6 hr after recovery in Ham's F-10 or human tubal fluid medium containing 10% human serum. The spermatozoa were washed, centrifuged, and layered before insemination. Five 2PN and six 3PN embryos at the pronuclear stage were fixed for transmission electron microscopy (TEM) 16–20 hr after insemination. Four 2PN and six 3PN embryos were further cultured to syngamy and fixed for TEM 25–26 hr after insemination. All 21 embryos were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), postfixed in 1% osmium tetroxide, dehydrated, and embedded in Araldite (15). Alternate series of thick (1 μ m) and thin (\approx 70 nm) sections were cut with glass and diamond knives. Thick sections were stained with toluidine blue, while thin sections were stained with alcoholic uranyl acetate and Reynold's lead citrate and examined with a Philips 301 electron microscope.

RESULTS

We first identified a centriole associated with a bipolar spindle while studying the fate of dispermic 3PN embryos during cleavage in 1987. This was an intriguing observation, which prompted us to examine more pronuclear ova for centrioles associated with both pronuclei and spindles at syngamy.

The pronuclear stage embryos examined had the usual fine structure, which has been described in detail in previous

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Abbreviations: MT, microtubule; PN, pronuclear; TEM, transmission electron microscopy.

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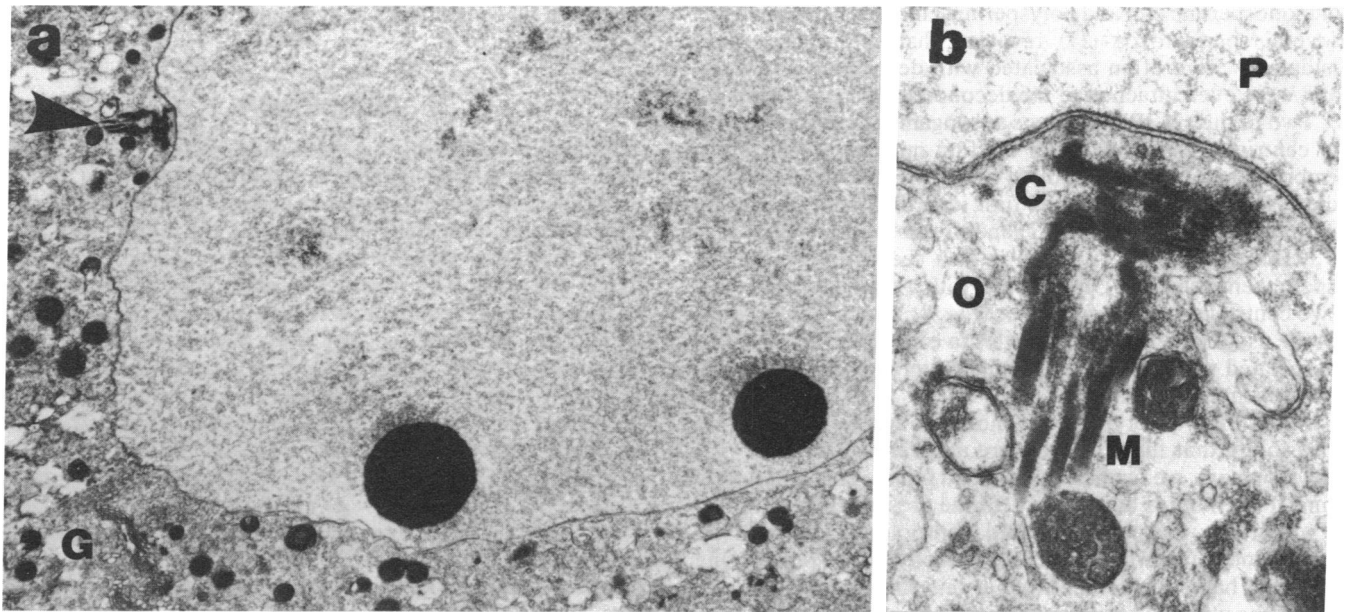


FIG. 1. A 3PN embryo fixed at the pronuclear stage. (a) Centriolar complex and sperm midpiece are closely associated with the nuclear envelope of one pronucleus (arrowhead). Two dense compact nucleoli are seen within the pronucleus. G, Golgi complex. ($\times 7000$.) (b) Centriolar complex at higher magnification. The proximal centriole (C) is sectioned longitudinally and is obscured by osmiophilic centrosomal material. Outer dense fibers are evident in the axonemal region of the midpiece (M). O, ooplasm; P, pronucleus. ($\times 70,000$.)

reports (15, 21). The 2PN embryos were normal in all respects, while the 3PN embryos were well-preserved and showed no signs of degeneration. Five of these embryos (two 2PN and three 3PN) examined in serial sections had flagellar

neck regions of spermatozoa, where centrioles are located, closely associated with male pronuclei (Fig. 1). Sperm tails and midpieces were also found in the vicinity of pronuclei in the ooplasm of other embryos. Furthermore, reexamination

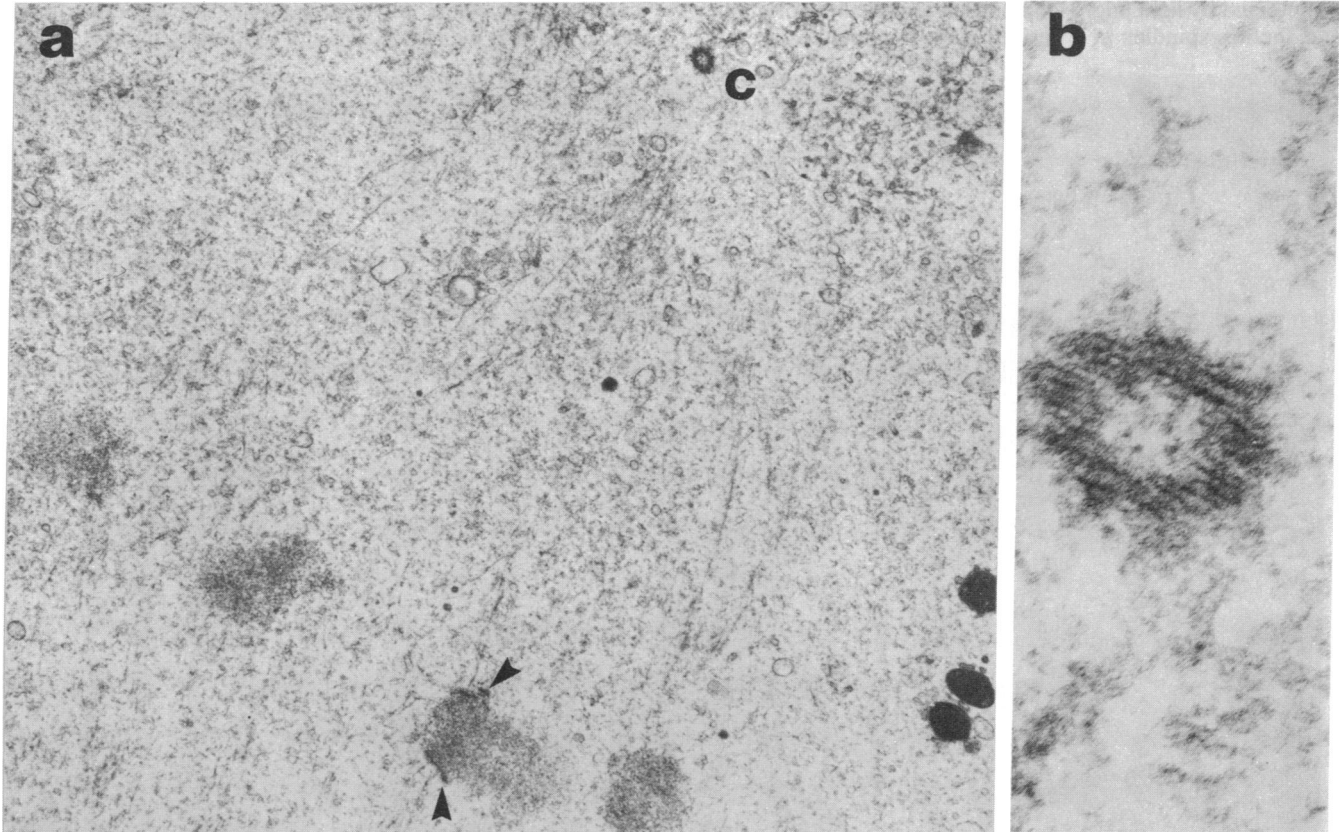


FIG. 2. Bipolar spindle developed from a 3PN embryo in syngamy. (a) A centriole (C) is visible at one pole of half a spindle depicted in this electron micrograph. Spindle MTs extend from the pole to chromosomes, connecting at kinetochores (arrowheads). The spindle zone is usually devoid of other organelles. ($\times 13,200$.) (b) Centriole in oblique cross-section at higher magnification. It presents the typical 9 + 0 structure, consisting of nine triplets of MTs arranged in a circle. ($\times 65,000$.)

of monospermic (16) and polyspermic embryos penetrated by several spermatozoa (17) revealed that sperm neck and midpieces were often associated with developing pronuclei and were even attached to the decondensing sperm nucleus.

Two of the six 3PN embryos at syngamy showed evidence of centrioles associated with the first mitotic spindle. Four embryos had bipolar spindles, while two had tripolar spindles. The centriole that was first identified was associated with a bipolar spindle (Fig. 2) and presented the typical 9 + 0 organization of MT, which is also seen in the proximal sperm centriole (15). One embryo with a tripolar spindle had two centrioles at one of its poles (Fig. 3), which were aligned in the usual manner at right angles to each other. These were associated with dense osmiophilic material characteristic of centrosomes (10, 11).

Since 3PN embryos are abnormal, and to exclude the possibility that the centrioles are associated with abnormal development, we further investigated four normal monospermic 2PN embryos at syngamy for the presence of centrioles. Two of the four embryos had centrioles surrounded by centrosomal material at one pole of each bipolar spindle. One of these had a single centriole at one spindle pole (Fig. 4), while the other had two centrioles at one pole detected at two levels of serial sectioning. Remnants of sperm tails and midpieces were also evident close to spindle poles. These results prove that centrioles are present after fertilization in both normally and abnormally fertilized oocytes. The failure to observe centrioles in all of the embryos examined at syngamy could be due to several reasons. (i) Centrioles are minute objects and could easily go undetected even by TEM. (ii) Serial sections may be lost during microtomy or sections may sit on grid bars obscuring spindle poles. (iii) Centrioles may be located in thick survey sections, where they cannot be detected by light microscopy. (iv) It is difficult to orientate and section spindles in a desired plane, as spindles are not

visible in whole embryos at syngamy, when viewed by light microscopy.

DISCUSSION

Although it is known that paternally derived centrosomes organize the mitotic spindles in sea urchin embryos (10, 11), it is generally believed, based on studies in the mouse (2, 22), that in mammalian embryos in general, the mitotic spindle is organized by maternally inherited centrosomes. The possibility that paternally inherited centrosomes could be organizing the mitotic spindle in human embryos was first suspected by us when we observed that most, but not all, dispermic human embryos cleaved directly and synchronously from one to three (19) or even four cells (23), instead of normal cleavage to two cells. Such a situation also occurs in dispermic sea urchin embryos (24). We thus proposed that for dispermic embryos to cleave to three cells, the mitotic spindles at the first cleavage division, as in the sea urchin, should have a tripolar spindle. The presence of a multipolar spindle in turn is suggestive (as in the sea urchin) of the mitotic spindle being organized by paternally inherited centrosomes or centrioles.

The centrioles found at spindle poles of human embryos in this study are most probably paternal in origin based on the following reasons. (i) The proximal sperm centrioles (Fig. 1) are closely associated with the male pronucleus during its entire maturation (16–18). (ii) The structure of the centrioles observed at spindle poles in normal monospermic embryos (Fig. 4) is very similar to that of the proximal sperm centriole, which in turn has a well-defined structure (13–15). (iii) The centrioles depicted in this study are often associated with osmiophilic pericentriolar material as is the case with sperm centrioles. (iv) Osmiophilic centrosomes are not demonstrable by TEM in meiotic spindles of human oocytes (6), which

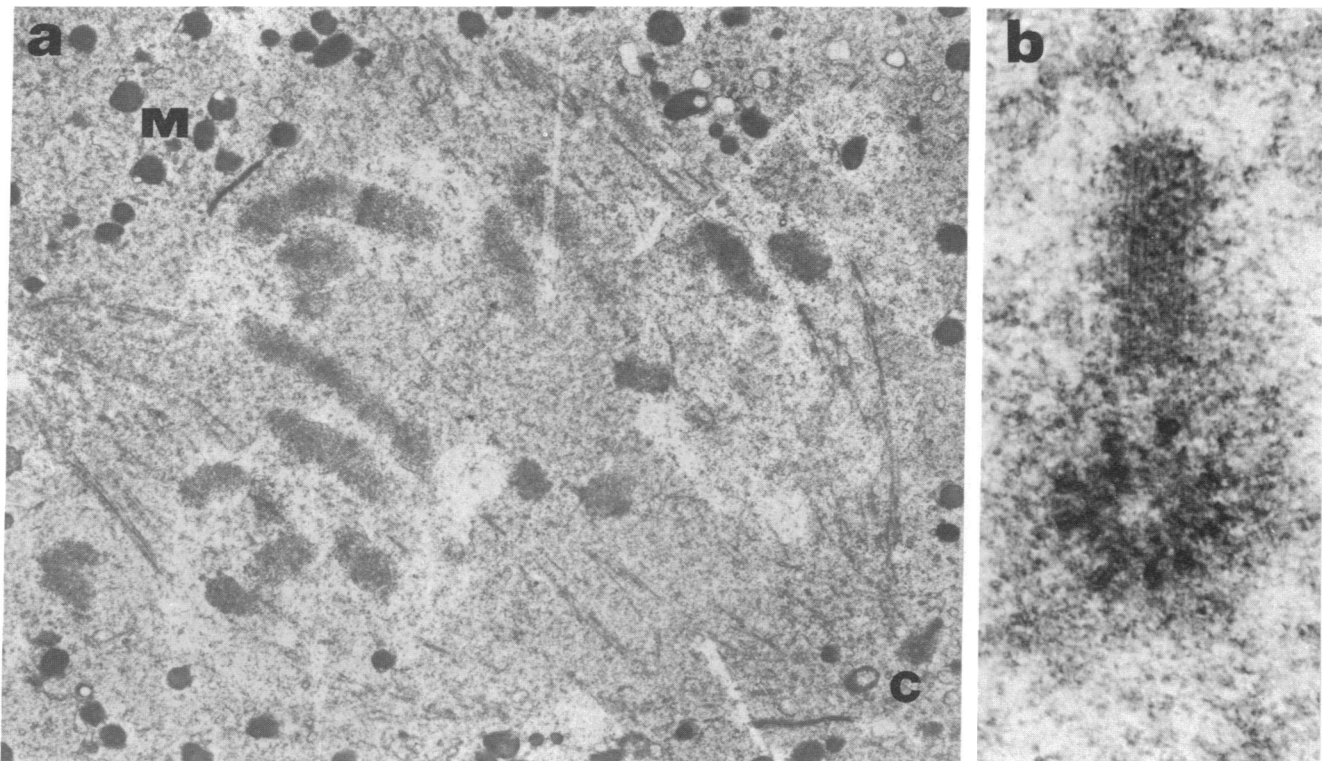


FIG. 3. Section of a tripolar spindle of a 3PN embryo at syngamy. (a) Two centrioles (C) masked by osmiophilic centrosomal material are seen at one of the three poles. MTs extend from each pole toward the chromosomes in the central region of spindle. M, mitochondria. ($\times 6000$.) (b) The two centrioles at higher magnification, aligned at right angles to each other. Both centrioles are associated with dense centrosomal material, which obscures their structure. ($\times 65,000$.)

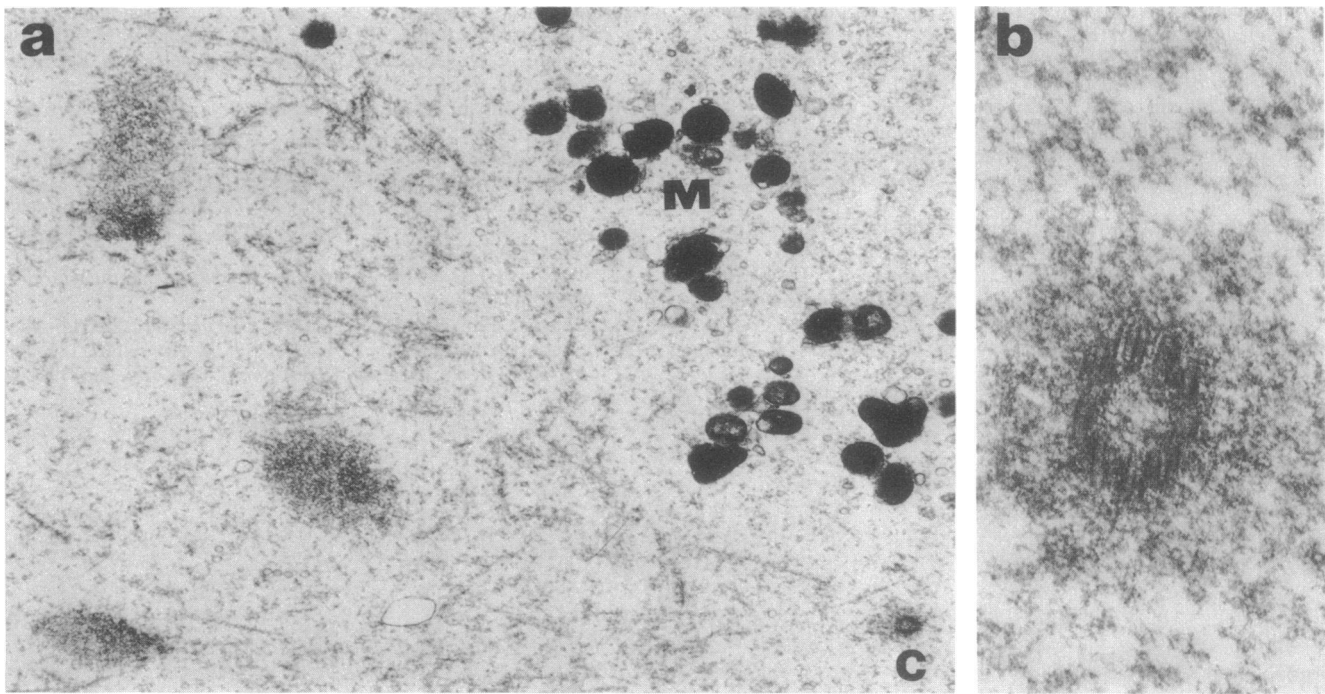


FIG. 4. Bipolar spindle developed from a normal 2PN embryo at syngamy. (a) Quarter of the spindle in longitudinal section is shown with a centriole (C) within an osmiophilic centrosome at one pole. Spindle MTs extend from the pole toward three chromosomes. M, mitochondria. ($\times 13,200$.) (b) Oblique cross-section of a centriole from another 2PN embryo at syngamy. Nine triplets of MTs are recognizable (compare with Fig. 2b). Osmiophilic centrosomal material surrounds the centriole, while some dense material is also evident within the centriole, which can also be demonstrated within the proximal centriole of the spermatozoon. ($\times 65,000$.)

instead end abruptly or are associated with clusters of minute vesicles (15). Osmiophilic sperm pericentriolar material is easily demonstrable. (v) Remnants of sperm tails and midpieces were seen in the vicinity of centrioles at spindle poles. (vi) Furthermore, the presence of centrioles associated with the flagellar neck piece of spermatozoa in pronuclear stage oocytes has also been reported in the human (25), pig, and sheep (26, 27).

Our study does not demonstrate the organization of bipolarity in mitotic spindles by centrioles associated with centrosomes as in the sea urchin (3, 10, 11). Considering the difficulties of locating centrioles and centrosomes at spindle poles by TEM, further studies by immunogold labeling are envisaged to test human fertilization with anti-centrosome antibodies (2), which should further elucidate the distribution and precise roles of both paternal and maternal centrosomes in human development. The centriole may help identify the paternal centrosome.

In all instances, centrioles (one or two) were detected only at one spindle pole. The association of these centrioles with the spindle does not seem to be accidental as there was always a close relationship between the sperm flagellar neck or midpiece with the male pronucleus during its formation (16, 17) and later during its association with the female pronucleus (18, 21). It is possible, although unlikely, that the paternal centriole may associate secondarily with spindle poles at syngamy after pronuclear disorganization. A maternal contribution of centrosomal material needs to be considered and further investigation of centrosomes in both human oocytes and zygotes is clearly warranted. Further study would also be required to determine whether the paternal centrosome nucleates subsequent mitotic spindles during early cleavage.

This study has demonstrated centrioles in at least one pole of the first mitotic spindle in normal and tripronuclear human embryos and suggests that centrioles are paternally derived. The observations show that the widely accepted model of

maternal origin of centrosomes, based on studies in the mouse, may not hold for mammals in general. Furthermore, this study also provides evidence of tripolar spindles in dispermic human embryos, thus establishing the mechanics of altered cleavage of such embryos to three cells. The birth of triploid and tetraploid babies further reinforces the complexity of the pattern of cell division and centrosomal inheritance in humans. The human situation is analogous to that of the sea urchin, which also demonstrates paternal inheritance of centrosomes with centrioles and multipolar cleavage in polyspermic embryos.

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