Histol Histopathol (1997) 12: 827-856

Histology and Histopathology

Invited Review

Mitosis in the human embryo: the vital role of the sperm centrosome (centriole)

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Summary. The pattern of sperm centrosomal (centriolar) inheritance, centrosomal replication and perpetuation during mitosis of the human embryo is reviewed with a series of electron micrographs. Embryonic cleavage involves repeated mitoses, a convenient sequence to study centriolar behaviour during cell division. After the paternal inheritance of centrioles in the human was reported (Sathananthan et al.,1991a), there has been an upsurge of centrosomal research in mammals, which largely follow the human pattern. The human egg has an inactive non-functional centrosome.

The paternal centrosome contains a prominent centriole (proximal) associated with pericentriolar material which is transmitted to the embryo at fertilization and persists during sperm incorporation. Centriolar duplication occurs at the pronuclear stage (interphase) and the centrosome initially organizes a sperm aster when male and female pronuclei breakdown (prometaphase). The astral centrosome containing diplosomes (two typical centrioles) splits and relocates at opposite poles of a bipolar spindle to establish bipolarization, a prerequisite to normal cell division. Single or double centrioles occupy pivotal positions on spindle poles and paternal and maternal chromosomes organize on the equator of a metaphase spindle, at syngamy. Bipolarization occurs in all monospermic and in most dispermic ova. Dispermic embryos occasionally form two sperm asters initially and produce tripolar spindles (tripolarization). Anaphase and telophase follows producing two or three cells respectively, completing the first cell cycle.

Descendants of the sperm centriole were found at every stage of preimplantation embryo development and were traced from fertilization through cleavage (first four cell cycles) to the morula and hatching blastocyst stage. Centrioles were associated with nuclei at interphase, when they were often replicating and occupied pivotal positions on spindle poles during mitosis. Sperm remnants were associated with centrioles and were found at most stages of cleavage. Centrioles were found in trophoblast, embryoblast and endoderm cells in hatching blastocysts. Pericentriolar, centrosomal material nucleated astral and spindle microtubules. Abnormal nuclear configurations observed in embryos reflect mitotic aberrations. The bovine embryo closely resembles the human embryo in centriolar behaviour during mitosis.

It is concluded that the sperm centrosome is the functional active centrosome in humans and is likely the ancestor of centrioles within centrosomes in foetal and adult somatic cells. The role of the sperm centrosome in embryogenesis and male infertility is discussed, since it is of clinical importance in assisted reproduction.

Key words: Sperm centriole, Fertilization, Human embyro, Mitosis, Ultrastructure

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Introduction

The main outcome of fertilization is the bringing together of maternal and paternal chromosomes to establish the embryonic genome in the embryo. However, recent research on human fertilization reveals that in addition to the father's complement of chromosomes, the sperm cell introduces into an oocyte a minute object called the centrosome that organises the first mitotic spindle of the embryo (Sathananthan et al., 1991a; Schatten, 1994; Simerly et al., 1995; Van Blerkom et al., 1995). The sperm centrosome consists of a distinctive body, the centriole with a well-defined microtubular structure surrounded by ill-defined, granular pericentriolar material (PCM) located in the sperm neck. Further research has revealed that the sperm centrosome/centriole complex duplicates at each cell cycle in synchrony with the chromosome cycle, and is perpetuated in early human embryos during cleavage up to the hatching blastocyst stage (Sathananthan et al., 1996). Thus the sperm centrosome is very likely the ancestor of centrosomes in fetal and adult somatic cells. Hence the human embryo not only inherits the paternal chromosomes from the sperm cell but also the vital

machinery that initiates cell division at the beginning of life.

Classically, each centrosome consists of 2 centrioles (diplosomes), aligned at right angles to one another surrounded by dense, osmiphilic PCM (Fawcett, 1981; Glover et al., 1993). The centriole, per se, is a controversial organelle and is an enigma in cell biology (Wheatley, 1982), because its function in cell division is debatable. Mammalian oocytes including the human and most plant cells have no centrioles and yet divide meiotically or mitotically. However, the PCM conforms to the centrosomal granular substance that nucleates microtubules (MT), asters and spindles, viewed by TEM (see reviews, Brinkley, 1985; Mazia, 1987; Paweletz and Mazia, 1989; Bray 1992; Kalnins, 1992; Glover et al., 1993). The centriole forms the heart of the centrosome when present, but is unable to nucleate MT, except as basal bodies of cilia (Bray, 1992). Occasionally, MT extend up to the centriole in embryos and somatic cells (Fawcett, 1981; Sathananthan et al., 1996). The sperm and zygote centriole and PCM (centrosome) seem to form one functional unit as revealed by its molecular structure, now been investigated (Schatten, 1994; Zoran et al., 1994). Both centrosomes and centrioles are self-reproducing entities in somatic cells and need to duplicate at each cell cycle in synchrony with the chromosome cycle (see Fawcett, 1981; Mazia, 1987).

Paternal inheritance of the centrosome has been shown to be the norm in most animals, including mammals, the exception being the mouse and perhaps some rodents (see Table 3, Schatten, 1994). Hence most animals obey Boveri's rule of paternal centrosomal inheritance, postulated over a century ago in the round worm and sea urchin (Boveri, 1887, 1901). Boveri called the centrosome the «cytocentrum» or «cell centre», which we know now organises the cytoskeleton of the cell, in particular, the mitotic apparatus (Fawcett, 1981). The centrosome is a cyclical, self-reproducing organelle (Mazia, 1987), a concept that Boveri introduced with the limited microscopic technology available at his time, which is all the more commendable. Today centrosomes or microtubule organising centres (MTOC) are demonstrable by fluorescent microscopy with specific antitubulin antibodies (Schatten, 1994; Simerly et al, 1995), confocal microscopy (Van Blerkom et al, 1995) and of course, by transmission electron microscopy (TEM), which reveals the precise structure of centrosomes, including centrioles (Sathananthan et al, 1991a, 1996). This tool has been invaluable in our research and we have been able to unravel the functional significance of the centriole at fertilization and early embryogenesis. Embryonic cleavage involves repeated mitoses and is a good sequence to study the behaviour of centrioles. However, oocytes and embryos are more difficult to process for TEM, due to their minute size (~120 microns). The human oocyte is fertilized 3 hours after insemination in vitro and the human embryo begins cleavage at about 20 hours and the first three cleavage divisions occur approximately at 36h, 48h and 56h after insemination. It becomes a blastocyst by day 6, when it implants in the uterine endometrium (Lopata et al., 1980; Sathananthan et al., 1986a, b, 1990, 1993; Sathananthan, 1993).

There has been a resurgence in mammalian centrosomal research in recent years, after its paternal inheritance was demonstrated in the human embryo (Sathananthan et al., 1991a). This review presents the recent work published on human and mammalian fertilization and embryogenesis with respect to centrosomal/centriolar inheritance, replication and perpetuation during early development. It will also focus on the mechanics of cell division during early cleavage of the human embryo as revealed by TEM and will attempt to clarify the vital role of the sperm centrosome /centriole in mitosis. The role of the sperm centrosome in male infertility will also be discussed, as this is of clinical importance.

Methodology

This review is supplemented with a series of micrographs portraying the process of human sperm centrosomal inheritance at fertilization and its perpetuation during cleavage (mitosis). The study is

Figures 1-57. Micrographs of human sperm and early human embryos, some showing centrioles.

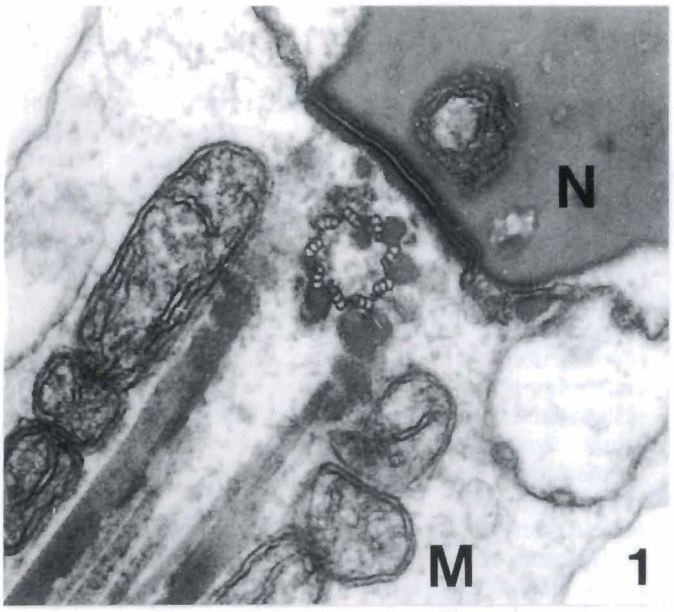
Fig. 1. Transverse section of proximal sperm centriole located beneath basal plate (neck region) showing typical pinwheel structure. Note dense material around and within centriole. N: sperm nucleus; M: midpiece. x 78,000. (Reproduced from Sathananthan et al., 1996).

Fig. 2. Longitudinal section of proximal sperm centriole with centriolar adjunct on left. Note centrosomal material surrounding (PCM) and within centriole. Remnants of the distal centriole MT are evident (arrow). N: sperm nucleus; M: midpiece. x 65,000. (Reproduced from Sathananthan et al., 1996).

Fig. 3. Expanding spermhead in ooplasm, still attached to midpiece (3 hours after insemination). The centriole is in the neck region beneath basal plate (arrow). x 11,000. (Reproduced from Sathananthan et al., 1986b).

Fig. 4. Developing male pronucleus in ooplasm (3 hours after insemination-polyspermy). Centriolar complex is attached to pronucleus (arrow). x 14,000. (Reproduced from Sathananthan, 1997).

Fig. 5. Centriolar complex («black box») closely associated with male pronucleus in a dispermic 3PN ovum. The proximal centriole (C) is in longitudinal section obscured by dense osmiophilic PCM. Outer dense fibres are evident in the distal centriolar region. M: midpiece; O: ooplasm; P: male pronucleus. x 70,000. (Reproduced from Sathananthan et al., 1991a).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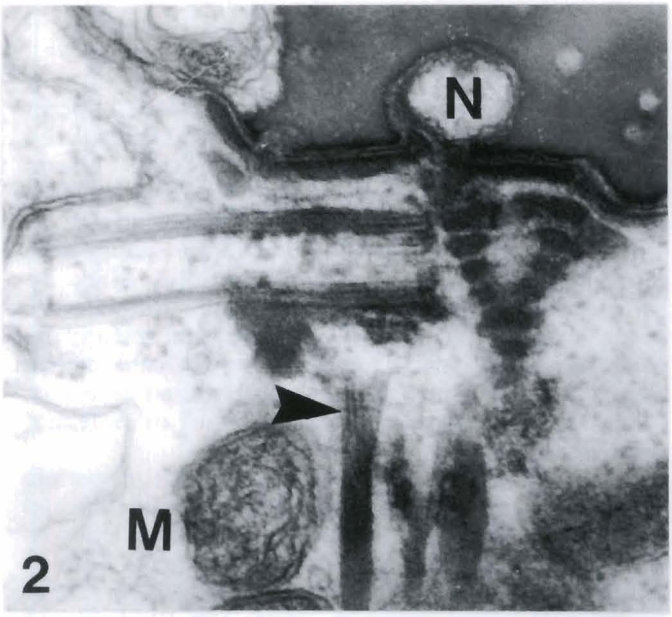
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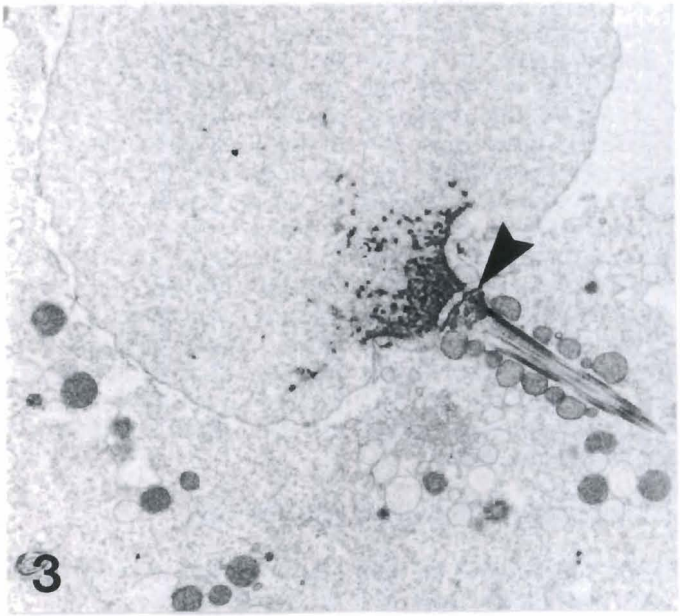
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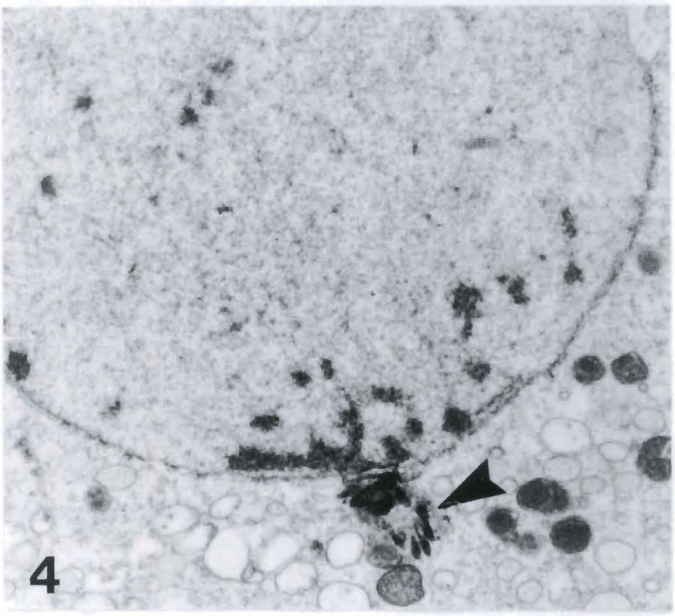
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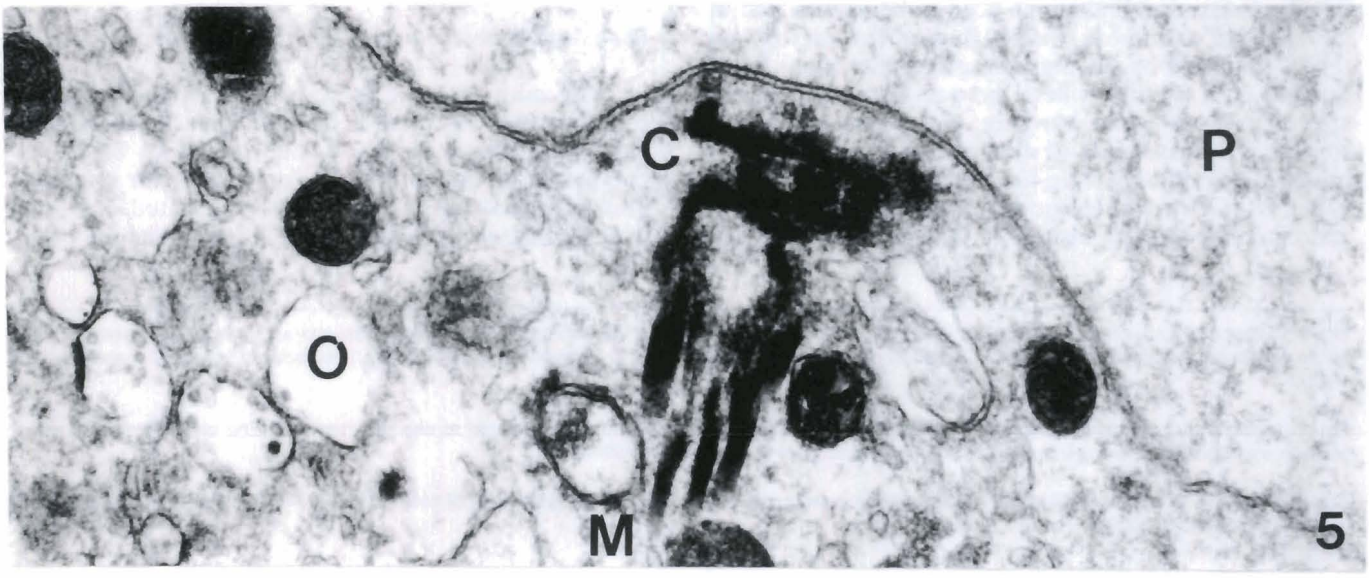
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based on the examination of several washed sperm pellets (n=30) mature oocytes (n=300) and fertilized ova and embryos (n=150) obtained from various collaborating IVF units, over many years of research (Sathananthan, 1985, 1994a, 1996a; Sathananthan et al., 1986a,b, 1990, 1993). Grids used in earlier studies were re-examined for centrosomes or centrioles. Since we reported human paternal centrosome inheritance (Sathananthan et al., 1991a), we have been researching centrosomes not only in human embryos (Sathananthan et al., 1996), but also in other mammalian embryos, which show either maternal (mouse) or paternal inheritance (marsupial and bovine).

Both normal monospermic and abnormal dispermic human embryos were examined for spindle structure and behaviour of centrosomes/centrioles. Dispermic embryos resemble normal embryos in many respects both in external morphology, cleavage and fine structure (Sathananthan and Trounson, 1985; Van Blerkom and Henry, 1991; Sathananthan et al., 1996), although they are triploid. They also inherit two sperm centrosomes that form two asters (Simerly et al., 1995), which makes it easier to detect them by TEM and usually organise bipolar (rarely tripolar) spindles (Sathananthan et al., 1991a, 1996).

All material was fixed routinely in 1% glutaraldehyde in 0.1M Cacodylate buffer (pH~7.2), postfixed in 1% aqueous osmium tetroxide, dehydrated in ethanol and acetone and flat embedded in Araldite. Alternating series of thick (1 micron) and thin (~ 70 nm) sections were cut and stained with either Toluidine blue or alcoholic uranyl acetate followed by Reynolds' lead citrate, respectively. Grids were examined by Philips and Jeol electron microscopes. For details of methodology see Sathananthan (1993).

Technical difficulties, limitations and advantages of TEM

The technical difficulties encountered in TEM and its advantages and limitations need to be appreciated in the study of centrioles. Unlike fluorescent microscopy (FM), employed by the Schatten group (Schatten, 1994; Simerly et al., 1995), where the oocytes are subject to special treatment for imaging, this study employed routine TEM methods of fixation and processing to preserve cell ultrastructure for comparison with somatic cells. With FM, whole oocytes, spindles, sperm asters and chromosomes are visualized, whereas in TEM only thin sections of oocytes and spindle components sectioned in various planes are viewed serially. Further, stabilization of MT using buffers containing Triton-X and enhancement of MT using Taxol was not employed in TEM, since they destroy cell ultrastructure. In fact, some of the MT may be lost during transient cooling to room temperature (Sathananthan et al., 1988; Pickering et al., 1990) during fixation for TEM. Most spindles and centrioles are sectioned obliquely and serial visual reconstructions are necessary in TEM. Spindles and centrioles may sit on grid bars, when they cannot be visualized at all, while centrioles and sperm tails are so minute that they could be easily missed by the observer. Occasionally thin sections may fold or be lost during staining. Further, centrioles cannot be seen in thick plastic sections by light microscopy. Apart from MT, centrosomes may be demonstrated by specific antibodies using FM. Our preliminary efforts of immunogold labelling of centrosomes and MT for TEM have been unsuccessful. Contrasting colour images of spindles, chromosomes and centrosomes can be produced by FM, while only black and white images are possible by TEM, which may be colour-enhanced by computer. Finally TEM is laborious, time-consuming and expensive, though rewarding. The advantages of TEM include preservation of fine structure and the precise location of centrioles/centrosomes, sperm asters and sperm components within cells. Centriolar structure is unmistakably well defined while centrosome material nucleating MT and chromosome attachment to MT (kinetochores) are clearly visible. Overall TEM seems to be complimentary to FM in centrosomal studies. Serial sectioning is the key to indentifying centrioles and our experiences may be useful for future research in centriolar biology.

Centrosomes in gametes and embyros

The centrosomes in human gametes and early embryos are portrayed by a series of micrographs (Figs. 1-57).

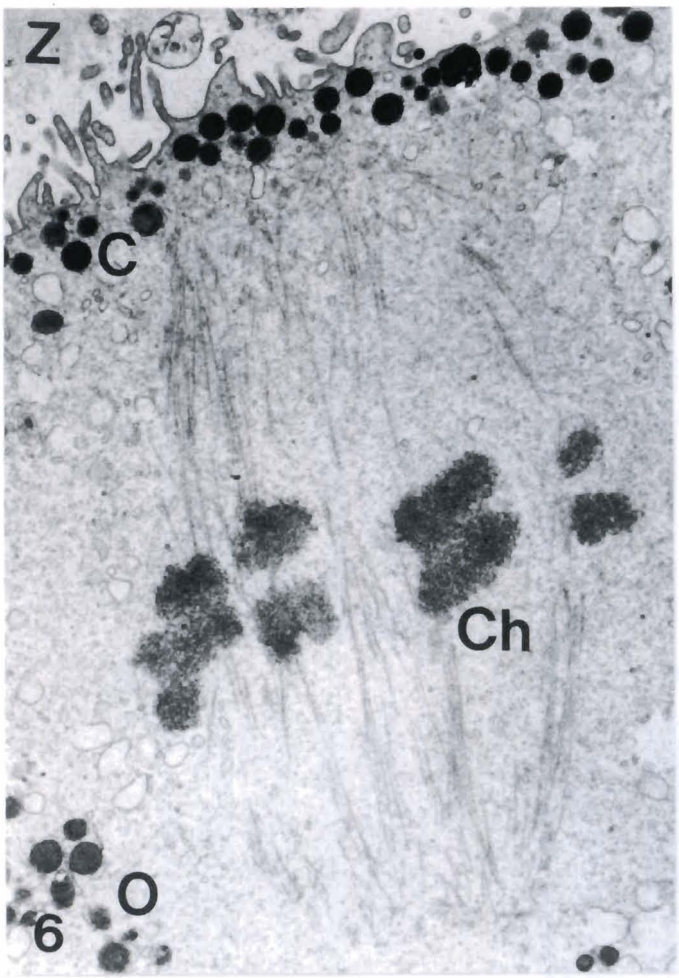
The paternal centriole (centrosome) - sperm cell

The male gamete has a well-defined proximal centriole (PC), located in the sperm neck (connecting piece) just beneath the basal plate hidden in a 'black box' composed of the capitulum (roof) beneath the basal plate, flanked laterally by 9 cross-striated columns, which interface with outer dense fibres of the midpiece.

Fig. 6. Metaphase II spindle of a mature human oocyte aligned at right angles to the surface. The spindle is barrel-shaped, anastral and does not show dense centrosomal material at the poles. C: cortical granules; Ch: chromosome; O: ooplasm; Z: zona. x 8,500. (Reproduced from Sathananthan et al., 1988).

Fig. 7. Metaphase II spindle of a mouse oocyte showing a distinct band of centrosomal material at one pole (arrows). The centrosome consists of granular and vesicular components. x 16,250. (Reproduced from Sathananthan, 1994a).

Fig. 8. Outer pole of spindle in figure 6. Note microtubules ending in inconspicuous specks (arrows) of dense material which could represent an inactive maternal centrosome. x 2,975



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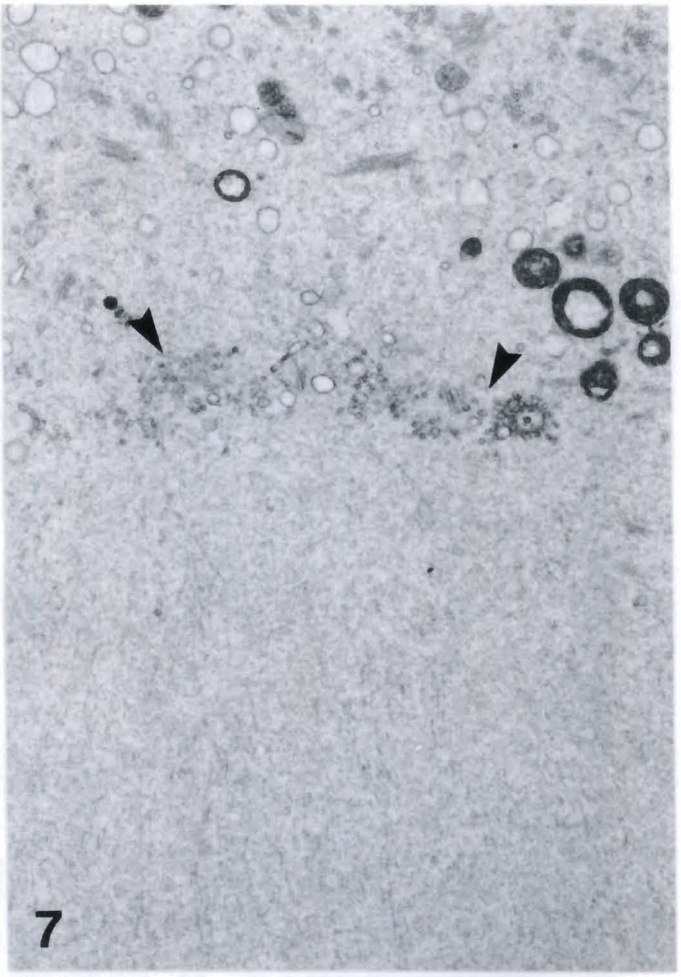

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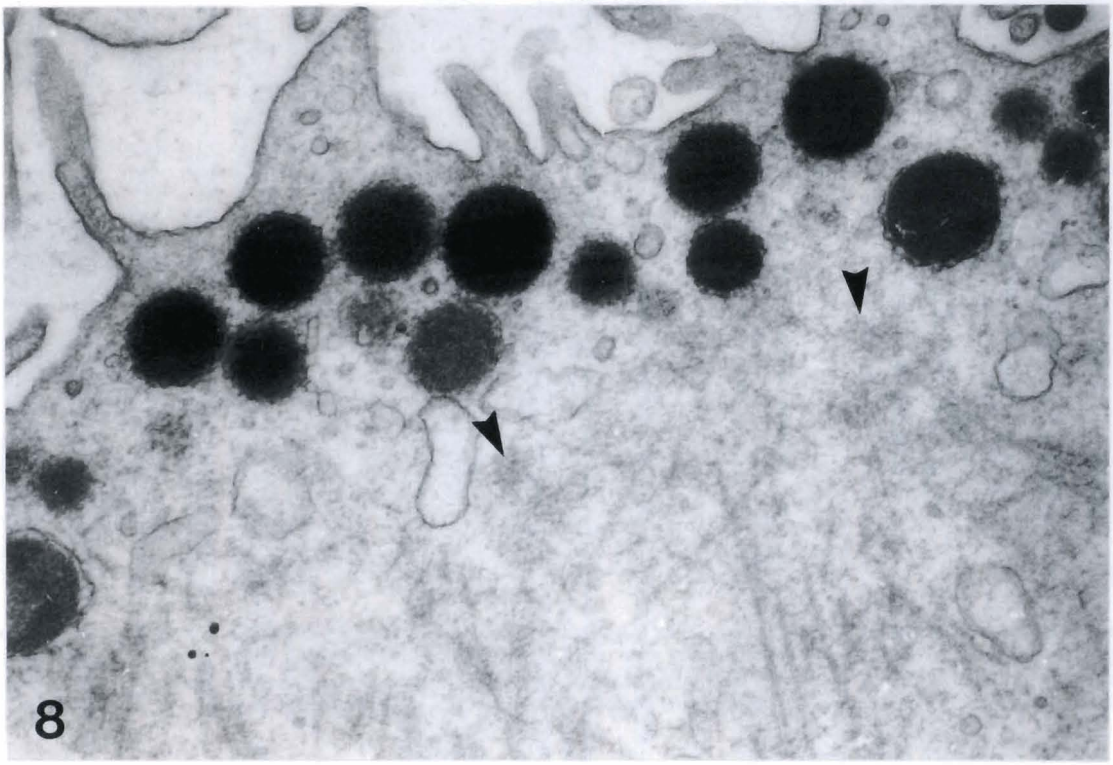

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Beneath the PC is the remnant of the distal centriole aligned at right angles to the PC and represented by a few MT, cut longitudinally and flanked against the electron-dense fibres in line with the outer doublets of MT of the axoneme. The central doublet of MT traverse through the distal centriole region to terminate in PCM

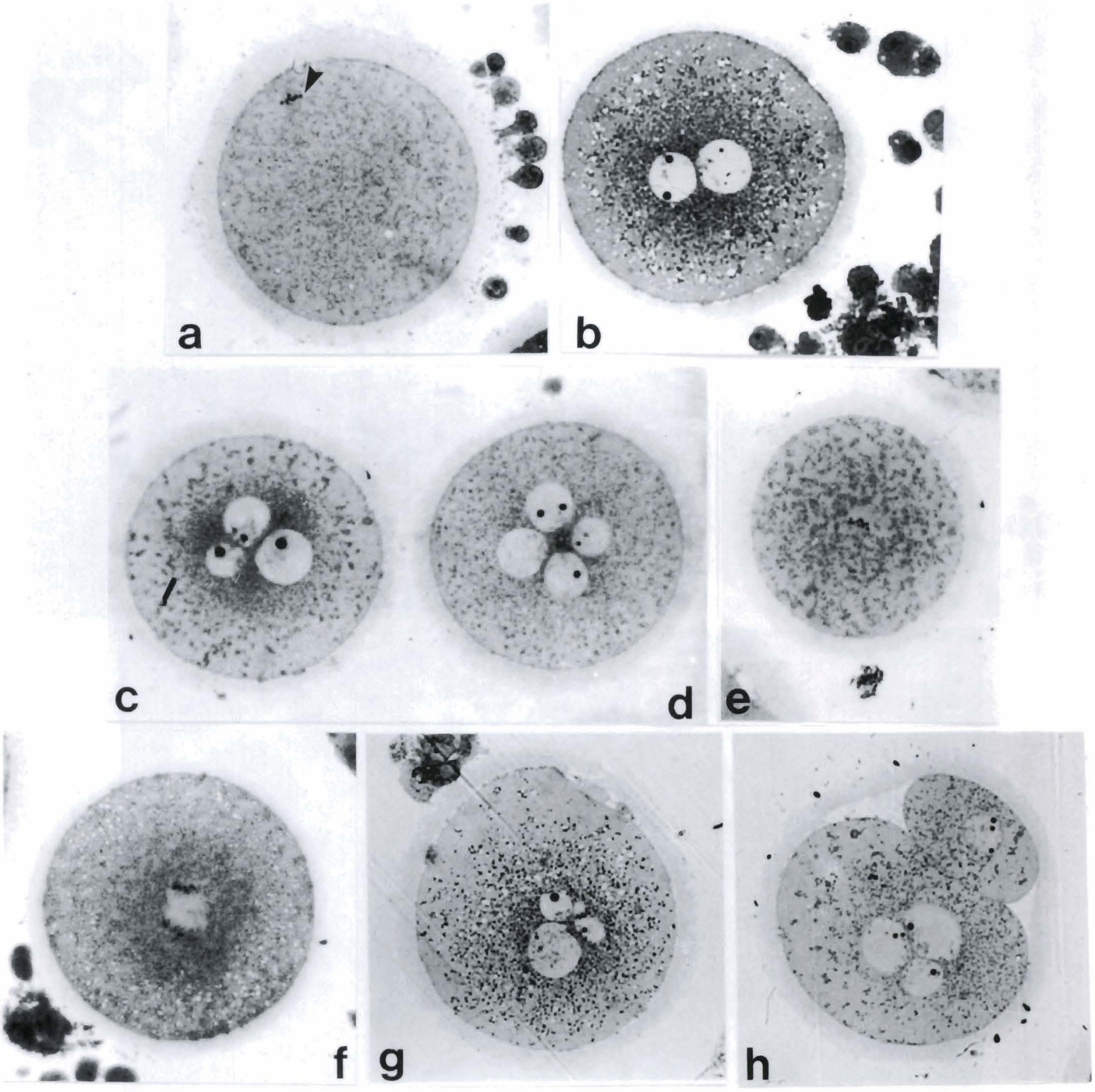


Fig. 9. Light micrographs of unfertilized and fertilized oocytes. **a.** Mature oocyte showing superficial metaphase II spindle (arrow). **b.** Normally fertilized ovum showing male and female pronuclei. **c.** Dispermic tripronuclear ovum. **d.** Polyspermic ovum showing four pronuclei. **e.** Normally fertilized ovum at metaphase of syngamy. Note spindle at the centre of ooplasm. **f.** Normally fertilized ovum at anaphase of syngamy. **g.** Fertilized ovum showing micronuclei. **h.** Two-cell embryo showing unequal blastomeres. Note three nuclei in one blastomere. x 400. (Reproduced from Sathananthan et al., 1993 ,1996).

located in the lower vault of the PC. The MT of the distal centriole show a disorganised doublet or triplet arrangement and was called the transitional centriole (Zamboni and Stefanini, 1970). These workers believe that since the distal centriole has a pair of MT traversing through its center it is not considered to be a basal body of the sperm flagellum. Most often the centrioles are cut obliquely but perfect cross and longitudinal sections could be obtained (Figs. 1, 2).

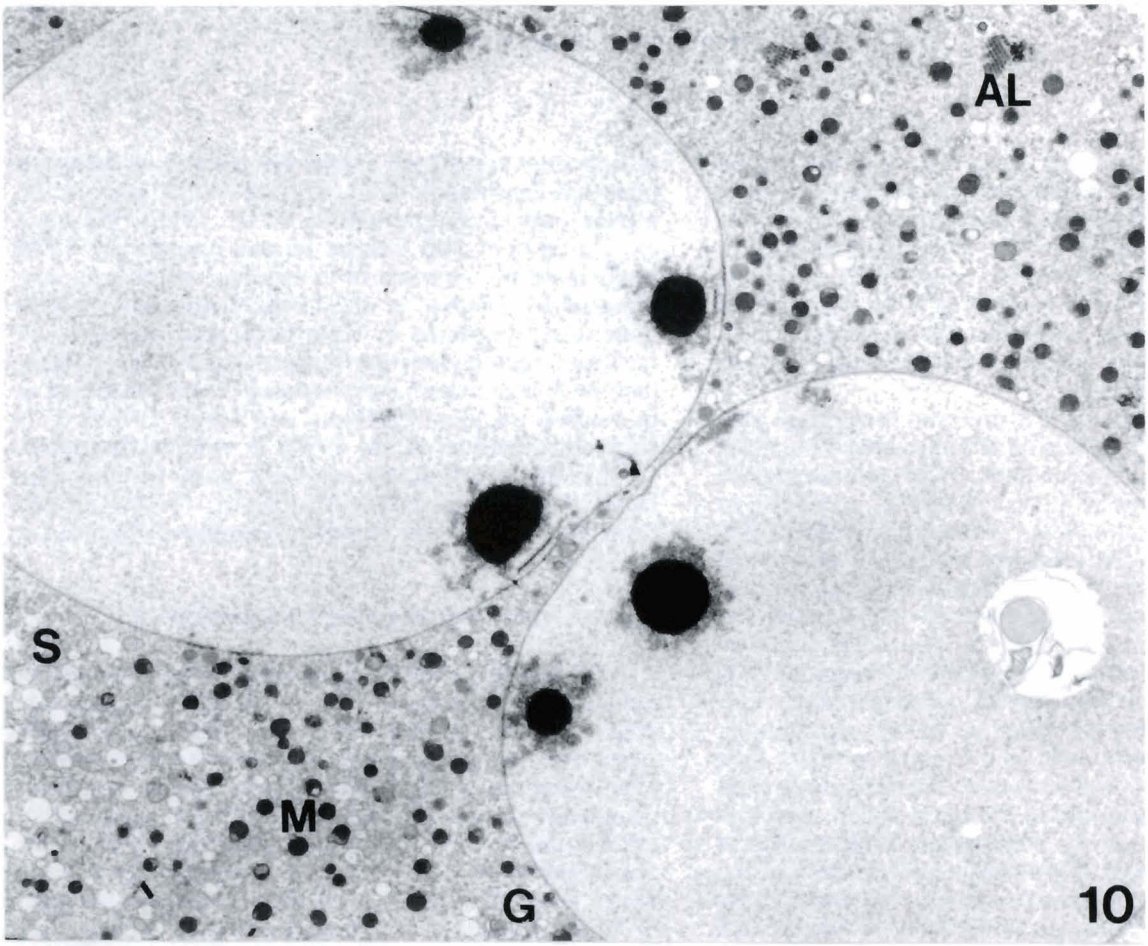
In cross sections, the PC presents the typical pin-wheel structure of 9 triplets of MT surrounded by a thin layer of moderately electron dense PCM, pressed against the striated columns (Fig. 1). The PCM conforms to granular centrosomal material defined by Mazia (1987) and Glover et al. (1993). Similar material is also found within the triplets of MT. The longitudinal section also reveals these features but the free end of the cylindrical centriole terminates in a centriolar adjunct composed of MT, arranged in a circle, which extends through a side opening of the 'black box' into the cytoplasm of the neck region (Fig. 2). There are variations in MT organization, particularly in sperm with low or zero motility (see section on clinical implications). Bovine sperm also show a proximal centriole in the neck region, which is aligned at an angle to the basal plate (Sathananthan et al., 1997a). For details of formation and structure of the neck components in human sperm, see reviews by Holstein and Roosen-Runge (1981) and de Kretser and Kerr (1994).

The maternal centrosome-mature oocyte

Detailed examination of mature human eggs, arrested at metaphase II of meiosis, over the years (Sathananthan, 1985, 1993; Sathananthan et al., 1991a, 1993) failed to reveal either centrioles or functional centrosomes associated with the maturation spindle (Fig. 6). The MII spindle of the mature oocyte is anastral, barrel-shaped and aligned more or less at right angles and is anchored to the oocyte surface (Sathananthan, 1985; Sathananthan et al., 1986a, 1988, 1993). Both spindle poles, however, do not show dense centrosomal material, in contrast to a dense distinct band, that is easily demonstrable by TEM in mouse oocytes (Sathananthan, 1994a; Sathananthan et al., 1992a, 1993). The mouse oocyte shows dense, granulo-vesicular material at both spindle poles (Fig. 7). The spindle is aligned parallel to the oocyte surface unlike in the human oocyte and a cortical band of microfilaments (MF) is found beneath the oolemma overlying the spindle. Such a band of MF is not so prominent in human oocytes, but appears during polar body abstriction at meiotic maturation (Sathananthan et al., 1986a, 1991b, 1993). The spindle MT in the human oocyte ends abruptly at the poles and the pole directed outwards is closely bound to the egg cortex terminating beneath 2 or 3 layers of cortical granules. However, inconspicuous specks of electron-dense material were occasionally found associated with terminating MT, which may represent a non-functional or reduced maternal centrosome (Fig. 8), unlike that seen in the mouse oocyte (Sathananthan et al., 1996). We have examined over 300 mature human oocytes by TEM confirming the absence of centrioles, which was also reported by Szöllösi et al. (1986). Centrioles are also absent at anaphase or telophase spindle poles of fertilised eggs completing maturation. It is very likely that the human maternal centrosome is non-functional as in starfish oocytes (Paweletz and Mazia, 1989) or is suppressed (Sluder et al., 1993) or reduced (Schatten, 1994). If centrosomes of both gametes become functional mosaicism could result following the formation of abnormal spindles in embryos (Palermo et al., 1994).

Paternal inheritance of centrioles at fertilization

The sperm centriole is introduced into the human oocyte at fertilization (Boveri, 1901). Monospermic, dispermic and polyspermic ova (Figs. 9-11) were examined for sperm centrioles soon after fertilization (Sathananthan et al., 1991a, 1996). Sperm centriolar complexes are closely associated with fertilising sperm during gamete fusion (Sathananthan and Chen, 1986; Sathananthan et al., 1986a,b) and also with expanding sperm heads at every stage of sperm incorporation right up to the formation of the male pronucleus (Figs. 3-5, 12). Sperm midpieces and tails, mitochondria and other remnants of the 'black box' are often found in close proximity to centrioles confirming their paternal origin without doubt. Well organized sperm asters, however, were not clearly visible in the early stages of sperm chromatin decondensation as shown by FM (Simerly et al., 1995), though a few MT were evident in the vicinity of spermheads. Neither were MT visible between migrating pronuclei prior to their association. The sperm centriole separates from the male pronucleus just prior to its replication, usually in the late pronuclear stage and duplication begins at this stage (Figs. 14,15). Each sperm centriole produces a sperm aster soon after sperm incorporation to organise the first mitotic spindle (Figs. 16, 24-27). The details of centriolar inheritance at fertilization was reported by Sathananthan et al. (1991a, 1996), while sperm asters and spindles were demonstrated by FM by Schatten (1994) and Simerly et al. (1995). There is no doubt, whatsoever, that the sperm centriole/centrosome is inherited by the human embryo, as was shown in these studies and that of Van Blerkom and Davis (1995), who injected the sperm centrosome into human oocytes to produce sperm asters. Hence the human follows Boveri's rule of paternal inheritance of centrosomes as has been reported in most other animals, including mammals (see reviews, Mazia, 1987; Paweletz and Mazia, 1989; Schatten, 1994). Boveri's initial discovery in the sea urchin has been confirmed elegantly by Paweletz and co-workers (1987a,b, 1989) by TEM. Due to their minute size and the technical difficulties encountered in their demonstration by TEM (see



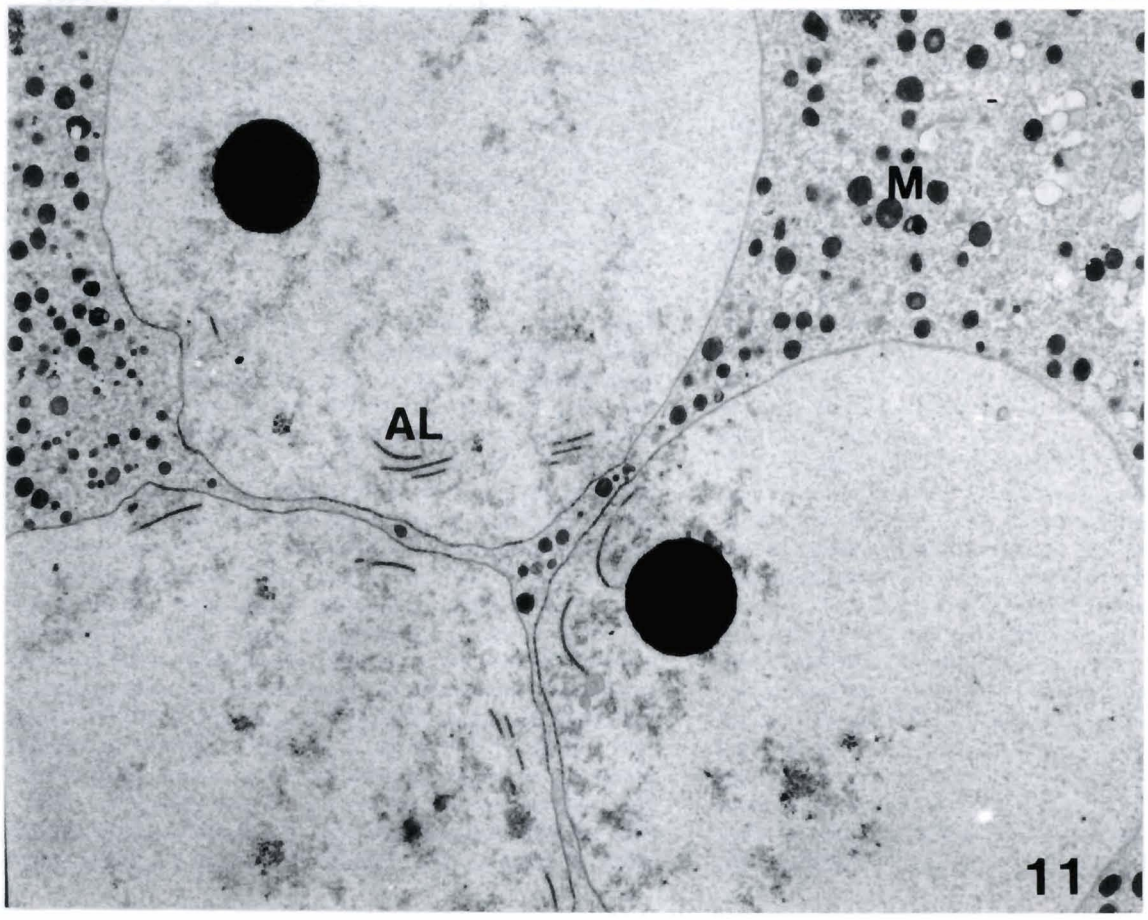


Fig. 10. Normally fertilized bipronuclear ovum. The male and female pronuclei are closely associated with one another in the centre of ooplasm. AL: annulate lamellae; G: golgi; M: mitochondria; S: SER. x 5,900. (Reproduced from Sathananthan, 1993).

Fig. 11. Dispermic ovum showing two male pronucleus closely and a single female pronuclei closely associated at the centre of ooplasm. AL: annulate lamellae; M: mitochondria. x 5,900. (Reproduced from Sathananthan, 1993).

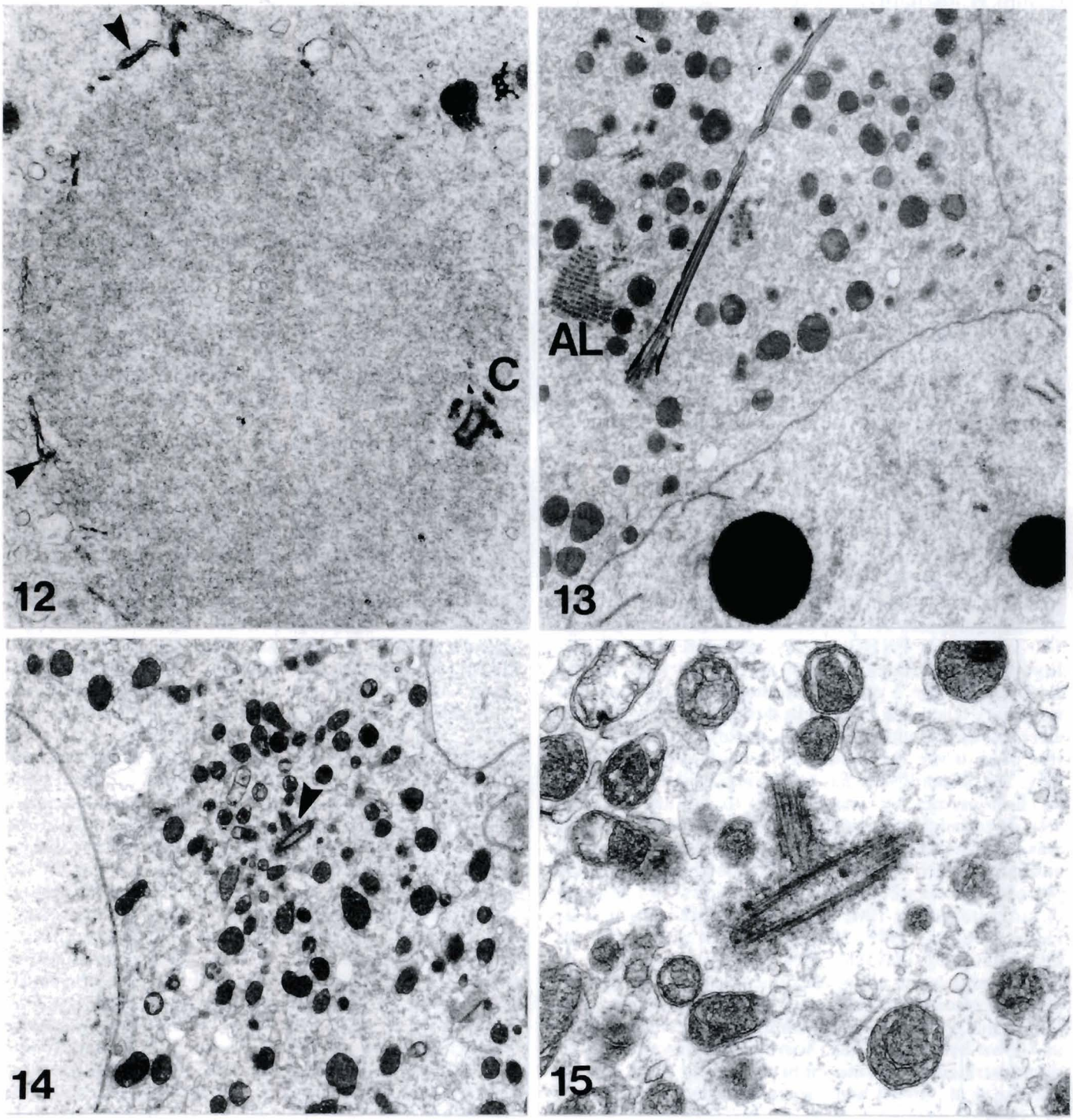


Fig. 12. Incorporated spermhead showing decondensed chromatin 3h after insemination. Note associated proximal centriole (C) and dismantling nuclear envelope (arrows). x 21,000. (Reproduced from Sathananthan and Chen, 1986).

Fig. 13. Two pronuclear ovum showing a sperm tail in longitudinal section directed towards the male pronuclei. AL: annulate lamellae. x 8,500

Fig. 14. Duplicating centriole (arrow) between two pronuclei of a dispermic embryo. x 8,500. (Reproduced from Sathananthan et al., 1996).

Fig. 15. The diplosomes in figure 14 are aligned almost at right angles to one another and surrounded by fine granular material (PCM). x 34,000. (Reproduced from Sathananthan et al., 1996).

technical difficulties), centrioles and associated centrosomes (PCM) are rarely reported in studies of fertilization. The human studies have evidently sparked off a resurgence of centriolar/centrosomal research in recent years in a number of large mammalian species both by TEM and FM.

The first to casually detect a centriole in a human embryo was Soupart (1980) followed by Sathananthan and Chen (1986), when its significance was not fully realized. Szöllözi and Hunter (1973) reported a centriole in the pig oocyte by TEM, while Crozet and co-workers elegantly demonstrated centrioles during sheep fertilization, at syngamy (Le Guen and Crozet, 1989; Crozet, 1990). Many reports of centrioles and centrosomes followed in bovine fertilization (Sathananthan et al., 1992, 1997b; Long et al., 1993; Navara et al., 1993, 1994); marsupial fertilization (Breed et al., 1994; Merry et al., 1995; Sathananthan et al., 1997c); rhesus fertilization (Hewitson et al., 1996). The bovine embryo closely follows the human pattern of sperm centriolar inheritance and is a good animal model to study centriolar behaviour.

Sperm asters, centrosomes and centrioles

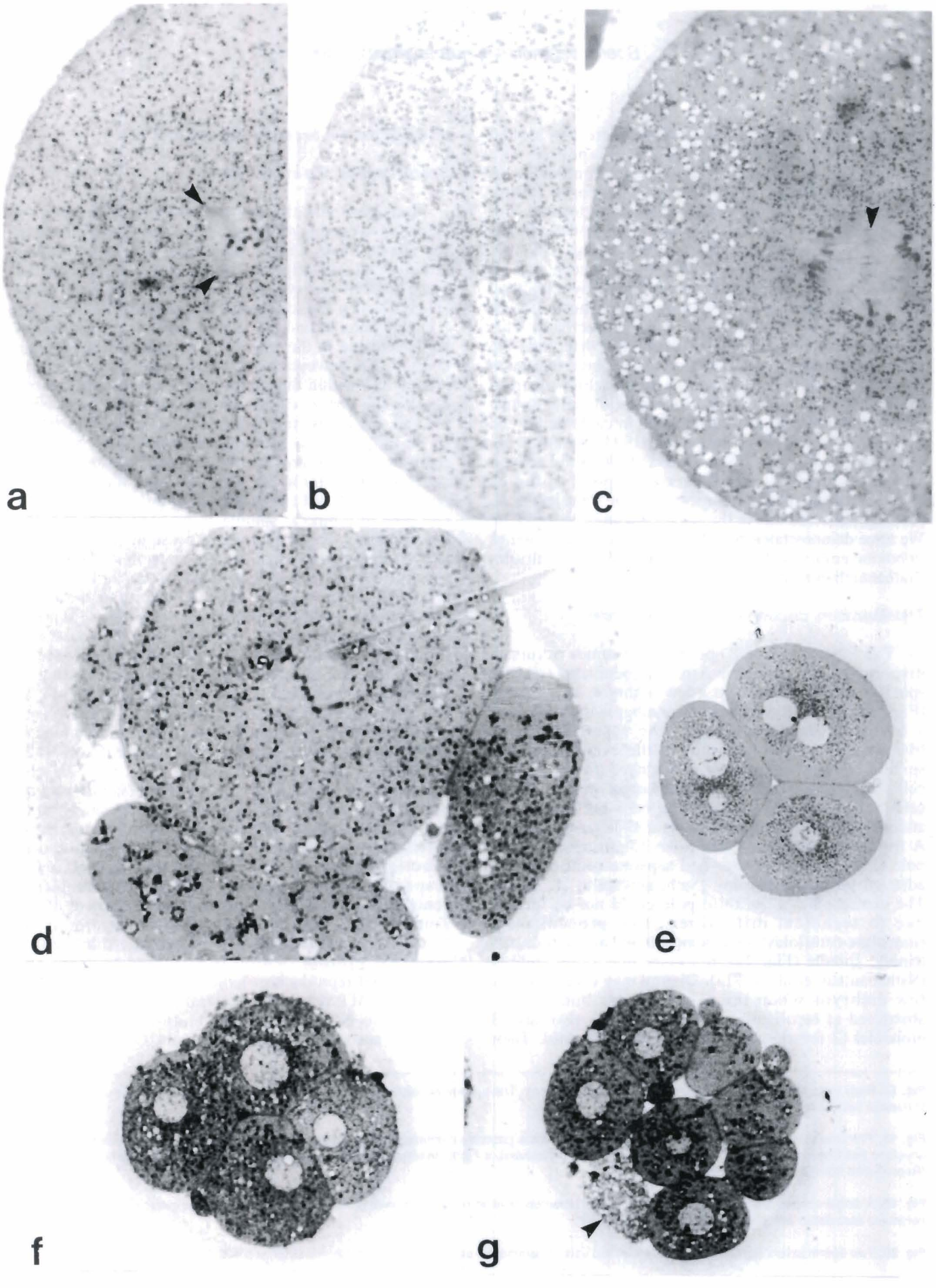
At the onset of syngamy (prometaphase of mitosis), the male and female pronuclear envelopes break down (PNBD), chromatin begins to decondense to form chromosomes and MT polymerize in their vicinity (Figs. 17, 18). When PNBD occurs, characteristic triple-layered membranes (Figs. 18, 19) are seen in the periphery of the 2 groups chromosomes (paternal and maternal). The male PN breaks down first and the sperm aster associated with sperm tails, makes its appearance at this pole, while the female PN is still partially intact (Fig. 18). When PNBD has been completed, the 2 groups of chromosomes are seen adjacent to one another (Fig. 19). Tripronuclear breakdown also occurs in a similar way in dispermic embryos and 2 sperm asters are formed associated with 3 groups of chromosomes (Fig. 20). Whole monoasters and double asters have been demonstrated by FM, associated with sperm tails (Simerly et al., 1995; Van Blerkom et al., 1995).Van Blerkom and Davies (1995) have been able to demonstrate sperm asters after microinjection of isolated sperm centrosomes into human oocytes and there is no doubt, that the sperm centrosome organizes these asters as shown below by TEM. Sperm asters have also been demonstrated in bovine embryos (Long et al., 1993; Navara et al., 1994) and rhesus embryos (Hewitson et al., 1996).

Each sperm aster, visualized by TEM, consists of 2 well-defined centrioles (diplosomes) and a tuft of astral MT originating from PCM (Fig. 21). The sperm centriole duplicates at interphase at the PN stage (Figs. 14, 15) and the typical structure of the centrosome is now established, which cannot be demonstrated by FM using antitubulin antibodies, where only the MTOC is demonstrable. The 2 centrioles are aligned at right angles to one another and are composed of the usual 9 triplets of MT surrounded by granular PCM (Fig. 21). The centriole in transverse section shows more PCM than the other in longitudinal section, which had varying amounts of PCM along its length. The former was regarded as the parent centriole, while the latter could be the daughter (Sathananthan et al., 1996). More PCM seems to be added on as the mitotic spindle is fully assembled, which could be of maternal origin (Sathananthan et al., 1996). Blending of centrosomes will be discussed later (see sperm centrosome in zygote).

Bipolarization: the first mitotic spindle

The essence of mitosis is the organization of a bipolar spindle (bipolarization) to ensure that the chromosomes are equally distributed to 2 daughter cells of similar size at the completion of each cell division (Fawcett, 1981; Mazia, 1987). This occurs at syngamy, when the maternal and paternal chromosomes are organized in homologous pairs on the equator of a bipolar spindle, at metaphase (Figs. 16, 24, 28-32). The single sperm aster splits (Schatten, 1994; Simerly et al., 1995) and the pair of centrioles (diplosomes) duplicate and move to opposite poles, establishing bipolarization (Fig. 24). This process is documented by TEM in a dispermic embryo with a bipolar spindle in several serial sections, which revealed evidence of centriolar duplication and possible relocation to the opposite poles (Figs. 28-32), thus establishing the definitive role of the sperm centrosome in the organization of the mitotic spindle (Sathananthan et al., 1996). The vast majority of spindles, whether after monospermic or dispermic fertilization are bipolar (Figs. 16, 24-26), whereas about 3% of spindles are tripolar (Fig. 16, 27). This is also the norm in subsequent cell divisions. The bipolar spindle at metaphase revealed 2 centrioles at one pole and a single centriole at the opposite pole and also 2 side centrioles away from the pole (Fig. 29). The latter are probably duplicated centrioles of the second sperm evidently not involved with the mitosis. Centrosomal material of moderate electron density was associated with polar centrioles (Fig. 32), while MT rarely extended up to the

Fig. 16. Light micrographs of fertilized ova and embryos. **a.** Dispermic ovum at prometaphase of syngamy. Note two sperm asters (arrows) associated with chromosomes. x 1,000. **b.** Dispermic ovum showing an eccentric bipolar spindle at metaphase of syngamy. x 1,000. **c.** Dispermic ovum showing a tripolar spindle at anaphase of syngamy. Note chromosomes at three poles and interbody (arrow). x 1,000. **d.** Three-cell embryo with one blastomere at metaphase. Note bipolar spindle. x 1,000. **e.** Dispermic 3-cell embryo with all blastomeres of almost equal size. This may have arisen from a tripolar spindle. Note binucleate cells. x 400. (Reproduced from Sathananthan et al., 1993). **f.** Normal frozen 4-cell embryo with equal sized blastomeres. Note normal nuclei. x 400. **g.** Normal frozen 12-cell embryo. One blastomere has lysed due to freezing (arrow). x 400. (f and g Reproduced from Ng et al., 1988).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centriole (Fig. 31). Multiple centrioles were also located in a 8-cell dispermic embryo at anaphase. The occurrence of supernumerary side centrioles in dispermic embryos could be a mechanism to ensure bipolarization. Two bipolar spindles were located in a dispermic bovine embryo (Navara et al., 1994). In the next stage the embryo enters anaphase (Figs. 16, 25), when chromosomes separate and move to each pole of either bipolar or tripolar spindles followed by telophase, when the nuclei are reformed and cytokinesis occurs producing either 2 or 3 blastomeres, respectively. This completes the first cell cycle. Interbodies determine the plane of cleavage of blastomeres and their remnants persist for some time between cells which go into interphase. The process of bipolarisation after sperm aster formation was also documented in cattle and in the rhesus monkey by FM (Long et al., 1993; Navara et al., 1994; Hewitson et al., 1996). Crozet (1990) and Le Guen and Crozet (1989) demonstrated bipolar spindles associated with centrioles in sheep, while Paweletz et al. (1987a,b) showed this in the sea urchin embryo by TEM. We have demonstrated both bipolar and tripolar spindles in bovine embryos at syngamy associated with centrioles (Sathananthan et al., 1992, 1997b).

Tripolarization: dispermy and tripolar spindles

The inheritance of 2 sperm centrosomes occurs in dispermy, when 2 sperm asters appear and tripolar spindles may be formed in a few embryos, at syngamy (Figs. 16, 20). The chromosome number is triploid and if a bipolar spindle is formed the resulting 2-cell blastomeres will be triploid. In the event a tripolar spindle appears the embryo will cleave directly into 3 cells, which may have a variable karyotype and if two cells and an extrusion results the cells are diploid, an attempt to restore normality (Kola and Trounson, 1989). A tripolar spindle had 2 centrioles (diplosomes) at one pole (Fig. 19) and another had separate centrioles at 2 adjacent poles, at syngamy (Sathananthan et al., 1996). The centrosome at the third pole could not be located due to technical difficulties. Two procentrioles (immature centrioles) were demonstrated at one pole of a tripolar spindle (Fig. 22) in a dispermic 1-cell embryo (Sathananthan et al., 1991a). Digyny may eventuate in a few embryos when the second polar body is not abstricted at fertilization. These embryos also have 3 pronuclei (2 female and 1 male) and are triploid. Their cleavage has not been investigated by TEM.

Sperm centrosome in the zygote

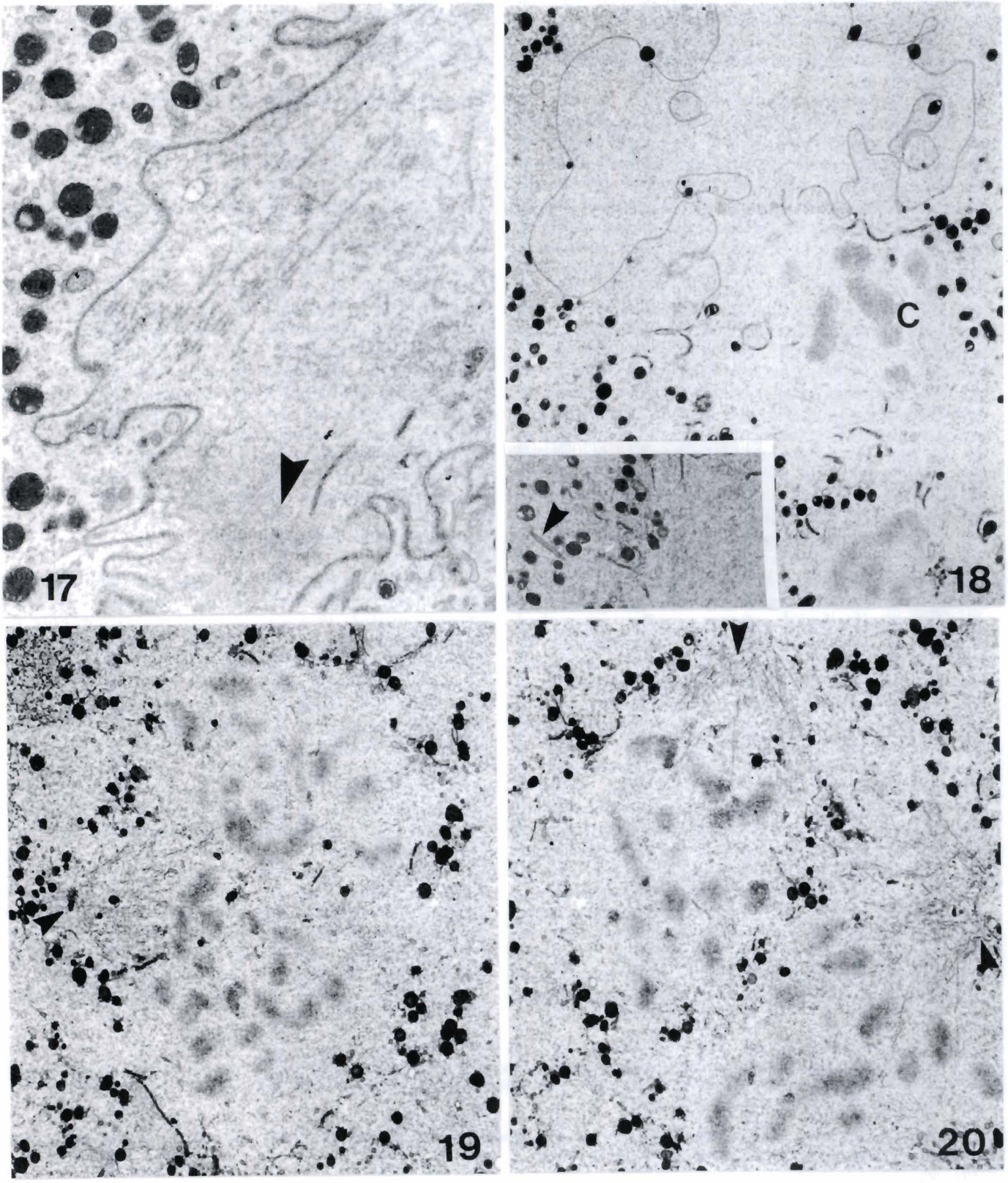
Lets compare the sperm centriole in the human zygote at syngamy with that of the proximal centriole in the sperm neck. The centriole has the same typical structure of MT triplets, clearly demonstrable in the astral centriole, at syngamy and later at the 8-cell stage (Figs. 21, 41). The centrosomal substance (PCM) forms a thicker halo around the zygote centrioles, which is not so discernable in the 'black box' beneath the basal plate (Figs. 1, 2). However, some dense material is seen both around and within the sperm proximal centriole pressed against the denser capitulum and segmented columns. Overall, the impression gained is that more PCM seems to be added on to the zygote centrosome after its replication. There are also diffuse specks or satellites of centrosomal material far removed from the centrioles, nucleating MT and an abundance of this was detected at one pole of a bipolar spindle at syngamy (Fig. 32). We have reported that there is more dense granular material (PCM) aggregating around the centrioles after sperm incorporation, which could be of maternal origin (Sathananthan et al., 1996). The human maternal centrosome is probably inactivated at metaphase II arrest or earlier during maturation and appears to be reactivated later at syngamy, when the first mitotic spindle is formed. Indeed, if we accept that there is a blending of maternal and paternal centrosomes after fertilization, as was also implicated by Schatten and co-workers (see Schatten, 1994), this could well be the morphological expression of such a process. The mother seems to be providing the re-inforcement for the 'castle' (centrosome), while the prince (centriole) is provided by the father. The molecular picture that is now emerging of the human sperm centrosome, before and after fertilization will give us more insights into the functioning of the sperm centriole and also the respective roles played by paternal and maternal centrosomes in human reproduction (Schatten, 1994; Zoran et al., 1994). The centrioles have centrin in the heart of the centrosome, while gamma-tubulin is a maternal protein, which is attracted towards the centrosomal reproducing element (centriole), which then nucleates MT resulting in astral growth. Thus, there appears to be a blending of maternal and paternal proteins according to this hypothesis. One of the chief

Fig. 17. Early male pronuclear breakdown at the onset of syngamy. The nuclear envelope is folded and dismantling. Note microtubules and condensing chromatin (arrow). x 12,750

Fig. 18. Pronuclear breakdown at the onset of syngamy. The male pronuclear envelope has disorganized (lower region), while the female pronuclear envelope has begun to dismantle. Chromosomes (C) have condensed. x 4,250. **Inset:** Sperm tail in a serial section at the lower pole (arrow). x 5,100. (Reproduced from Sathananthan, 1997).

Fig. 19. A single sperm aster associated with two sets of chromosomes at the completion of pronuclear breakdown in a dispermic ovum. Note a pair of centrioles associated with the sperm aster (arrow). x 5,100

Fig. 20. Two sperm asters (arrows) associated with two sets of chromosomes derived from a dispermic ovum. x 5,100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functions of the centriole seems to be centrosomal reproduction (see functions of centrioles).

Replication and perpetuation of sperm centrioles in embryos

We have traced the sperm centriole in human and also bovine embryos during the first week of development to show its continuity during mitosis (Sathananthan et al., 1996, 1997a,b). Over 150 human monospermic and dispermic embryos and 300 bovine embryos were examined for centrioles in serial sections by TEM in closely knit series. Centriolar behaviour during the first cell cycle has been described above and this section will cover subsequent cell cycles to show its replication and perpetuation. Centrioles were found in at least one blastomere at every stage of pre-implantation embryonic development, from the 2-cell stage to the hatching blastocyst. Centrioles occurred singly or in pairs (diplosomes) often detected in serial sections or were evidently duplicating when closely attached to one another. Details of centriolar structure and distribution in the first four cell cycles and later embryos were reported by Sathananthan et al. (1996) and are portrayed in the micrographs presented in this review with reference to mitosis (Figs. 33-52). During interphase, centrioles are situated close to blastomere nuclei (Figs. 33, 46), sometimes attached to the nuclear envelope. In morulae and blastocysts they are associated with Golgi membranes, as well (Figs. 46-52). Most centrioles were closely associated with mitotic spindles, when they occupied precise, pivotal positions at spindle poles. If, however, the spindle is oblique in section their pivotal placement may not be obvious. Though the incidence of mitotic cells in cleaving embryos was very low (<3%), embryos cleaving asynchronously (3-cell, 5-cell, 7-cell) had at least one blastomere in mitosis. The majority of spindles were bipolar and resemble those seen at syngamy (Figs. 24-32). Spindles of a few embryos derived from dispermic embryos were also disorganized, as revealed in serial sections. Most embryos had varying numbers of cytoplasmic fragments in their perivitelline spaces or between blastomeres and many had at least 1 multinucleated blastomere (Sathananthan et al., 1982, 1986a, 1990, 1993). These abnormalities were particularly evident in embryos developed from 3PN ova and in embryos showing retarded or arrested development, which also present varying numbers of cytoplasmic fragments. The microstructure of normal and abnormal human embryo structure is reviewed in chapters, atlases and CD-ROMs (Sathananthan, 1993; Sathananthan et al., 1986a, 1993; Sathananthan and Edwards, 1995).

Evidence of sperm remnants in embryonic cells

Remnants of sperm are important clues to the identity of sperm centrioles or centrosomes. Sperm tails were evident in 1-cell embryos and in some blastomeres at all stages of early development from the pronuclear stage to the morula (Figs. 13, 18, 43). Sperm tail axonemes were found close to centrioles associated with spindles or with nuclei of early embryos, especially at the PN stage and at syngamy. These embryos showed remnants of sperm dense fibres and mitochondria, as well. Sperm mitochondria are distinctly different from maternal mitochondria. At later stages sperm axonemes were located randomly in blastomeres, while two 8-cell blastomeres had dense fibres associated with centrioles (Fig. 43). Sperm tails are easily demonstrable by FM in human, bovine and rhesus embryos (Navara et al., 1994; Schatten, 1994; Asch et al., 1995; Simerly et al., 1995; Hewitson et al., 1996) and also by confocal laser scanning microscopy (Van Blerkom et al., 1995).

Centrioles and centriologenesis

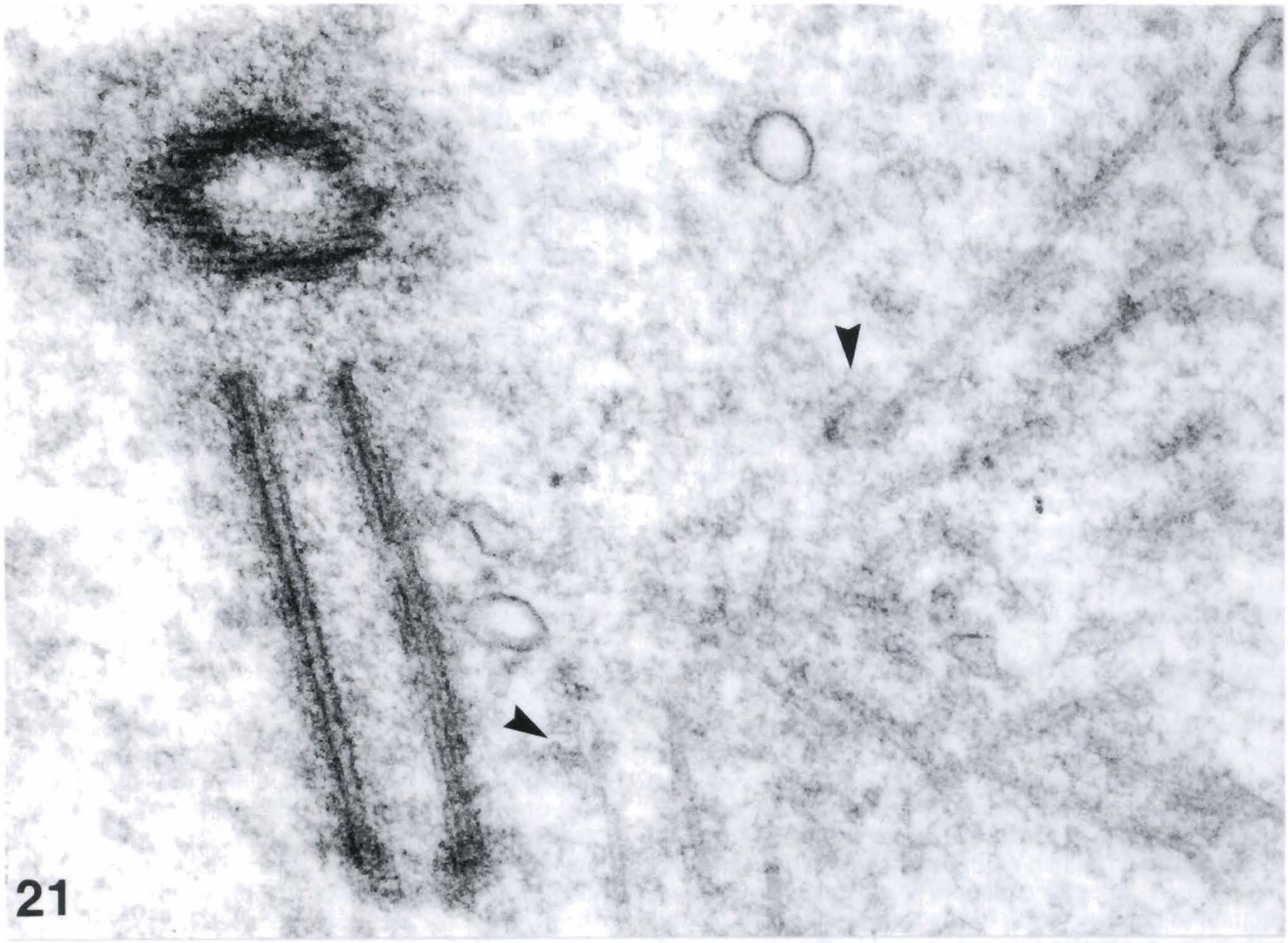
Typically, two centrioles, aligned at right angles to one another was surrounded by PCM nucleating MT. Each centriole was well-defined and 0.2 micron in diameter and up to 0.7 micron in length and with the surrounding PCM measured about 0.4 micron in diameter, making it more visible. Centrioles presented the typical pin-wheel structure in cross section (Figs. 21, 31, 41) exhibiting the '9+0' organization of MT triplets, while the PCM was finely granular and moderately osmiophilic, conforming to centrosomal substance (Mazia, 1987). Centrioles were often sectioned obliquely, though classical longitudinal and transverse sections were occasionally encountered in embryos (Figs. 21, 23, 41).

Boveri (1901) theorized that the centrosome is a self-duplicating cyclical organelle. The replication of centrioles has been observed in the human embryos mostly at interphase, beginning at the pronuclear stage prior to syngamy and at later embryonic interphases. In replication, the daughter centriole often arises as a perpendicular growth of the parent (see Fawcett, 1981)

Fig. 21. Sperm aster at one spindle pole at prometaphase (Fig.18) showing duplicated centrioles. The parent is cut transversely with dense PCM, while the daughter is in longitudinal section with less PCM, which is unevenly distributed. The astral MT terminate in dense specks of PCM (arrows). x 85,000. (Reproduced from Sathananthan et al., 1996).

Fig. 22. Tripolar spindle in a dispermic ovum at syngamy. Two procentrioles are located at one pole (arrow). Chromosomes seem to be separating toward poles. x 6,000. (Reproduced from Sathananthan et al., 1991a).

Fig. 23. The two procentrioles (diplosomes) in figure 22 aligned at right angles to one another masked by dense PCM. Microtubules are barely visible in the procentrioles. x 65,000. (Reproduced from Sathananthan et al., 1991a).



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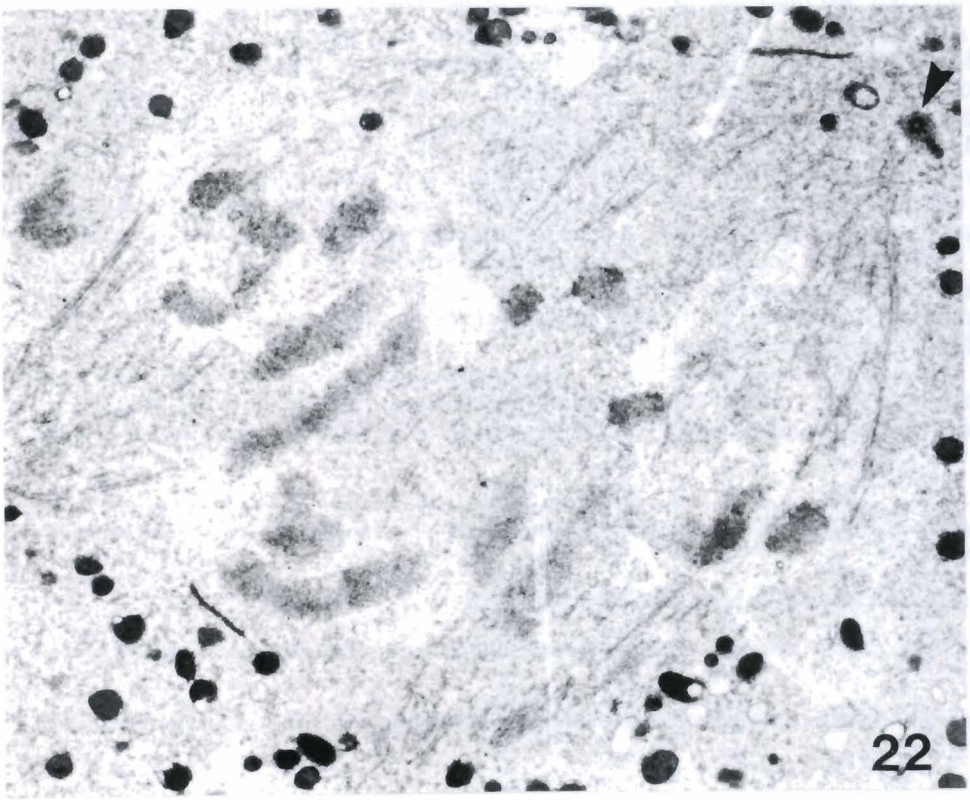

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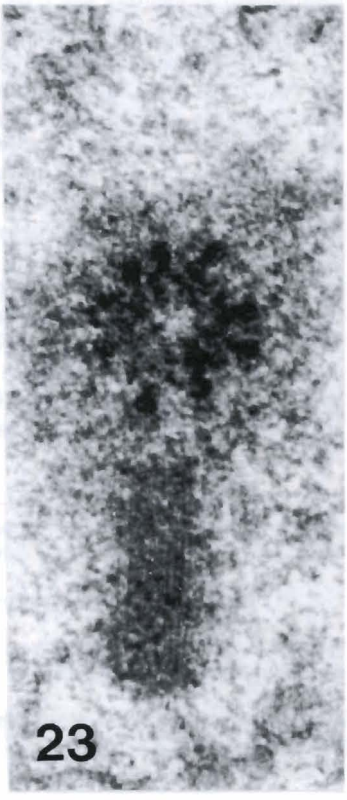

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and is seen to progressively grow and acquire PCM as they migrate towards the poles of the mitotic spindle (Figs. 21, 37-39). Immature centrioles have less centrosomal material and do not nucleate MT (Stubbfield, 1968; Rieder and Borisy, 1982). There is also dense material within each centriole similar to that seen outside it, as was also seen within the sperm neck centriole. However, figure 23 shows a new centriole at a tripolar spindle pole at syngamy, which appeared as an annular condensation of dense material devoid of MT (Sathananthan et al., 1991a). These, referred to as procentrioles, elongate by accretion of tubulin material to its free end and the pin-wheel arrangement of MT triplets gradually appears in this dense ring (Fawcett, 1981; Bray, 1992). Classically, in cell division, two diplosomes (2 double centrioles) are formed from an original pair of centrioles and take up positions at opposite poles of the mitotic spindle (Sathananthan et al., 1996). We have observed two centrioles, sometimes one, at a spindle pole at syngamy and at later cleavage stages and in two instances a single centriole at the opposite pole. It is possible that due to technical difficulties outlined earlier we are not detecting one of the pair of centrioles at each pole. However, we found three centrioles at one bipolar spindle pole in a bovine embryo (Sathananthan et al., 1997b), strongly indicating replication of diplosomes in mitosis. One has to be very fortunate to see all four polar centrioles in a single section by TEM. It is likely that centriolar duplication occurs in early human embryonic cells very much like that seen in somatic cells and diplosomes so formed migrate to opposite poles of the spindle (Fig. 58). These embryonic centrioles are very probably the ancestors of those found in foetal and adult somatic cell.

This behaviour of centrioles in centrosomes during the formation of a bipolar mitotic figure (bipolarization) is remarkably similar to what occurs in the cell cycles of somatic cells as reviewed by Mazia (1987); Paweletz and Mazia (1989) and is not very different from the early pictures visualized by Wilson (1895). A similar but not identical picture emerges in early sea urchin development (Paweletz et al., 1987a,b). The sea urchin sperm has two functional sperm centrosomes, spermhead centrosome and mitochondrial centrosome, both associated with centrioles. These separate to opposite poles of the bipolar spindle during the first mitosis (bipolarization), which has been elegantly traced by TEM. The human sperm, however, has probably only one functional centriole, the proximal, located in the 'black box' and is surrounded by PCM. Evidently it gives rise to the initial sperm aster. The inheritance of the 'black box' containing the proximal centriole was clearly shown earlier (Sathananthan et al., 1991a) at the pronuclear stage and is also shown in figure 5. Recently, we have also detected the 'black box' containing the proximal centriole associated with the male pronucleus in the bovine embryo (unpublished data). Hence there is little doubt that the proximal centriole is still functional in the embryo. The most salient finding in humans is the replication of the sperm centriole prior to syngamy and the relocation of diplosomes to opposite poles of a bipolar spindle at syngamy (Figs. 28-32, 58), which establishes bipolarization and the definitive role of the sperm centrosome in the organization of the first mitotic spindle. The detection of separate centrioles at two adjacent poles of tripolar spindles at syngamy (Sathananthan et al., 1996) further confirms the pivotal role of the sperm centrosome in cell division (tripolarization). Paternally derived centrosomes and tripolar spindles were also reported in the sea urchin embryo (Paweletz and Mazia, 1989). It is very likely that centrioles in the human embryo originated from two sperm at fertilization, since the embryo arose by dispermy. Further, two sperm asters were found in dispermic embryos (Fig. 20), which was also reported in dispermic embryos by immunofluorescence, whilst multiple sperm asters were found in polyspermic embryos (Simerly et al., 1995). However, we were not able to locate diplosomes (double centrioles) at each of these poles, nor the centrosome at the third pole, as in the case of the bipolar spindle at syngamy. It appears that two sperm centrosomes have been active in the tripolar spindle, which will permit one cell to divide into three cells, as reported by Kola and Trounson (1989). Supernumerary centrioles seen on sides of bipolar spindles in 1-cell and 8-cell dispermic embryos (Figs. 29, 45) very likely represent sperm asters, since they nucleate MT from their PCM. It appers that these sperm centrosomes are not actively involved in the mitotic process (bipolarization).

Functions of sperm centrioles

The functions of centrioles per se is a much debated topic in cell biology (Wheatley, 1982). It still remains a central enigma as to its role in cell division, since it is

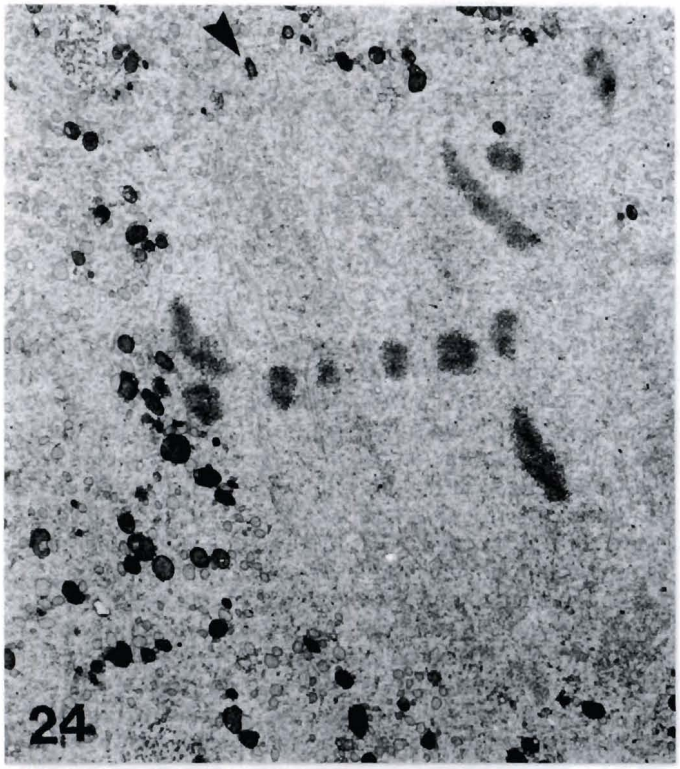
Fig. 24. Typical bipolar spindle at metaphase of syngamy developed from a dispermic ovum. A sperm centriole is seen at one pole (arrow). A chromosome is outside the spindle zone. x 5,100

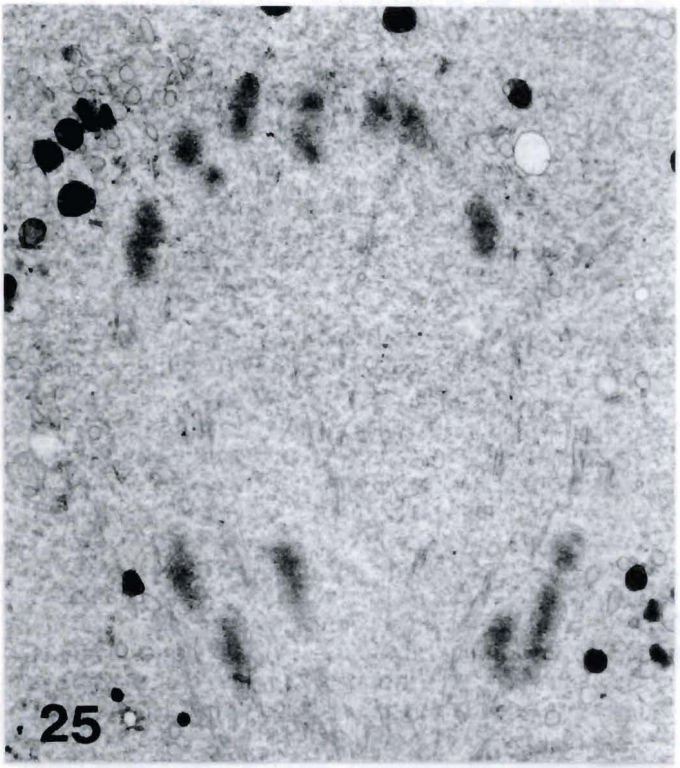
Fig. 25. Bipolar spindle with chromosomes at anaphase derived from a dispermic ovum. x 6,800

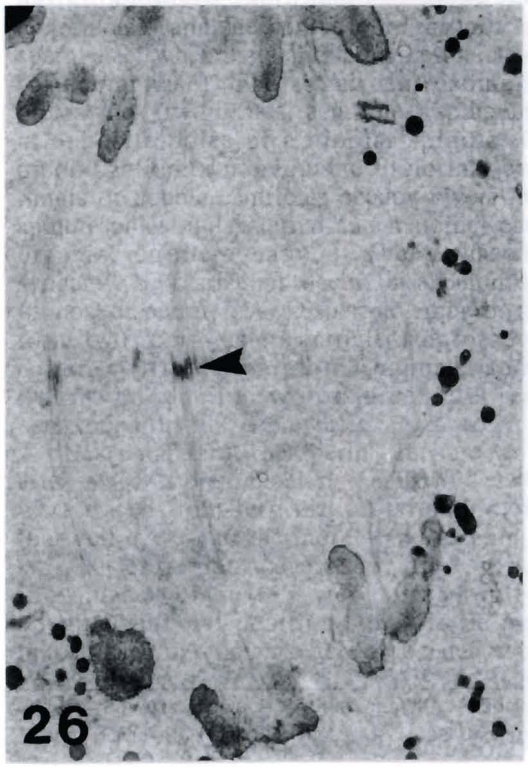
Fig. 26. Bipolar spindle derived from a normal 2PN ovum at early telophase. Nuclear envelopes are developing around each chromosome. Note interbody at equator (arrow). x 5,100

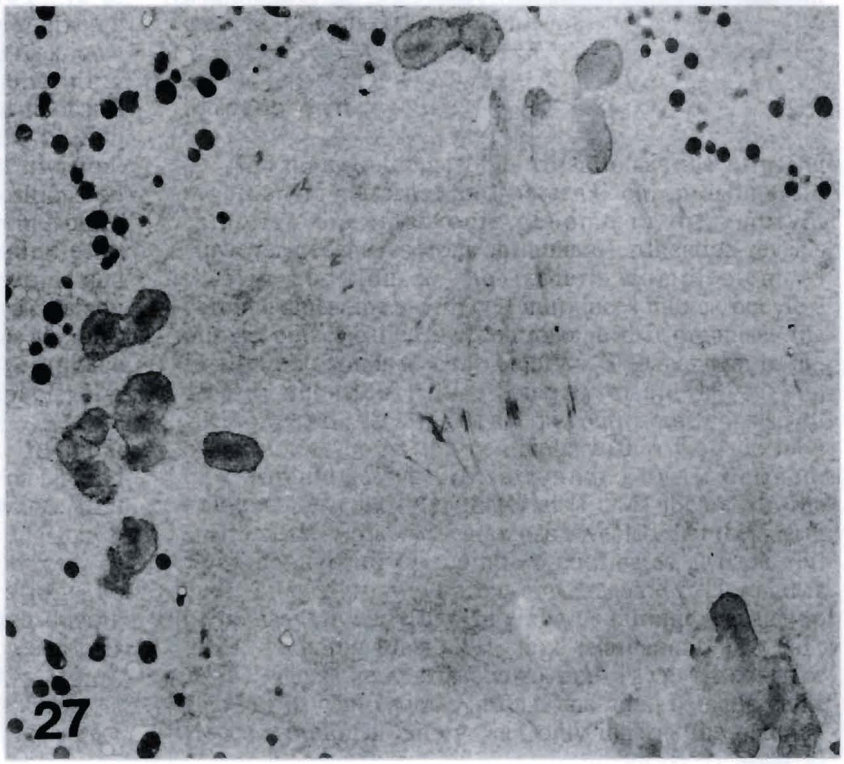
Fig. 27. Tripolar spindle developed from a 3PN dispermic ovum at early telophase (see figure 16c). The chromosomes and interbody are similar to that seen in figure 26. x 5,100

now universally accepted that the PCM (centrosomal material) is the chief MTOC (see Kalnins, 1992; Glover et al., 1993). The question that needs to be answered is what are the functions of the centriole in embryonic cell cycles? Surely, it is futile to think that it's doing nothing, sitting prominently at spindle poles during each cell









division and duplicating regularly at each interphase. It is clearly seen to form the core or nucleus for the dense centrosomal substance (PCM). One of the functions attributed to centrioles is its possible role in centrosome reproduction (Sluder and Rieder, 1985), which is clearly the impression gained by our observations on centriole replication in embryos. Not only does the centriole replicate at each cell cycle together with the PCM, in synchrony with the chromosome cycle, it also serves as a template or cytoskeleton to progressively condense centrosomal material to surround both centrioles (diplosomes). This is best seen in the growth of daughter centrioles after replication (Fig. 21). It must also be realised that PCM is rather diffuse, forming satellites in the vicinity of centrioles. Mazia (1987) believed that centrioles, when present, serve as useful advertisements of centrosomes and help in their detection. This is very true and the TEM is an invaluable tool to do this detective work. He also thinks that the centriole is a passenger and a dispensable one at that. This is not the impression gained in early human development for the reasons given above. Occasionally, spindle MT have been seen to extend right up to the centriole and this relationship has also been reported in somatic cells (Fawcett, 1981). An analogous situation where centrioles (basal bodies) nucleate MT in the absence of centrosomes exists in cilia (see Bray, 1992). If we further accept that there is a blending of paternal and maternal centrosomes after fertilization around the centriole, then its presence is vital at fertilization and early embryonic cleavage. After all, inheritance of the sperm centriole initiates normal development. Above all, the primary and most important function of the sperm centriole seems to be the introduction of the sperm centrosome intact into the oocyte to organize the sperm aster. Thus, the sperm centrosome incorporating centrioles seems to be the mechanism for handling over of the mitotic potential to the developing embryo at fertilization from one generation to another and the centriole is evidently the prominent organelle involved in this transmission. This seems to be the case in most animals, which show paternal centro-somal inheritance with concomittant inactivation or reduction of the maternal centrosome (see Schatten, 1994). It is thought that only one centrosome, paternal or maternal, needs to be functional to ensure normal development (Palermo et al., 1994). The paternal centrosome seems to be the dominant centrosome in most animals, including humans. This review has attempted to throw some new light on the role of the centriole in cell division. Further, TEM studies on parthenogenetic, digynic and dispermic human embryos will help us elucidate the precise roles of paternal and maternal centrosomes in embryonic development. This would be greatly enhanced if we could use specific antibodies to identify these centrosomes at the ultrastructural level, which is a difficult task to accomplish with the technology now available.

Abnormal cleavage: possible mitotic aberrations

Human embryos constantly present several abnormal morphological features such as aberrant cleavage patterns, retarded or arrested development. These could arise after both normal and dispermic fertilization, which may be expressed as abnormal asters and spindles, abnormal nuclear configurations (micronuclei and multiple nuclei), uneven-sized blastomeres and cytoplasmic fragmentation. Many of these defects could be explained in terms of mitotic aberrations apart from suboptimal embryo culture conditions, which also could influence embryonic cleavage. Such defects have been documented in several studies (see Sundstrom et al., 1981; Sathananthan, 1984, 1990, 1991, 1993; Trounson and Sathananthan, 1984; Sathananthan et al., 1982, 1986a, 1990, 1993). Several groups have also shown a high incidence of chromosomal abnormalities, particularly aneuploidy, triploidy and mosaicism (see reviews Kola and Trounson, 1989; Fishel, 1993; Munne et al., 1994). Recently the focus has been on the centrosome/centriole complex, which has clinical implications in infertility (see clinical implications).

Morphologically, one of the earliest aberrations of cell division is the failure to abstrict a second polar body (digyny) after sperm penetration, which of course, is a meiotic maturation defect. Another is abnormal sperm incorporation after penetration, where sperm fail to undergo complete nuclear decondensation and form a normal vesicular male pronucleus. At the pronuclear stage (interphase of the first cycle), the male and female pronuclei may fail to fully incorporate chromatin during their formation (Sathananthan, 1990), leading to the origin of several micronuclei (Figs. 53, 54). Such embryos show late nuclear formation or produce smaller pronuclei compared to normal pronuclear ova.

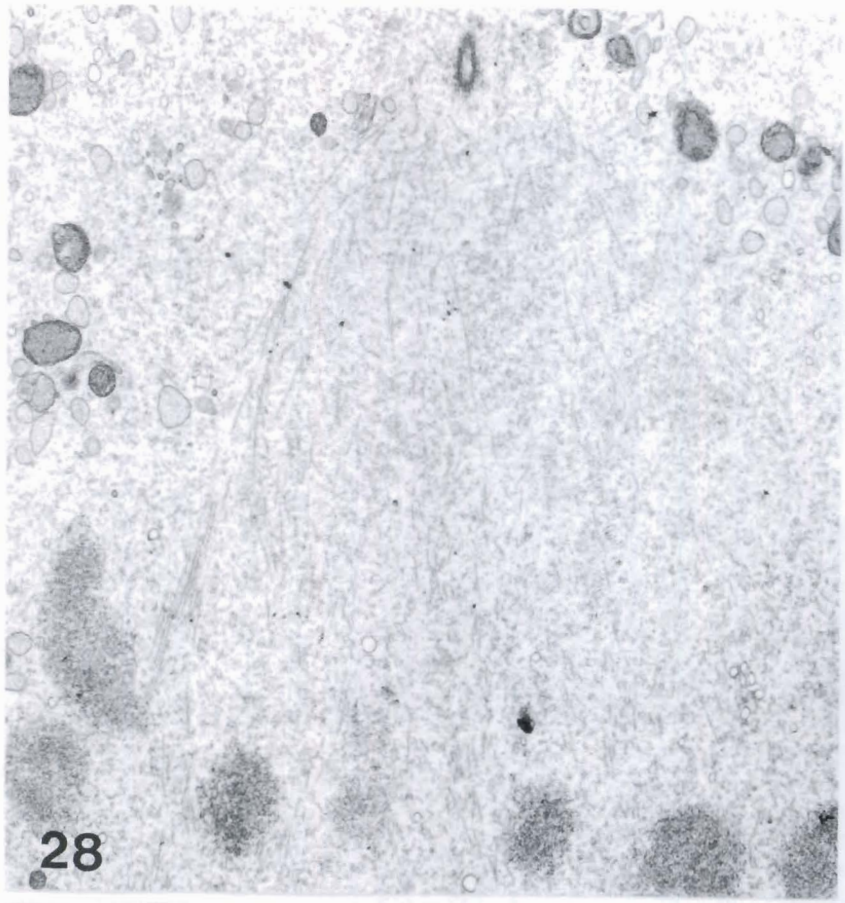
Fig. 28. Upper half of the bipolar spindle shown in figure 24. The centriole is cut obliquely at the pole. x 12,750

Fig. 29. Other centriole at same pole cut transversely in a serial section. x 1,2750. **Inset:** Two side centrioles of the same spindle associated with microtubules. x 34,000

Fig. 30. Serial section of the centriole seen in figure 28. Note microtubules associated with PCM. x 34,000

Fig. 31. Centriole in figure 29 showing the pinwheel arrangement of microtubule (MT) triplets and a branching MT extending right upto the centriole (arrow). Note osmiophilic centrosomal material outside and within centriole. x 170,000

Fig. 32. Centriole in transverse section (arrow) at opposite pole of bipolar spindle (Fig.28). x 12,750. (Figs. 28-32 Reproduced from Sathananthan et al., 1996).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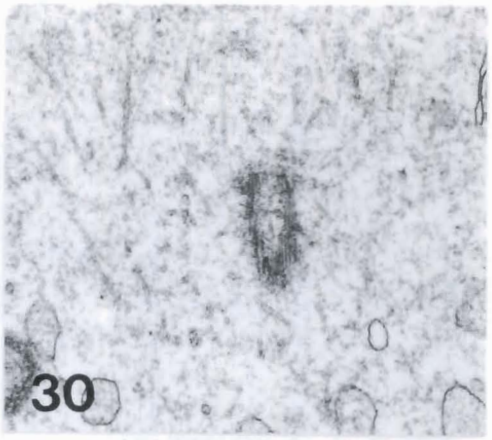
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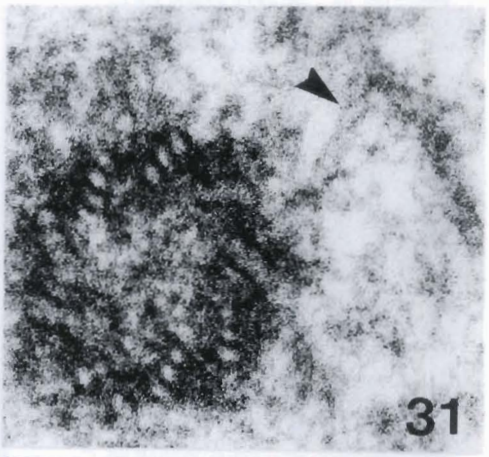
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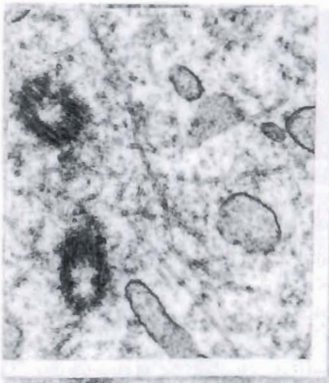

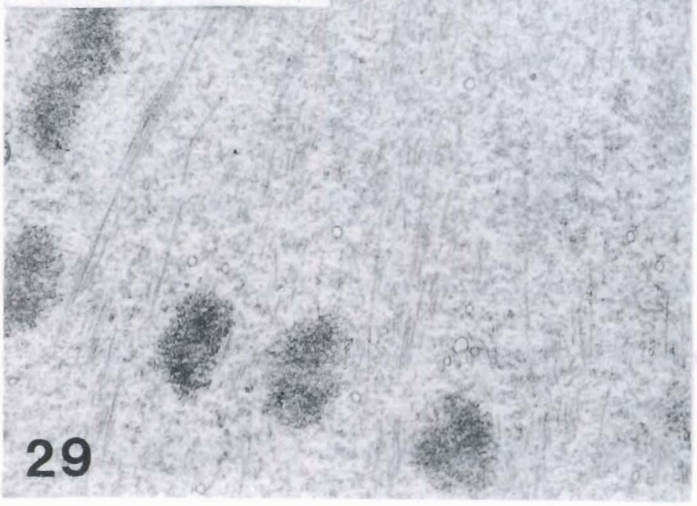
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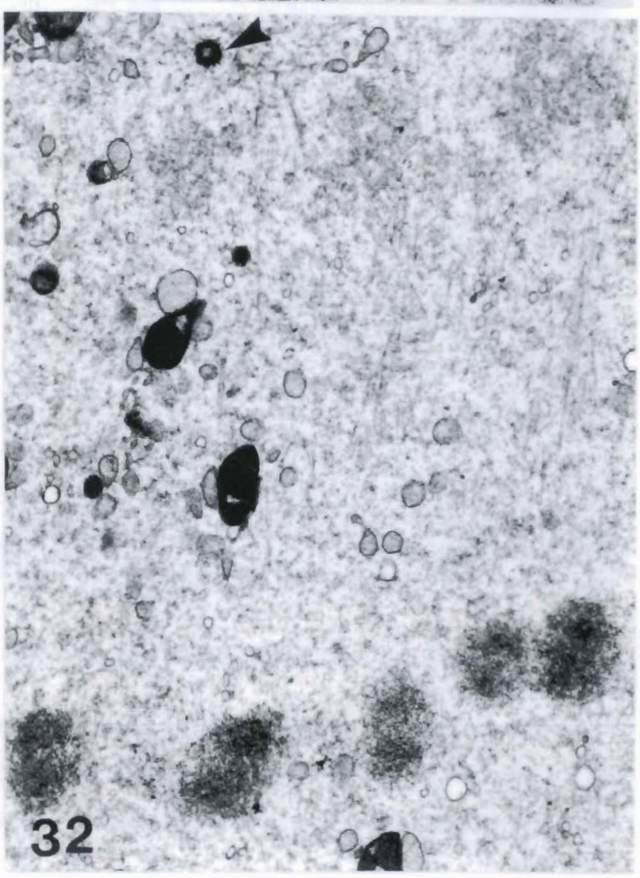
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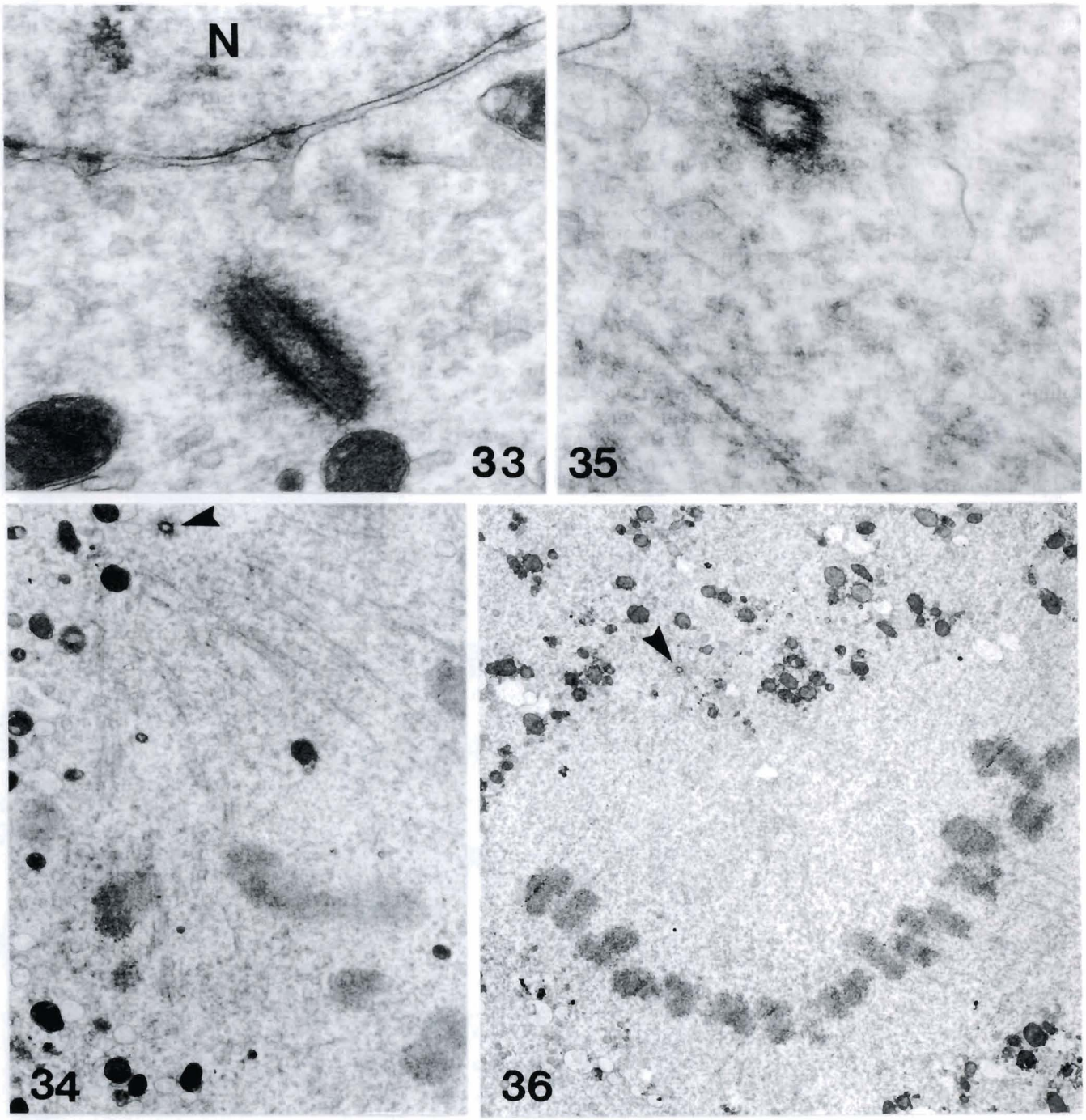


Fig. 33. Two-cell blastomere with a centriole cut longitudinally located close to its nucleus (N). Note dense PCM. x 42,500. (Reproduced from Sathananthan et al., 1996).

Fig. 34. Two-cell blastomere with a centriole (arrow) at one pole of a bipolar spindle. x 8,500. (Reproduced from Sathananthan et al., 1996).

Fig. 35. Centriole (Fig. 34) at higher magnification associated with MT. x 34,000

Fig. 36. Uneven 2-cell dispermic embryo showing a centriole (arrow) and an array of chromosomes. This spindle was probably tripolar. x 5,100

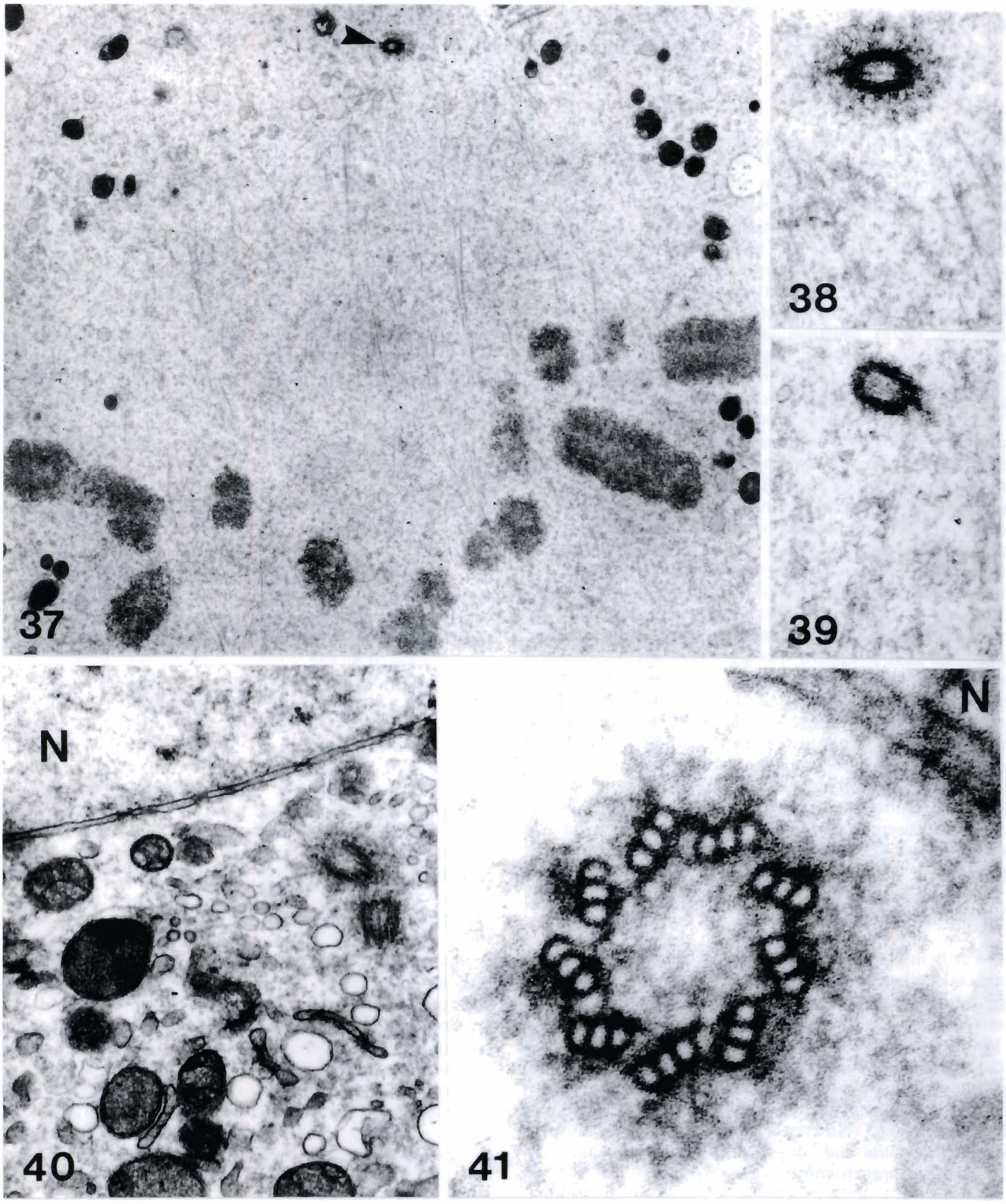


Fig. 37. Four-cell blastomere with a centriole (arrow) prominently located at one pole of a bipolar spindle. The chromosomes are in metaphase. x 8,500. (Reproduced from Sathananthan et al., 1996).

Figs. 38 and 39. Serial sections of same centriole (Fig. 37). Note halo of PCM in one and not in the other. x 34,000. (Reproduced from Sathananthan et al., 1996).

Fig. 40. Five-cell blastomere at interphase. Diplosomes (double centrioles) are located adjacent to nucleus (N). x 34,000. (Reproduced from Sathananthan et al., 1996).

Fig. 41. Centriole of a 8-cell blastomere in perfect transverse section, located close to its nucleus (N). Note characteristic ring of MT triplets associated with moderately dense centrosomal material both around and within the centriole. x 255,000. (Reproduced from Sathananthan et al., 1996).

Micronuclei could form in blastomeres at every stage of embryonic cleavage (2-cell, 4-cell, 8-cell) in both normal and dispermic embryos. Micronuclei are good indicators of aberrant mitosis and the blastomere nuclei may have chromosome aberrations (aneuploidy) and the embryo formed could be a mosaic of normal and abnormal cells. Another common defect in earlier embryos is the presence of 2 or 3 large nuclei (Figs. 55, 56) in a blastomere (see Sundstrom et al., 1981; Sathananthan et al., 1990, 1993). Multinucleated embryos usually have enucleated cytoplasmic fragments, as well (Fig. 57). These have been documented in several studies and appear in normal as well as retarted or arrested embryos. The degree of fragmentation is a useful indicator of embryo viability and is used to grade embryos in IVF laboratories (Trounson and Osborn, 1993). While fragments are visible, nuclear aberrations cannot be detected in living embryos in IVF laboratories. Another variation is the size of blastomeres in some embryos. If there is an eccentric spindle one might expect unequal blastomeres after cytokinesis. Embryos with equal blastomeres during the first four cell cycles are normally regarded as been good. However, cleavage may be asynchronous leading to unequal sized blastomeres. The speed of cell division during cleavage is also an important criterion in assessing embryos for embryo transfer in IVF. Embryos that are retarded in mitosis or arrested at some stage of cleavage are normally considered not suitable for transfer. For a review of embryo assessment in assisted reproduction see Trounson and Osborn (1993). Many of these abnormal aspects of embryo behaviour could be finally attributed to mitotic aberrations during cleavage and ultimately to centrosomes and centrioles that organize the mitotic apparatus.

Parthenogenesis

Parthenogenesis or 'virgin birth' may be natural or artificial and occurs in many animal species, when the egg initiates full development and results in a sexually mature adult (see Kaufmann, 1983; Schatten, 1994). This also occurs in mice, which have a dominant maternal centrosome (Schatten et al., 1991). Artificial parthenogenesis could be induced in human eggs after Ca^{2+}-ionophore activation, when they divide upto about the 8-cell stage (Winston et al., 1991). Later cleavage becomes irregular and abnormal. It seems plausible that the maternal centrosome is reactivated by activating agents such as Ca^{2+}-ionophore inducing the maternal centrosome to form MTOC. This was also speculated by Paweletz and Mazia (1989), although artificial parthenogenesis is a rare occurrence in the sea urchin (Schatten, 1994). Clearly we need to investigate parthenogenetic activation and also digyny in human oocytes by TEM to determine the mechanics of cleavage and contribution of maternal centrosomes, in the absence of the sperm centrosome. This would be greatly enhanced if we could use specific antibodies to identify maternal centrosomes at the ultrastructural level. It is also necessary to investigate the fate of single pronuclear eggs occasionally encountered in human IVF. Many of these oocytes have one polar body and arrest at the PN stage. We are now investigating parthenogenesis by TEM in a separate study.

Sperm centrosomes/centrioles and infertility: Clinical implications

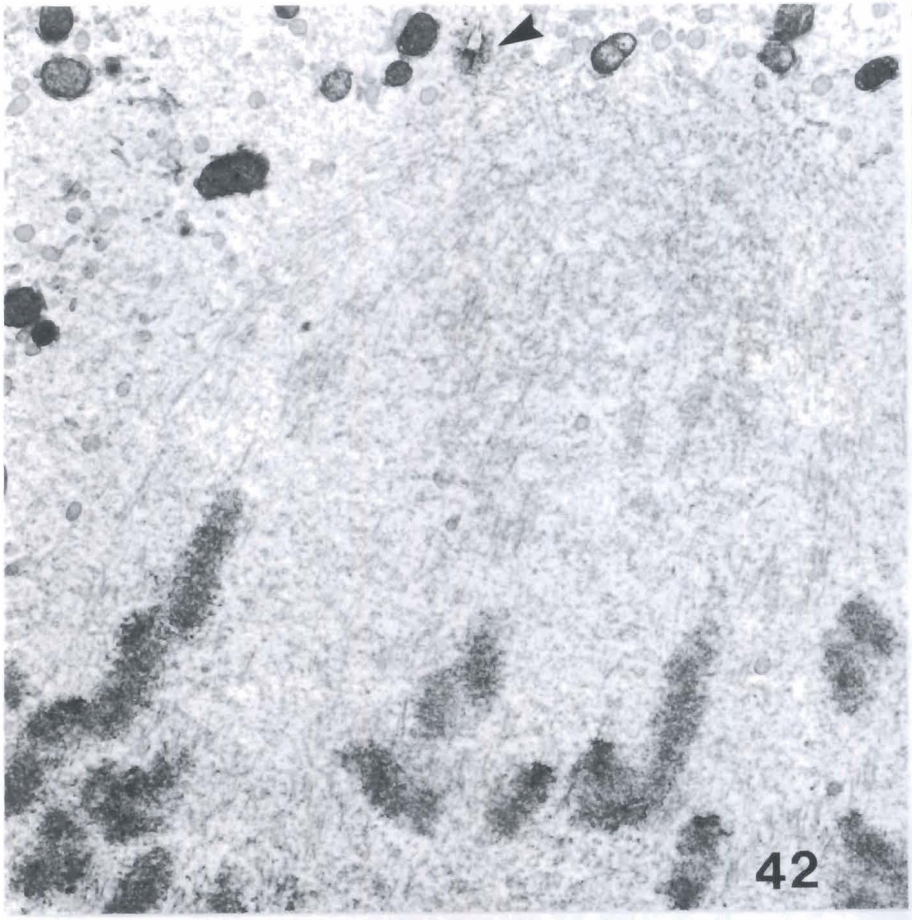
Continuing TEM studies on washed sperm pellets from men with immotile sperm with no forward progression, poorly motile and highly motile sperm show that there are more centriolar defects in immotile sperm than in motile samples, which led us to postulate a new hypothesis on male infertility (Sathananthan, 1994b, 1996a,b). This hypothesis was first presented at the XIII World Congress in Obstetrics and Gynaecology, Singapore (Sathananthan, 1991) and later at the 51st Symposium of the Society of Developmental Biology, USA, in Seattle, Washington (Sathananthan, 1992). According to this hypothesis, if a defective sperm centriole/centrosome is inherited by an embryo at fertilization it may lead to abnormal cleavage and produce aberrant embryos, a new dimension in the assessment of male infertility. One of the first to implicate the sperm centriole in retarded development was Edwards (1956a,b), where mouse sperm were irradiated by X-rays or ultra-violet light in vitro, prior to fertilization. The sperm proximal centriole is believed to be functional and the kinetic center of sperm motility (Zamboni and Stefanini, 1970), while the distal centriole which gave rise to the sperm tail during spermiogenesis persists as a remnant or in a modified form in ejaculated sperm (see Holstein and Roosen-Runge, 1981; de Kretser and Kerr, 1994). The significance of centriolar defects is being evaluated by TEM in both ejaculated sperm and testicular biopsies, since both proximal and

Fig. 42. Eight-cell blastomere at metaphase with a centriole (arrow), at one pole of a bipolar spindle. The spindle is cut slightly oblique. x 10,500. (Reproduced from Sathananthan et al., 1996).

Fig. 43. Centriole in figure 42 at higher magnification, apparently sectioned obliquely at the junction between proximal centriole (above) and axoneme (below). Note dense PCM around centriolar complex and spindle MT (arrow). x 105,000. (Reproduced from Sathananthan et al., 1996).

Fig. 44. Dispermic 8-cell blastomere showing two separated centrioles (arrows) at one pole of an anaphase spindle. C: chromosome. x 34,000

Fig. 45. Three supernumerary centrioles on one side of the anaphase spindle (Fig. 44), close to the same pole. x 34,000. (Both reproduced from Sathananthan, 1997).



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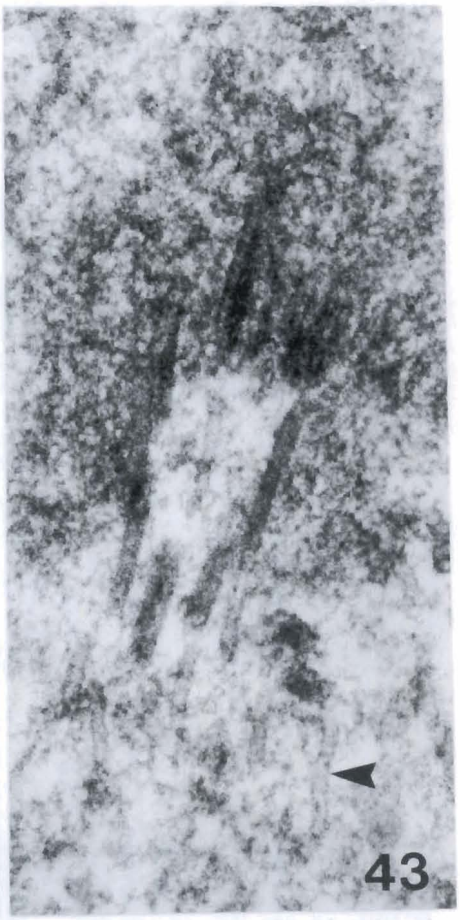

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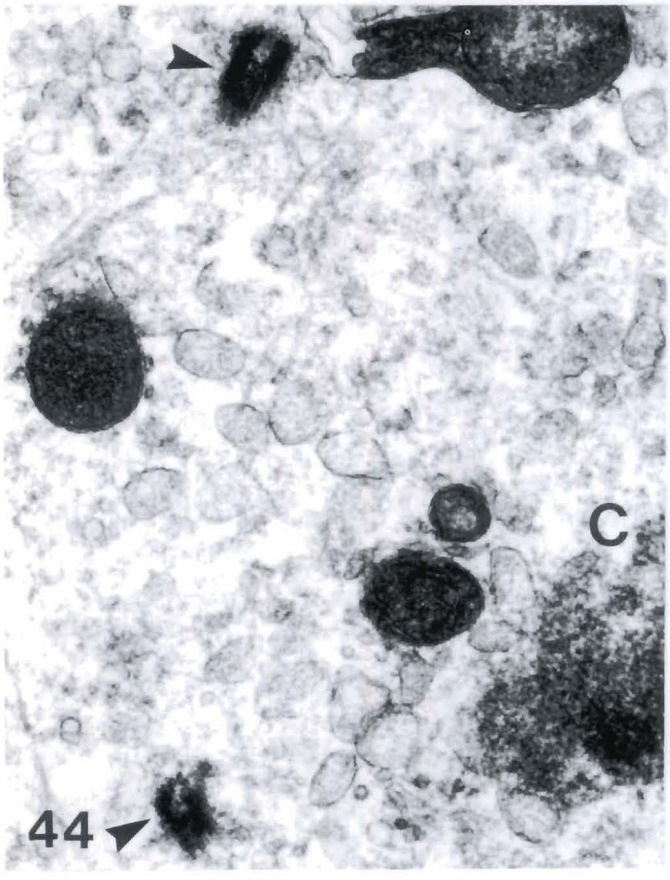

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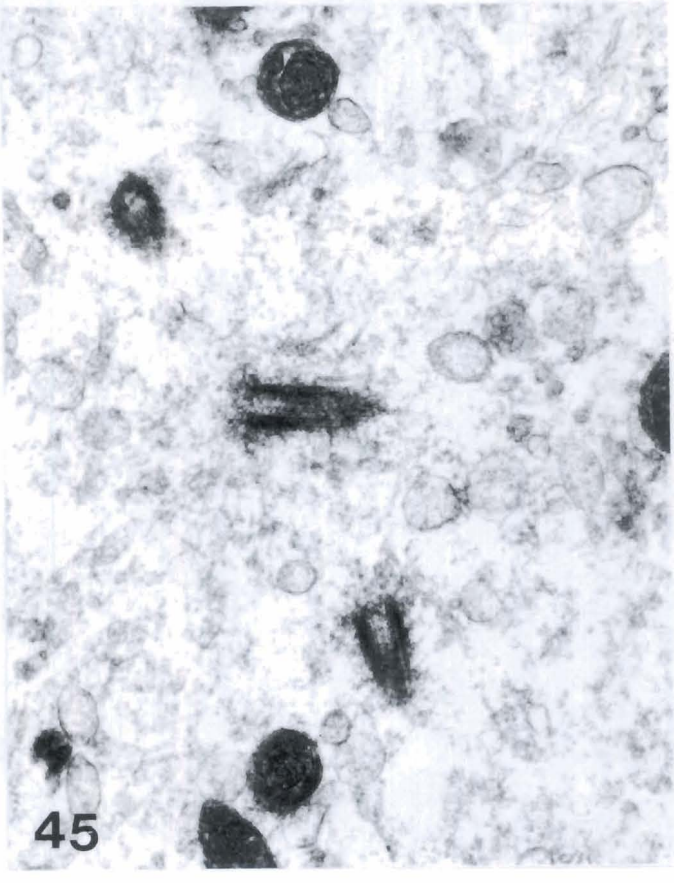

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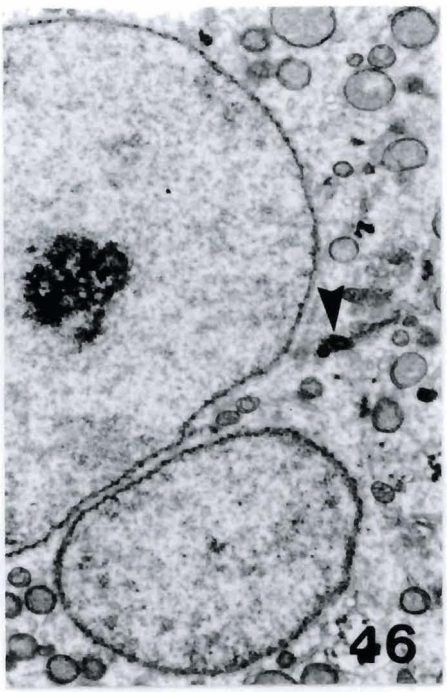
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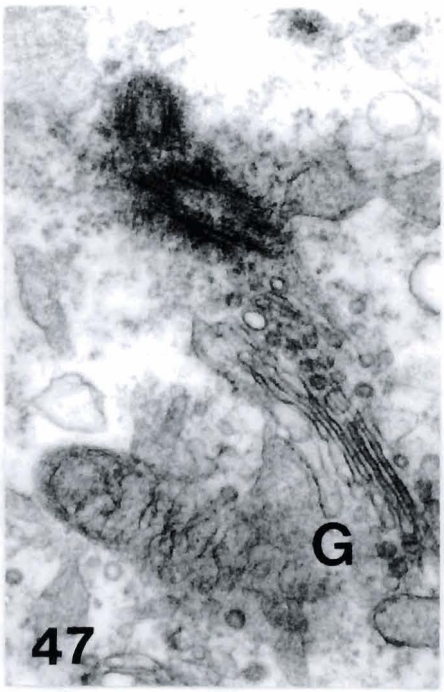
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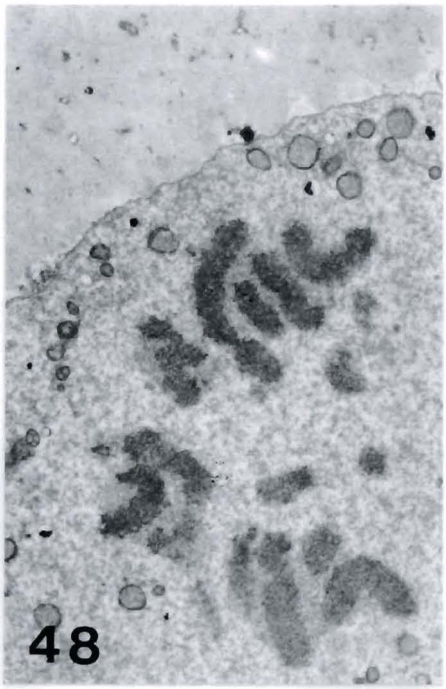
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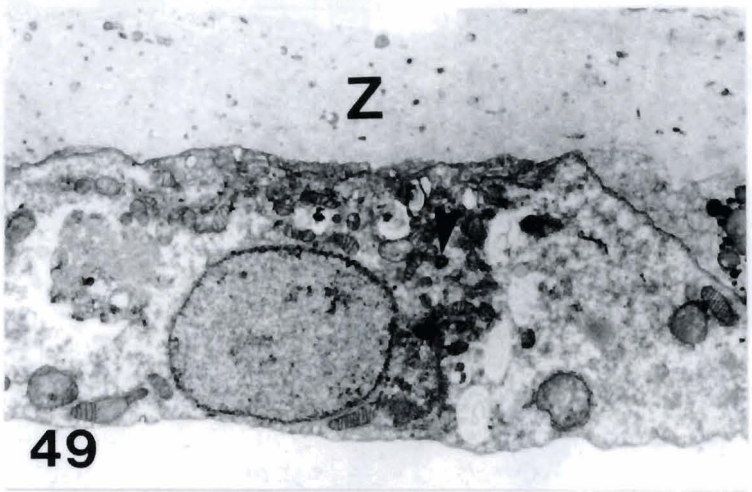
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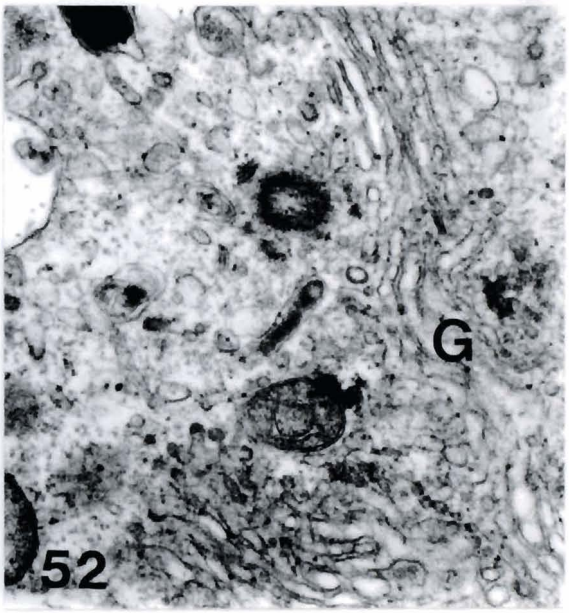
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Fig. 46. Binucleate inner cell of a morula showing duplicating centrioles between nuclei (arrow). x 6,800. (Reproduced from Sathananthan et al., 1996).

Fig. 47. Centrioles closely associated with Golgi membranes (G). x 34,000. (Reproduced from Sathananthan et al., 1996).

Fig. 48. Outer cell of a morula showing chromosomes at metaphase. x 5,100

Fig. 49. Trophoblast cell of a hatching blastocyst with a centriole (arrow) situated near nucleus. Z: zona pellucida. x 5,100

Fig. 50. Centriole in figure 49 at higher magnification. x 34,000. (Both reproduced from Sathananthan et al., 1996).

Fig. 51. Embryoblast cell of a late blastocyst with a centriole (arrow) near nucleus associated with Golgi membranes (G). x 5,100

Fig. 52. Centriole in figure 51 at higher magnification and Golgi membranes (G). x 34,000. (Both reproduced from Sathananthan et al., 1996).

distal centrioles originated from parent centrioles in spermatids, which were active during spermiogenesis. Centriolar MT structure is difficult to assess both in sperm and in embryos, where they are often sectioned obliquely. Centriolar defects in poor quality sperm include total loss of centrioles or MT triplets (half centrioles) or disorganization of triplets and variation of PCM, which is harder to assess (Sathananthan, 1992, 1996a,b). An interesting parallel is the aberrant organization of MT in basal bodies of tracheal cilia in respiratory disorders such as in immotile cilia syndrome (Lungarella et al., 1985). A human model to investigate centriolar dysfunction in both sperm and cilia could be immotile cilia syndrome (Palmblad et al., 1984), where abnormalities of sperm tail axonemes are also common in addition to lack of dynein arms. Several reports, however, are in the literature on axonemal defects of the sperm flagellum in the infertile male (Hancock and de Kretser,1992). Since the sperm centriole and the surrounding PCM form an integral unit, as revealed by both TEM and its molecular architecture (Schatten, 1994; Zoran et al.,1994), defects of centriolar function could be attributed to either or both of these components and hence the sperm centrosome as a whole should be responsible to organize the mitotic apparatus in the zygote. This becomes further complicated, if we accept the hypothesis that there is a blending of paternal and maternal centrosomes in the zygote at syngamy (Schatten, 1994; Sathananthan et al., 1996). There is some FM evidence in human oocytes that fail to fertilize in vitro, of defective centrosomes, sperm asters, aster detachment from spermheads and arrested sperm incorporation, even though the eggs were penetrated (Asch et al., 1995). Images of defective sperm incorporation are also encountered in 'failed fertilized' oocytes by TEM (Van Blerkom, 1989; Sathananthan, 1990). Our TEM studies also show that many oocytes that show no pronuclei after IVF are arrested at syngamy when the first mitotic spindle is formed, but sperm aster size and centrosomes are difficult to assess by this method. Small sperm asters, detached sperm asters and defects in male chromatin decondensation have also been reported in rhesus 'fertilization failures' (Hewitson et al., 1996). There is also some evidence in cattle, where the centriolar complex is paternally inherited, that sperm aster size differs with sperm quality (superb, average, subfertile) influencing fertilization and live birth rates (Navara et al., 1994; Schatten, 1994). Thus there is increasing evidence to support our hypothesis of sperm centriolar/centrosomal involvement in embryonic development (Sathananthan, 1991, 1992, 1994b).

Future directions in centrosomal/centriolar research

The success of intracytoplasmic sperm injection (ICSI) as a viable technique for assisted reproduction will now enable us to test the hypothesis on the role of the sperm centriole/centrosome in male infertility. Indeed, there are clinical indications that fertilization and pregnancy rates are lower when sperm with zero motility are used for ICSI (Nagy et al., 1995). We can now inject a sperm with zero motility or one with no forward progression from severe male factor patients (oligo-asthenoteratospermia) and assess the outcome of fertilization and determine any arrest of embryonic development thereafter, since the sperm centriole is replicated and perpetuated in early human embryos (Sathananthan et al., 1996). This could also be

Figures 53-57. Abnormal nuclear configurations in early embryos.

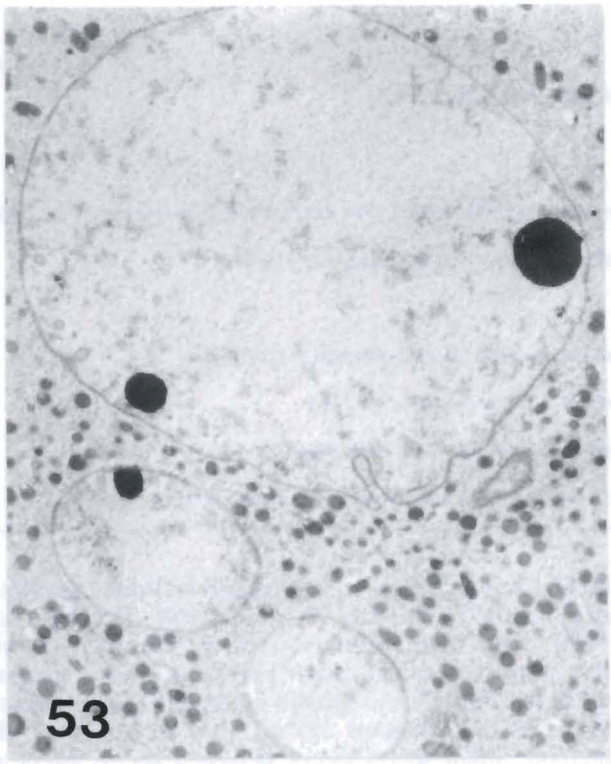
Fig. 53. Two micronuclei associated with a pronucleus of a bipronuclear ovum. x 4,250

Fig. 54. Micronuclei in a dispermic tripronuclear embryo. The nucleoli are dense and compact resembling those of the pronucleus (Fig. 53). x 5,100

Fig. 55. Binuclear 2-cell blastomere also showing dense compact nucleoli. x 4,900. (Reproduced from Sathananthan et al., 1993).

Fig. 56. Inner cell of a morula showing two nuclei with reticulated nucleoli. x 3,500

Fig. 57. Enucleated cytoplasmic fragments (F) in a 4-cell embryo with normal blastomeres. Note cellular debris (arrow). x 4,080. (Reproduced from Sathananthan et al., 1993).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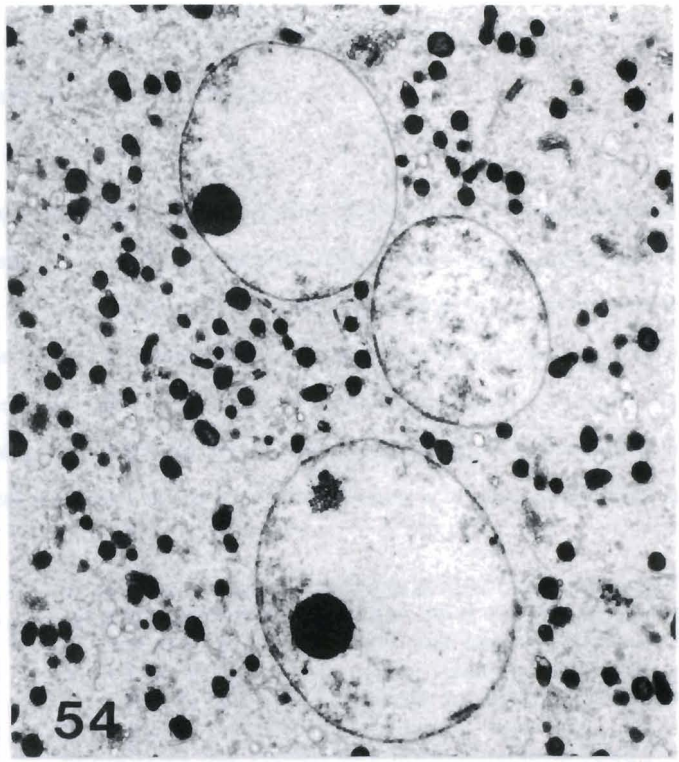
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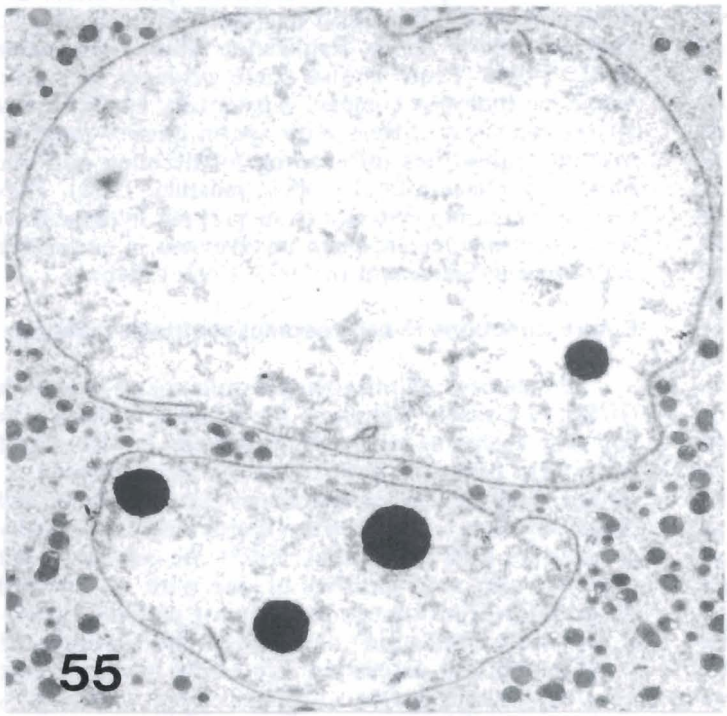
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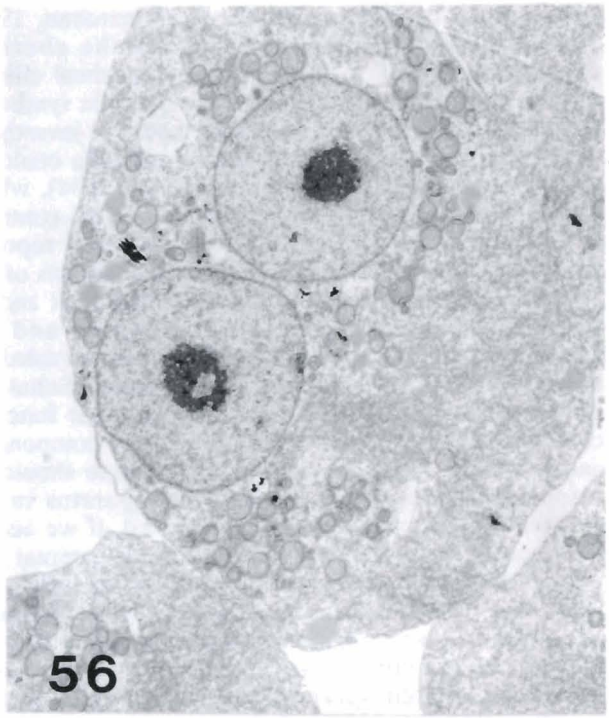
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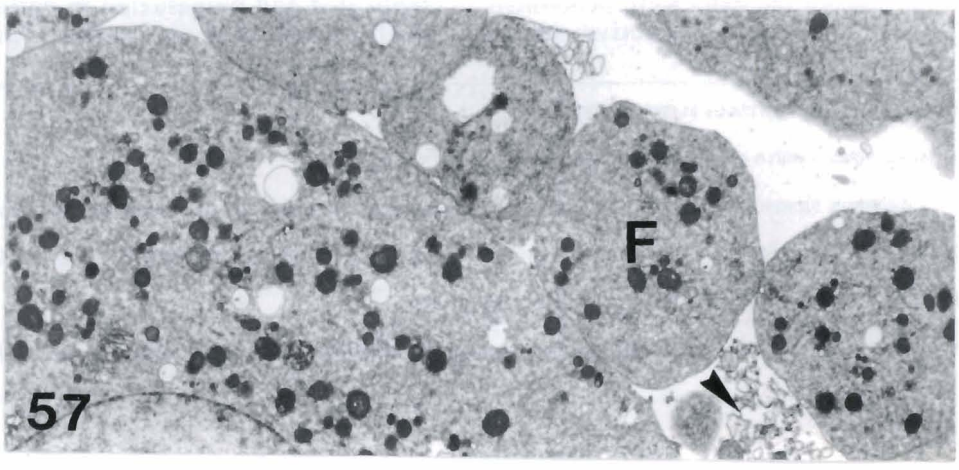
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applicable to the bovine model which closely follows the human pattern of centriolar inheritance and perpetuation in embryos (Sathananthan et al., 1997b). Bovine oocytes are easily obtained from ovaries from abattoirs and could be matured in vitro, fertilized and developed into viable embryos (Trounson, 1992). Hancock and de Kretser (1992) have shown significant axonemal defects in sperm of men with severe asthenospermia and this type of investigation could be extended to centriolar structure. Some caution needs to be exercised in the use of sperm with no motility or very low motility with no forward progression for ICSI, since it might prevent fertilization or compromise embryo development. If embryo development is retarted or arrested or if embryos cleave irregularly, it could be a good indicator of mitotic spindle aberrations very likely involving centriolar/centrosomal dysfunction. This could also lead to chromosomal and nuclear aberrations so common in early human embryos (Kola and Trounson, 1989; Munne et al., 1994). We envisage using the bovine model to complement our human work.

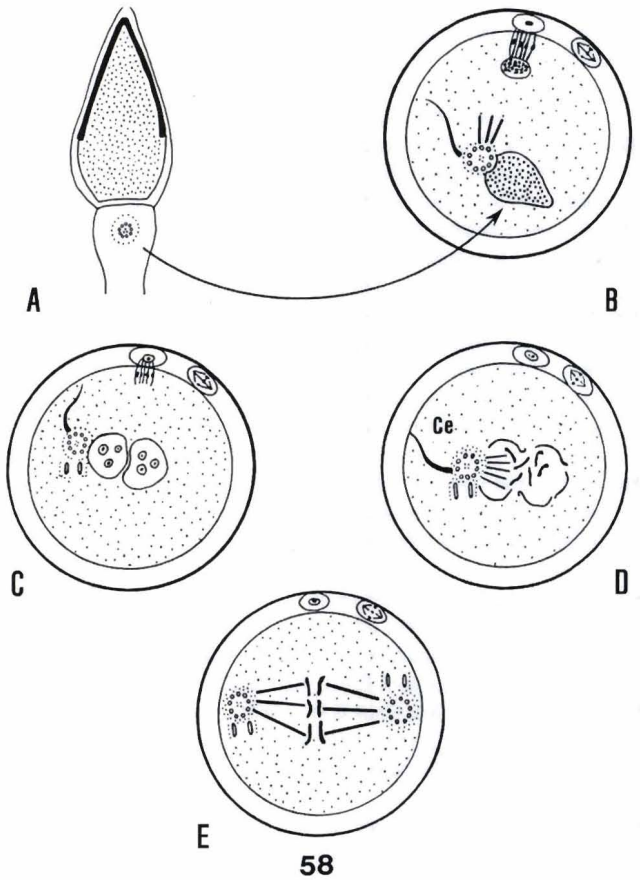

Fig. 58. Diagrams showing inheritance, duplication and relocation of the sperm centrosome (centriole) during human fertilization visualized by TEM. **A.** Sperm proximal centriole in the sperm neck, associated with PCM (fine dots). **B.** Sperm incorporated into the ooplasm at fertilization. The developing sperm aster is shown associated with the centrosome. (This sperm aster was not documented by TEM). Sperm chromatin is decondensing and the sperm midpiece and tail are attached. The second polar body is being abstricted into the perivitelline space beneath the zona pellucida, beside the first polar body and the female pronucleus is forming at the inner pole of the second maturation spindle. **C.** Duplicating sperm centriole associated with the male pronucleus at the bipronuclear stage, when male and female pronuclei are in close association. **D.** Sperm aster and centrosome with duplicated centrioles organizing one pole of the first mitotic spindle (prometaphase). The male and female pronuclear envelopes are disorganizing and the chromosomes are condensing. **E.** Bipolarization: The first bipolar mitotic spindle at metaphase of syngamy showing duplicated centrioles (diplosomes) at either pole. Note increase in PCM around centrioles (bipolarization). (Reproduced from Sathananthan et al., 1996).

Unresolved questions

The fate of distal centriole is unknown. Was it involved in sperm motility like the proximal centriole? Is it reactivated or does its MT contribute to the formation of the sperm aster soon after fertilization? Does the centriolar adjunct contribute to the sperm aster? What's the fate of the 'black box', sperm mitochondria and other components of the sperm flagellum? Further, when is the maternal centrosome inactivated in oogenesis? Is it at the germinal vesicle stage or at metaphase II; when meiotic arrests occur, or in between? Is the maternal centrosome reactivated at telophase II of meiotic maturation, when the second polar body is abstricted? Does the sperm centriole influence this maturation division, which occurs only after sperm entry? These questions must be answered to fully appreciate the respective roles of paternal and maternal centrosomes in human development.

In conclusion, oocyte activation (onset of embryonic development) in the true sense of the phrase should now precisely mean initiation of mitotic cleavage after the entry of the sperm centriole/centrosome into the egg, at fertilization. The other aspects of activation are completion of egg maturation and the cortical reaction that elicits a block to polyspermy (see Sathananthan et al., 1993). Hence activation now has a deeper meaning than mere onset of embryonic cleavage, since we know the real mechanics of initiation of somatic cell division in the human zygote.

Acknowledgements. Professors A. Trounson, D. de Kretser and S.S. Ratnam are specially commended for their continued encouragement in supporting fundamental research at Monash and Singapore universities. Dr. Y. Munesinghe and Ms C. Morgan assisted in the preparation of the manuscript. The review was funded by the National Health and Medical Research Council of Australia. This review is dedicated to the late Professor Daniel Mazia of Stanford University, who encouraged me to do centriolar research.

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