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The human sperm centrosome is responsible for normal syngamy and early embryonic development

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As early as 1887, it was postulated that the mature oocyte possesses all of the elements necessary for embryonic development with the exception of an active division centre, and that the spermatozoon contains such a centre, but lacks the substrate in which to operate. This division centre is called the centrosome. The precise definition of this structure is still a subject for debate. It consists of two centrioles in a perpendicular arrangement and pericentriolar material, and is considered to be responsible for nucleation of microtubules and the formation of the mitotic spindle. There is a paternal pattern of inheritance of the centrosome in humans; thus, human oocytes lack centrioles but the spermatozoa carry two. At gamete fusion the sperm tail is incorporated into the ooplasm, and the centriolar region forms the sperm aster while the sperm head is decondensing; this aster acts to guide the female pronucleus towards the male pronucleus. The centriole duplicates during the pronuclear stage, and at syngamy centrioles are found at opposite poles of the first cleavage. The centrosome has several implications for human infertility. It is possible that immotile or nonprogressively motile spermatozoa may possess centriolar abnormalities or an absence of centrioles. Similarly, antisperm antibodies against centrioles may be responsible for mitotic arrest. One way of solving this problem would be the use of donor centrosomes. To this end, we have assessed the ability of embryos injected with physically separated sperm segments (head only, head and tail separated or isolated tail) to develop normally. Fluorescent *in situ* hybridization revealed an almost universal mosaicism in these embryos, suggesting that physical disruption of the spermatozoa compromises the ability of the centrosome to function in the zygote. Thus far, centrosome donation with centriole-carrier flagellae obtained by this dissection method does not appear to be feasible.

The centrosome: division centre or enigma?

The study of cell replication has occupied scientists for centuries and one of the most intriguing aspects of cell division is the organized distribution of genetic material that accompanies it. Nowhere else is this mechanism so crucial as in the zygote. More than a century ago it was postulated that the mature oocyte possessed all of the elements necessary for embryonic development with the exception of an active 'division centre', and although the spermatozoa was believed to contain such a centre, it was thought to lack the protoplasmic substrate in which to operate (Boveri, 1887).

Whereas the chromosomes of somatic cells simply duplicate and then separate during division, each gamete must contain only one half of the diploid complement in order to ensure normal fertilization, restoration of diploidy, and subsequent embryonic development. The key structure in the organized or directed distribution of this genomic material is the mitotic spindle. This is generated by the centrosome, which determines the intrinsic polarity and orientation of microtubule assembly and so ensures an even distribution of chromosomes during division.

The definition of the term 'centrosome' has been somewhat vague for many scientists, as has the precise nature of this 'central body'. One of the first attempts to define the centrosome was made by Boveri (1887, 1901), who described it as a polar corpuscle containing centrioles. This description was later replaced by the more functional definition of a microtubule organizing centre (MTOC) (Pickett-Heaps *et al.*, 1984). A distinction must be made between the two terms, since the centrosome is necessarily a MTOC but a MTOC need not to be a centrosome because one can be generated independently from centriolar structures. In defining its function, Bornens *et al.* (1990) proposed that the centrosome be considered as the structure responsible for two basic events: the nucleation of microtubules and the formulation of an efficient mitotic spindle.

In this manuscript we discuss the various attempts to elucidate the definition of the centrosome, and we examine its composition and changes during the cell cycle, its role, its inheritance and its significance for human infertility.

Composition of the centrosome

Ultrastructure

In most cells, the centrosome comprises two morphologically distinct centrioles (a pair of cylinders arranged perpendicularly) and the pericentriolar material (PCM) from which the aster and spindle fibres are generated (Fig. 1). Centrioles are integral to the definition and identification of centrosomes,

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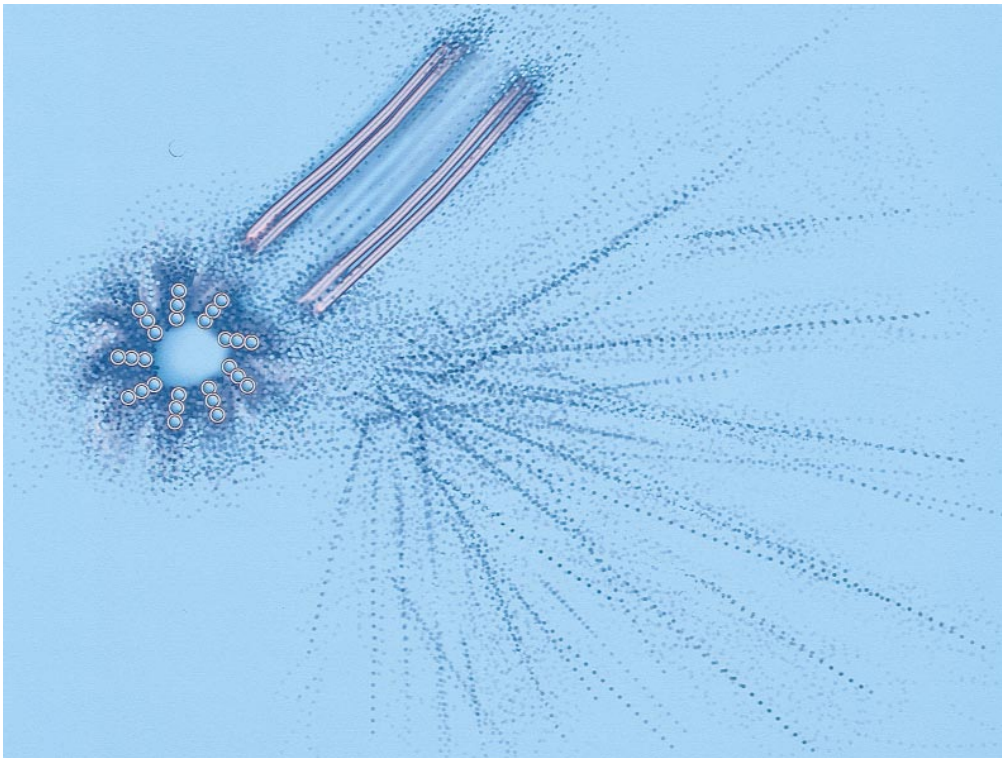

Fig. 1. Structural representation of centrioles in their characteristic spatial configuration.

since they do not seem to be present in the meiotic spindle of reproductive cells. They display the classic 9+0 pattern of nine triplet microtubules (without the central pair of microtubules characteristic of the axoneme) and they undergo a semiconservative duplication, that is, each daughter cell retains one of the mother's centrioles and a newly formed daughter centriole (Kochanski and Borisy, 1990). While earlier reports suggested that mammalian zygotes lack centrioles, there is now evidence that centrioles are present at the spindle poles during the first mitotic division in zygotes from various species (Le Guen and Crozet, 1989), including humans (Sathananthan *et al.*, 1991). There is little doubt that centrioles are indeed an essential component of the centrosomal structures of the cell, although the functional significance of these microstructures has yet to be fully elucidated.

While human oocytes apparently lack centriolar structures (Sathananthan *et al.*, 1991), human spermatozoa have two distinct centrioles. The proximal centriole is located within the connecting piece next to the basal plate of the sperm head, and has the pin-wheel structure of nine triplets of microtubules surrounded by electron dense material and flanked by nine cross-striated columns. The distal centriole is located perpendicular to the proximal centriole aligned with the axis of the flagellum, and this gives rise to the axoneme during spermiogenesis (Sathananthan *et al.*, 1991, 1996) (Fig. 2).

Molecular composition

An extensive list of proteins and antigens associated with the centrosome has been compiled (Kimble and Kuriyama, 1992; Kalt and Schliwa, 1993; Rose *et al.*, 1993); these have been identified through the use of specific antibodies against centrosomal components (Kuriyama, 1992).

The centrosome is most likely composed of a structural matrix (PCM) within which functional and regulatory molecules are embedded. These molecules can be divided into four different categories: components present in the centrosome at all times during the cell cycle; those detectable only during mitosis; those localized at the centrosomal site during mitosis and elsewhere in the cell during the rest of the cell cycle; and those acting as regulators (Kalt and Schliwa, 1993).

Centrosomal component

Cell cycle. The composition of the centrosome changes somewhat at different phases of the cell cycle. Among the proteins located exclusively in the centrosome throughout the entire cell cycle, γ-tubulin and the group of Ca^{2+}-modulated proteins typified by centrin have been studied in great detail. γ-Tubulin is the only centrosomal component known to be present in all species: it is situated around the centrioles and occupies an area in the pericentriolar material. γ-Tubulin plays an important role in the initiation of microtubule nucleation and is essential to centrosome function (Oakley *et al.*, 1990). However, although human sperm centrosomes have detectable concentrations of γ-tubulin, this protein is not apparent until centrosome priming and exposure of the spermatozoa to cell-free cytoplasmic extracts obtained from mature *Xenopus laevis* oocytes (Schatten, 1994; Zoran *et al.*, 1994). At a functional concentration, the γ-tubulin component initiates the assembly of the microtubules for the sperm aster and attracts the additional maternal γ-tubulin needed for enlargement of the sperm aster and association with

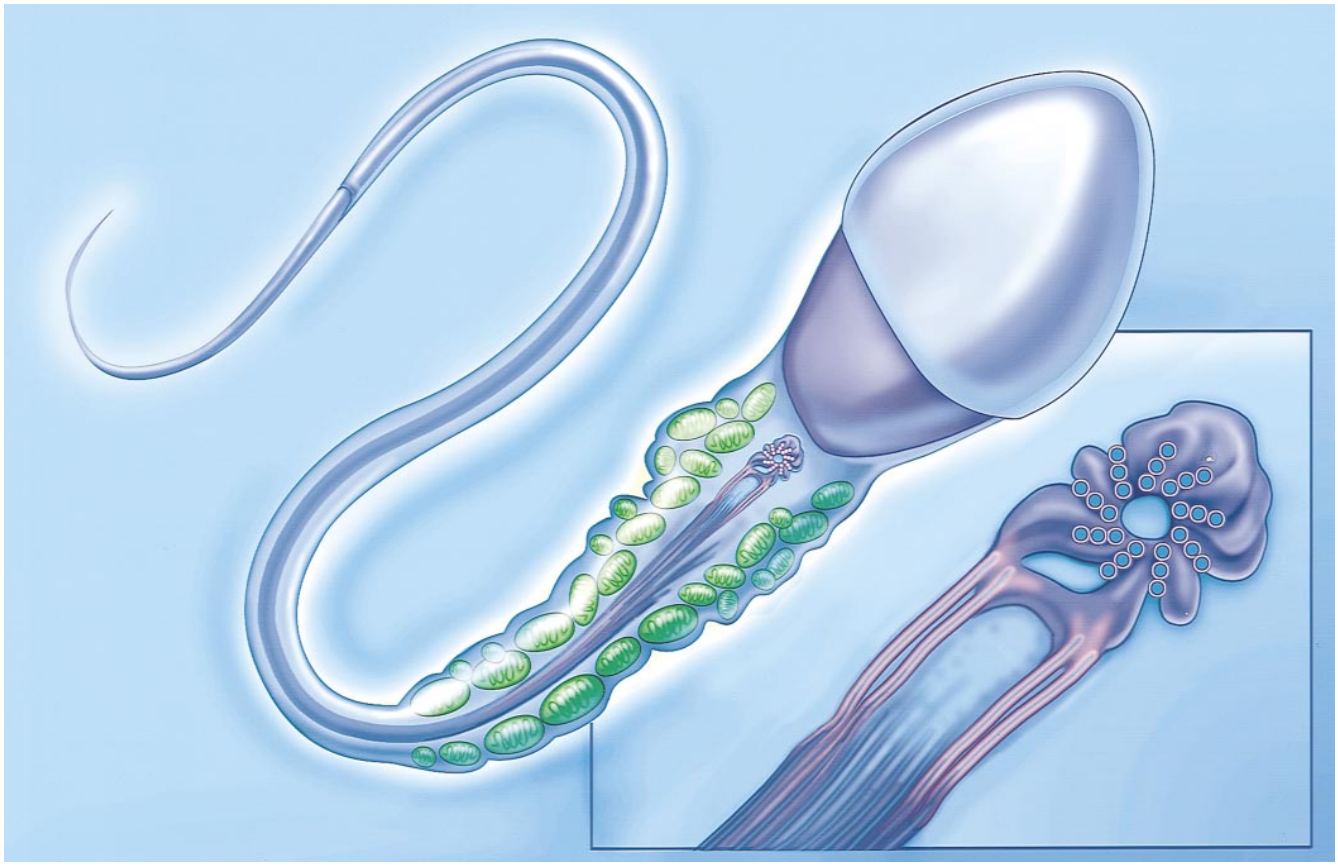

Fig. 2. Proximal and distal centrioles of the human spermatozoon.

the female pronucleus. Other permanent components of the centrosome are the Ca^{2+}-modulated regulatory proteins: centrin, pericentrin and a 62/64 kDa doublet. These are characterized by contractile properties, and thus may play a major role in cell and nuclear division (Salisbury *et al.*, 1984). In addition, some studies support the involvement of these proteins in nucleation, that is, the seeding of microtubules, since antibodies against the 62/64 kDa doublet component inhibit aster formation around isolated centrosomes (Moudjou *et al.*, 1991). The Ca^{2+}-sensitive contractile elements represent a protein group that is essential not only for maintaining the correct disposition of the mitotic apparatus, but also for regulating microtubule nucleation. The interaction between centrin in the sperm centrosome and intracellular calcium released in the oocyte as a result of activation may result in excision of the sperm tail from the proximal centriole, which remains connected to the sperm nucleus (Schatten, 1994).

Mitosis only. In somatic cells, the two centrosomal proteins detectable only during mitosis are prophase-originating polar antigen (POPA) (Sager *et al.*, 1986) and centrosomal protein α (CSPα) (Ellem, 1990). POPA was identified by a human autoantibody from a patient with a particular collagen disease (Sager *et al.*, 1986). Since the antibody reacts with spindle poles in cells recovering from nocodazole, POPA could be involved in microtubule nucleation or spindle function (Sager *et al.*, 1986). CSPα was detected with an antibody against a peptide homologous to a segment of human transforming growth factor α. The function of this protein is unclear, although its appearance at mitosis suggests a role in this process.

Mitosis and interphase. Among the proteins located at the centrosome during mitosis and elsewhere in the cell during interphase, the nuclear mitotic-apparatus protein (Nu Ma) is one of the most ubiquitous. On the basis of its association with the chromosomes, Nu Ma is most likely involved in chromatin organization, possibly as a structural component of the nuclear matrix (Compton and Cleveland, 1993). Nu Ma may also play a role in the nucleation of microtubules, based on its association with the mitotic spindle.

Centrosomal components as regulators. Other cellular proteins found at the centrosome may act as regulators by stabilizing microtubule arrays or inducing mitosis-specific modifications of the centrosome, such as the increased presence of epitopes. A 50/51 kDa protein and a phosphoprotein specifically recognized by the MPM-2 (mitotic protein monoclonal) antibody are representative of this group. The 50/51 kDa protein is thought to act as a nucleation site for microtubules, since it is found in the aster-forming fraction of granules in the PCM (Toriyama *et al.*, 1988). Some regulatory kinases interact with the centrosome during mitosis and their activity is reflected by an increased presence of epitopes that react with an antibody to the mitosis-specific phosphoprotein MPM-2 in the centrosome

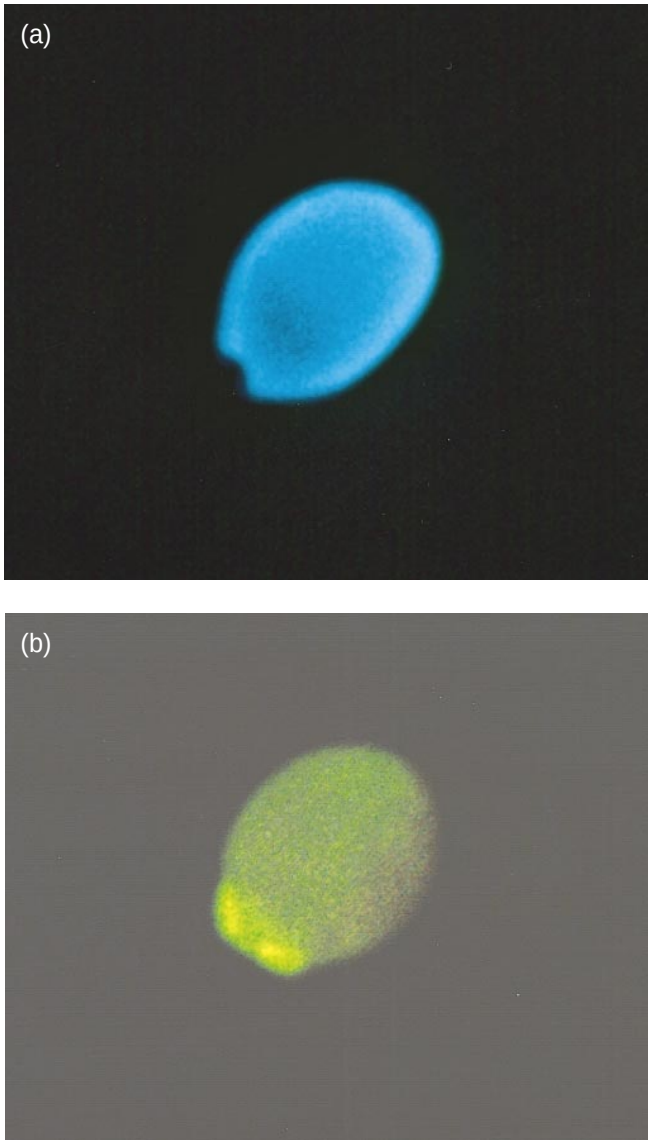

Fig. 3. Immunofluorescent labelling of human sperm heads isolated by mechanical dissection. The centriolar/centrosomal structures were labelled using MPM-2 (mitotic protein monoclonal) antibodies. By the use of 4′,6-diamino-2-phenylindole (DAPI) as a counterstain and a triple-bandpass filter set, it is possible to observe absence (a) or presence (b) of centrosome labelling.

region (Vandre *et al.*, 1984). Thus, it seems that the centrosome possesses some components that are subject to regulation by phosphorylation. The phosphorylation of the sperm centrosome, along with the nucleation of γ-tubulin, leads to nucleation of microtubules, which is required for sperm aster formation (Schatten, 1994).

Although many studies have been performed to ascertain whether nucleic acids are represented among the centrosomal components (Smith-Sonneborn and Plaut, 1967; Berns *et al.*, 1977; Peterson and Berns, 1978), their involvement is still unclear. Since the data obtained using specific dyes for DNA or RNA indicate that DNA is probably not found in the centrosome (Vorobjev and Nadezhdina, 1987), the centrosomal nucleic acid is most likely RNA (Peterson and Berns, 1978). However, additional studies are needed to identify this RNA positively, and define its role in centrosomal replication.

Labelling, identification and localization of the centrosome

Identification of MTOC components responsible for aster and spindle generation has long been a goal of cell biologists. Now, using biochemical and immunological techniques, it has been possible to identify proteins that are structural components of the centrosome. Thus far, however, the majority of these proteins have been isolated as bands on an electrophoretic gel or with fluorescent markers.

Several methods for identifying centrosome components have been attempted (Kimble and Kuriyama, 1992). We have endeavoured to visualize the centriole/centrosome in human spermatozoa under light microscopy by three indirect fluorescent labelling methods using: rabbit polyclonal centriole-specific antibodies; rabbit polyclonal antibodies reacting with the phosphoprotein MPM-2 of the centrosome; and mouse monoclonal antibodies against MPM-2 (Dako Corp., Carpinteria, CA). Both types of rabbit antibody yielded a nonspecific labelling of the whole midpiece or flagellum or both structures. By contrast, the mouse monoclonal antibodies against MPM-2 appeared to be specific and reliable, and point-labelled a centrosome in 63% of the spermatozoa tested or stained. Single or double fluorescent signals were evident at the junction between the head and the midpiece (Colombero *et al.*, 1996a). Additional labelling was performed on spermatozoa in which the head was dissected from the tail, either by ultrasound or physically with a glass pipette. Spermatozoa processed by sonication displayed an unpredictable disruption of the sperm flagellum rendering centriolar assessment unreliable. However, in physically dissected spermatozoa, precise labelling of the proximal centriole was observed with an approximately 50% incidence of labelling in the isolated head (Fig. 3) and a similar incidence confined to the dissected flagellum (Fig. 4). We conclude that the human sperm centrosome can be labelled reliably with monoclonal antibodies against the MPM-2 protein, and that after mechanical separation of heads from tails, approximately 50% of flagella carry a proximal centriole. Thus, it may be possible to use isolated tails with a proximal centriole as donor centrosomes in patients presenting with sperm centrosome dysfunction.

Role of the centrosome

Although many functions have been attributed to the centrosome (Schatten, 1994), two functions best define this organelle: nucleation of microtubules and mitotic spindle formation (Bornens *et al.*, 1990).

Centrosomes arise from pre-existing centrosomal structures, and in their formation they appear to duplicate at interphase, most likely during the S phase. The duplicated centrosome is in fact generated by a doubling of the centrioles, which then separate during mitosis. However, the control of centrosome duplication and spindle formation during fertilization differs from that in somatic diploid cells. The divisions occurring during meiosis are thought to reduce the centrosome equivalency, as well as the number of chromosomes. In the development of

fertilized eggs, there must be specific mechanisms in the gamete or zygote to control centrosome inheritance. If centrosomes from both gametes were retained and remained functional, the zygote would enter the first mitotic division with two sets of centrosomes and four centrioles, resulting in the generation of abnormal multipolar spindles, and so aneuploidy and mosaicism (Sluder *et al.*, 1989). Such problems do in fact occur after dispermy even when the male pronucleus is removed. The centrosome of one of the gametes is downregulated to avoid this abnormal development, and in most species it is the fertilizing spermatozoon that introduces the functional centrosome (Schatten, 1994).

As noted earlier, an essential function of the centrosome is to organize the microtubular network, the components of which are derived from the egg. With the mouse as a notable exception, in many mammals after fertilization, the sperm centrosome nucleates the aster, a radial microtubule-containing structure (Fig. 5). The sperm aster is typically the most prominent microtubule structure found in the zygote and operates to promote the mutual approach and final apposition of the male and female pronuclei. Thus, the oocyte contributes proteins essential for the formation of the microtubule structures and, consequently, for the sperm aster, which is derived from the sperm centriole and directs female and male pronuclear movement towards the centre of the cell. It has been suggested that the tail of the spermatozoon enters in folds within the cytoplasm of the oocyte in a way that exposes its centriolar structure to the female pronucleus. The ability to form the sperm aster is maintained even when an isolated sperm tail is injected into an oocyte (Van Blerkom and Davis, 1995).

Although mitotic spindle formation is dependent on the centrosome, recent studies have demonstrated that human oocytes do not possess centrioles or other centrosomal elements (Sathananthan *et al.*, 1996). The metaphase II spindle is located peripherally, is anastral and, as with other mammalian species (Zamboni and Mastroiani, 1966; Navara *et al.*, 1994), its long axis is perpendicular to the oocyte surface (Pickering *et al.*, 1988). However, previous reports compared the orientation of the spindle with that in mice, that is, parallel to the oolemma (Sathananthan *et al.*, 1986). Occasionally, the terminating microtubules are surrounded by traces of electron dense material, which may represent the downregulated, nonfunctional maternal centrosome (Sathananthan *et al.*, 1996) (Fig. 5).

After fusion of human gametes, the sperm tail is invariably incorporated into the ooplasm, and the centriolar region often remains in close contact with the decondensing sperm nucleus, maintaining this relationship after male pronucleus formation. The aster is initially formed while the sperm head is decondensing and later is thought to draw the female pronucleus towards the centre of the egg. The sperm centriole duplicates during the pronuclear stage, and at syngamy one or two centrioles are located at opposite poles of the first mitotic spindle. (Sathananthan *et al.*, 1991; 1996) (Fig. 6). The mitotic spindle is fusiform and is generally almost centrally located (Simerly *et al.*, 1995; Sathananthan *et al.*, 1996), having never been identified in the cytocortex (Fig. 4). The sperm tail is generally found in close proximity to one of the poles of the spindle. Centrioles are detectable from the one-cell to the eight-cell stages of embryonic cleavage, and even in the hatching blastocyst (Sathananthan *et al.*, 1996).

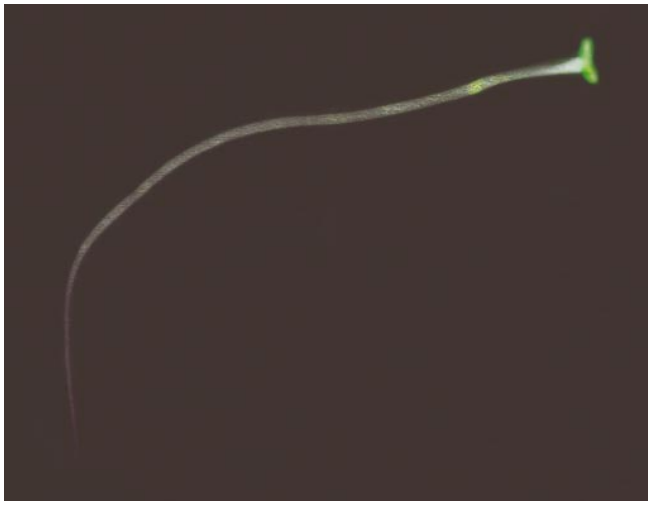

Fig. 4. Immunofluorescent staining of a human sperm tail isolated by mechanical dissection. Centrosome labelling is evident at the dissected portion of the midpiece.

As a unique tubule-nucleating centre, the centrosome is closely associated with the nucleus, and in fact has been shown to be physically connected to it in several animal cells (Bornens, 1977) and even in the early stages of spermiogenesis (Phillips, 1974). In many lower eukaryotic cells, a definite structure binds the centrosome to the nucleus (Heath, 1981); this structural association has obvious functional implications for cell polarity as well as for mitosis (Omura and Fukui, 1985). It may also be linked to the acquisition of properties associated with cell movement. The nucleus and centrosome relationship is of significant interest in assisted reproduction, specifically when round spermatid nuclei are used to provide the male genome via intracytoplasmic injection. This procedure entails the microinjection of a spermatid nucleus surrounded by a minute amount of cytoplasm into an oocyte cytoplasm. However, centrosome integrity may not always be maintained and its possible disruption may explain the failure, thus far, to achieve viable offspring with this technique (Sofikitis *et al.*, 1995).

Centrosome inheritance

Although Boveri's theory of paternal inheritance of the centrosome is applicable for most animal species, including sea urchins (Wilson and Matthews, 1895), sheep (Crozet, 1990), pigs (Szollosi and Hunter, 1973), rabbits (Longo, 1963) and cows (Long *et al.*, 1993), mice appear to be an exception. Schatten *et al.* (1986) reported that the mouse oocyte has the ability to generate multiple MTOCs that imitate centrosome function; but the role, if it has one, of the sperm centrosome in this species is unclear. Rat oocytes may have a similar ability, since the mature rat spermatozoon does not display a centriole at all (Woolley and Fawcett, 1973).

There is now little doubt that in humans only the male gamete possesses an active centrosome. Extensive analysis by transmission electron microscopy (TEM) has demonstrated the presence of centrioles in spermatozoa and in fertilized oocytes at syngamy, and their absence from metaphase II oocytes,

Fig. 5. Haploidization of the human oocyte and reconstitution of the 2n genome after sperm penetration; (a) represents the meiotic spindle during the extrusion of the first polar body, while (b) represents the meiotic spindle for the extrusion of the second polar body, sperm aster formation, replication of the centriole at pronuclear stage and mitotic spindle after syngamy.

confirming the paternal inheritance of the centrosome in humans (Sathananthan *et al.*, 1991). Furthermore, fluorescence *in situ* hybridization (FISH) assessment of chromosome distribution has revealed that the sperm centrosome is the sole structure responsible for organization of the first mitotic division in human embryos (Palermo *et al.*, 1994). In that study, the ploidy and chromosomal distribution were assessed in blastomeres of embryos generated from four groups of zygotes: (1) dispermic zygotes; (2) dispermic zygotes from which a single pronucleus was removed; (3) monospermic zygotes derived from intracytoplasmic sperm injection (ICSI) which retained the second polar body (and were therefore digynic); and (4) monospermic digynic zygotes from which a single pronucleus was removed. The results showed that dispermic zygotes do not possess bipolar spindles and are mosaic. When a single pronucleus was removed from these dispermic zygotes, the mosaic status was not corrected, illustrating that abnormal fertilization patterns are related to extranuclear material, that is, the formation of abnormal or multipolar spindles. Furthermore, monospermic digynic embryos became triploid, indicating that chromosomes arising from the three pronuclei are all organized at syngamy in a single bipolar spindle. In contrast to dispermy, removal of one pronucleus from such zygotes restored the embryo to a normal diploid state, compatible with embryo survival and normal development.

In humans, the fate of the centrosome in the maternal germ cell line is unclear. The centrosomes of human oocytes may be suppressed at syngamy or, more likely, the centriolar structures may be lost during maturation of the oogonia. Nevertheless, there persists some sort of centrosome-like microtubule-nucleating structure, which accommodates meiotic spindle formation and sustains the early cleavage of a parthenogenetically activated oocyte. Further evidence that a maternal MTOC can support spindle formation, embryo development up to implantation and even organogenesis has been demonstrated by Ozil (1990) using rabbit oocytes. In this particular study, oocyte activation was induced by the use of electrical stimuli and exposure to cytochalasin B to generate gynogenetic zygotes, which displayed a second pronucleus arising as a result of the failure to extrude a second polar body. Whether a comparable maternal spindle-organizing centre can be formed in human oocytes in the absence of the sperm centriole needs to be investigated. Human parthenogenetic embryos usually do not develop past the eight cell-stage (Winston *et al.*, 1991), whereas mouse parthenotes may develop much further and even implant – an outcome consistent with maternal inheritance of the centrosome in this species (Schatten *et al.*, 1991). Whether these differences are due to the sensitivity of these different species to culture conditions or to their intrinsic ability to generate effective MTOC remains to be explored.

Two theories have been proposed to explain the behaviour of the centrosome during normal fertilization and parthenogenetic activation. The first posits that the spermatozoon possesses a replicating centrosomal structure that adheres to maternal γ-tubulin and other proteins at fertilization, generating spindle fibres. Parthenogenesis in this model would be

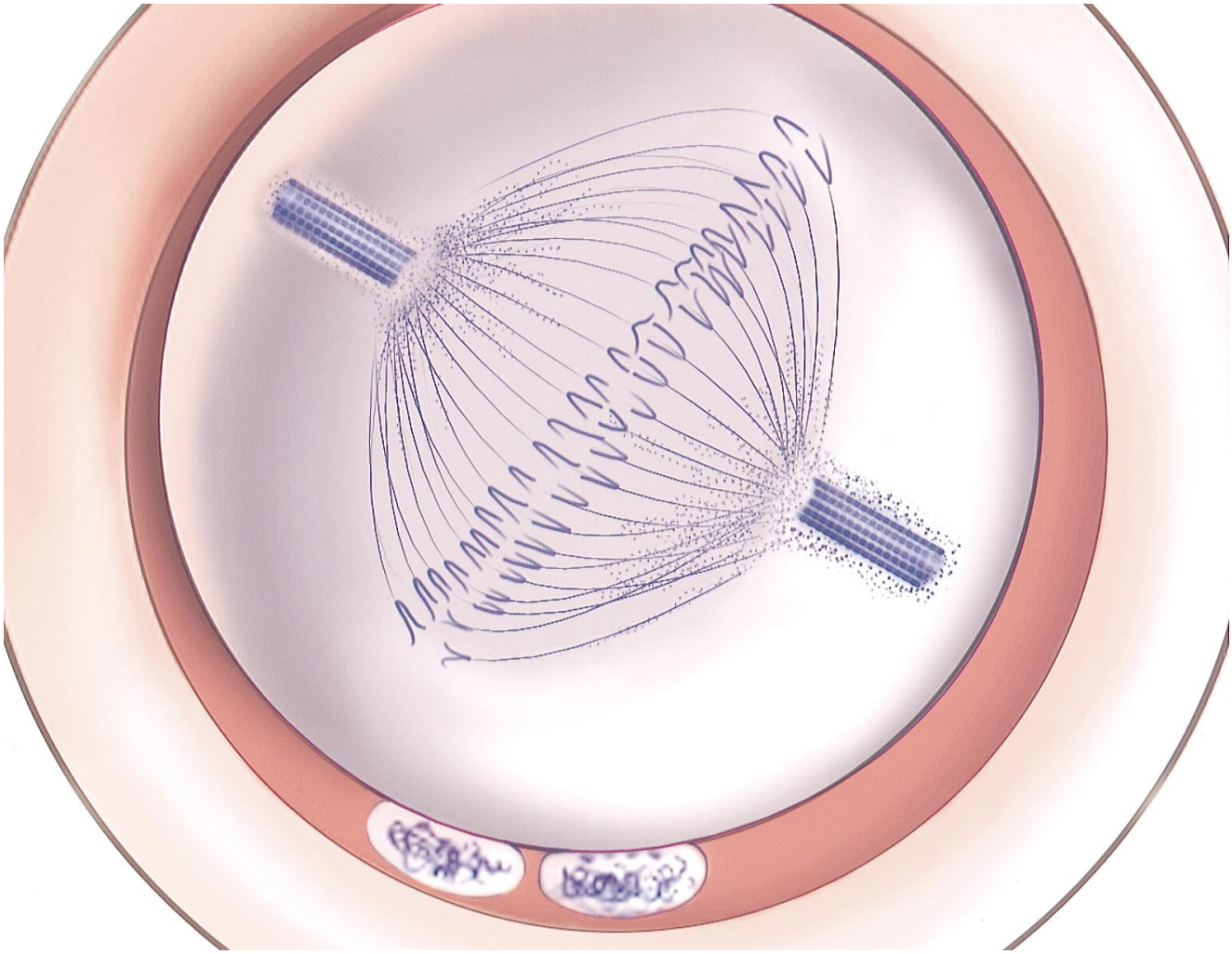

Fig. 6. Detailed representation of a mitotic spindle with centrosomal structures at the poles in the human zygote.

resolved by renaturation of remnants of the maternal MTOC, frayed during oogenesis. In a second model, the spermatozoon introduces a scaffold where the maternal interlocking pieces meet their complements, resulting in the preferential formation of microtubules. During parthenogenesis, the interlocking maternal proteins could join with one another or with the remnants of a primordial template, building at a slower rate a rudimentary bipolar mitotic spindle (Schatten, 1994).

The centrosome: its role in infertility

The study of the centrosome opens an exciting new chapter in the study of human infertility, since defective centrosomes may be responsible for fertilization arrest as well as certain types of male infertility (Schatten, 1994).

Intracytoplasmic sperm injection (ICSI) (Palermo *et al*., 1992) has facilitated the successful treatment of male patients with subfertile spermatozoa. The distal centriole gives rise to the axoneme during spermiogenesis (Holstein and Roosen-Runge, 1981). Therefore, it is interesting that preliminary studies on human sperm centrioles showed a greater incidence of centriolar abnormalities or absence of centrioles in immotile or nonprogressively motile spermatozoa when compared with normally motile sperm cells (Sathananthan, 1994).

Another dysfunction associated with the centrosome could occur in vasectomized patients who generate antisperm antibodies against centriolar structures; the spermatozoa of these patients decondense and zygotes with two distinct pronuclei are produced, but the pronuclei may be unable to undergo syngamy. A similar abnormality has been observed in the timing of the pronuclear apposition observed after the microinjection of extremely abnormal spermatozoa isolated from severely compromised semen samples. These zygotes are typically unable to participate in syngamy or to generate normal embryos and, in such cases, one option may be the use of donor centrosomes (Schatten, 1994). When flagellae of human spermatozoa prepared by sonication were injected into mature human oocytes, individual centrosomes were indeed able to effect aster formation, suggesting the feasibility of centrosome donation in cases of defective centrosome (Van Blerkom and Davis, 1995).

Additional interest in the centrosome has arisen from the need to treat patients who demonstrate flagellar defects (Tucker *et al*., 1996). In some cases, the isolated sperm head is

injected into the cytoplasm (Mansour *et al.*, 1995), presumably carrying a centrosome, since delivery of a healthy baby has been reported with this approach (Tucker *et al*, 1996). Future research involving the injection of isolated nuclei is underway as a result of increasing interest in the potential of immature sperm forms for example, elongated and even round spermatids. The injection of such immature spermatogenetic cells from azoospermic men represents the only possibility of them achieving a successful paternity. Therefore, when sperm nuclei or immature spermatogenetic cells are injected, meticulous prior assessment of the technique for retention of centrioles will be needed to ensure their presence during the injection of these cells.

Through FISH assessment, we have now evaluated the developmental potential of embryos injected with dissected spermatozoa (Colombero *et al.*, 1996b). Fresh oocytes were donated by patients undergoing ICSI at our centre. Each of the oocytes (n = 77) was injected with an isolated sperm head, with a separated head and tail together, or with a sperm tail alone. After injection, the oocytes were observed for cleavage for up to 72 h, and 17 embryos were analysed by FISH for chromosomes X, Y, 18 and 13/21. Of those oocytes injected with isolated sperm heads, 61% displayed two pronuclei. Of the oocytes injected with a separate head and tail, 64% developed two pronuclei. Finally, a sperm tail only was injected into each of nine oocytes, only two of which developed two pronuclei but a single polar body. However, FISH analysis revealed that only one out of five embryos created by the injection of an isolated sperm head had a normal diploid constitution, indicating in principle that a functioning centrosome can occasionally remain with the head nucleus after physical separation. All embryos arising from oocytes injected with separated heads and tails were chromosome mosaics. We also observed a mosaic constitution in the embryos derived from injection of isolated tails. When whole spermatozoa were injected into human oocytes, the frequency of mosaicism was not higher that 20%, similar to the occurrence of mosaicism in oocytes fertilized with standard *in vitro* insemination. Consequently, it appears that the sperm centriole is often damaged as a result of physical dissection. Another possibility is a disruption in the pericentriolar material, inimical to normal microtubule nucleation. A final possibility is that separation of the head and tail may activate the distal centriole to generate its own aster, resulting in abnormal multipolar spindles, similar to those observed in cases of polyspermic zygotes.

Thus, overall, our studies so far suggest that injected isolated sperm components and dissected spermatozoa both result in oocyte activation and pronuclear formation at a rate comparable with that of intact spermatozoa, but that the migration and syngamy of the pronuclei are not properly achieved the outcome being the generation of abnormal embryos. An almost universally abnormal chromosome distribution within the blastomeres indicates that mechanical dissection of the spermatozoa compromises centrosomal function in the zygote. Such abnormal findings, obtained after injection of a separated head and tail or an isolated tail, indicate that centrosome donation by this dissection method is not yet feasible. However, a more gentle preparation of centriole-containing tails, perhaps based on the chemistry of the head and tail junction (Young and Cooper, 1983; Bedford and Hoskins, 1990) may perhaps resolve this problem.

Conclusions

For very practical reasons, in recent years, there has been a renewed interest in the centrosome, particularly in the field of reproductive medicine. The centrosome has been an enigma in cell biology for decades, and its role, replication mechanism and even its definition to a degree remain controversial. We consider it essentially as a functional entity constituted by two centrioles in a perpendicular arrangement surrounded by pericentriolar material.

There is evidence that the poles of the meiotic spindles of human oocytes do not possess a centrosome, and centrioles have not been observed in human oocytes. Thus, its participation in the formation of the meiotic spindle remains uncertain and is beyond the scope of this discussion. By contrast, the involvement of the centrosome in mitotic spindle formation is clear.

An issue that has been amply elucidated is the mechanism of centrosome inheritance. The spermatozoon introduces the reproducing element into the oocyte in most species, including humans, with the mouse and perhaps some other rodents representing possible exceptions. Cleavage during human parthenogenesis can be explained by reactivation of a maternal MTOC structure that functions in a similar fashion to that of a mouse oocyte, generating a mitotic spindle-like structure.

The widespread experience now from ICSI raises the possibility that some spermatozoa so injected may carry abnormal centriole/centrosomes, particularly those spermatozoa that display a poor motility. In general, however, the high incidence of normal pregnancies and the many normal babies born argue against any dysfunction of the sperm centrosome in these subfertile spermatozoa. Although the evidence is limited, the absence of a centrosome may be a factor in the pregnancy failure after injection of round spermatid nuclei, as compared with the success obtained with intact spermatids.

In cases of defective centrosomes, donor centrosomes provided by the injection of isolated sperm tails appear to represent an option. However, according to our initial findings with mechanical sperm dissection, this has not been feasible thus far. Other means of sperm head and tail separation that do not eliminate or damage the centrosome must be developed for this approach to become a practical proposition.

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