Paternal Centrosomal Dynamics in Early Human Development and Infertility

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Purpose: Our purpose was to demonstrate the dynamics of the human sperm centrosome during fertilization and cleavage.

Methods: Human gametes, fertilized oocytes, and preimplantation embryos were examined by transmission electron microscopy.

Results: The functional sperm centrosome containing a typical centriole (proximal) is inherited at fertilization and forms a sperm monoaster. It then replicates and is perpetuated during cleavage. It organizes the mitotic apparatus at each stage of cleavage up to the hatching blastocyst stage. Bipolar spindles are formed in all monospermic and most dispermic embryos. Occasionally, two sperm asters and tripolar spindles are formed in dispermic embryos. Centrioles are associated with pronuclei and nuclei at interphases when they duplicate and occupy pivotal positions at spindle poles during mitoses. The maternal centrosome is not functional.

Conclusions: The human embryo shows paternal centrosome inheritance and perpetuation like most other animals. Inheritance of defective centrosomes may lead to abnormal cleavage and contribute to infertility.

KEY WORDS: sperm centrosome (centriole); mitosis; embryo; infertility; ultrastructure.

INTRODUCTION

Recently we showed that centrosomes containing centrioles are paternally inherited at fertilization (1). This is true of most animals including mammals, with the exception being the mouse, in which it is mater-

nally inherited (2). Thus most animals including mammals follow Boveri's rule of paternal inheritance postulated over a century ago in the round worm and sea urchin (3,4). Theodore Boveri's predictions were that the sperm provides the active division center, the ripe egg possesses all of the elements necessary for development, the sperm and egg are complementary structures and their union at syngamy thus restores to each the missing element necessary to development. He further predicted that the centrosome is a cyclical structure-permanent reproducing organ of the cell, centrosomes are part of a "central apparatus" or "microcentrum" in cells, they are "dynamic centers" of cells and the true division organs of the cell, and centrioles are contained within centrosomes. We have further demonstrated that the sperm centrosome formed a sperm aster, replicated and perpetuated during each cell cycle, and organized mitotic spindles in cleaving human embryos (5,6). It was further postulated that the sperm centriole might play a role in human infertility in being involved in early embryonic cleavage (7-10). This paper reviews our findings in the human with reference to recent reports on centrosomal behavior in humans as well as in higher mammals (2) and provides further evidence that the sperm centrosome (centriole) might indeed have a significant role to play in normal and abnormal embryonic development, thereby influencing fertility.

TECHNIQUES

These studies are based on the examination of human embryos at fertilization and during the early preimplantation period during the past two decades, where over 200 normal bipronuclear and abnormal tripronuclear cleavage embryos were examined by routine transmission electron microscopy (TEM) (5,11). Early cleavage-stage embryos were examined by serial

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sectioning up to the hatching blastocyst stage. In addition, centrosomes were examined in several mature oocytes and sperm pellets to characterize maternal and paternal centrosomes. Electron microscopy is the only method currently available to reveal centrioles within centrosomes in embryos. Most other studies use confocal fluorescent microscopy (FM) to demonstrate chromosomes and microtubules (MTs) by immunocytochemistry (2,12). These images elegantly demonstrate whole asters, spindles, and chromosomes, while TEM demonstrates the fine structure of the centrosome in serial sections. These two techniques are complementary in the visualization of nearly all components of meiotic and mitotic figures in oocytes and early embryos. The most difficult object to demonstrate by FM seems to be the centrosome, which can be easily visualized by TEM due to the presence of the centriole, which has a unique structure.

SPERM CENTROSOME (CENTRIOLE)

Human sperm have a functional proximal centriole (PC) showing the typical pinwheel structure of nine triplets of MTs seen in cross section, surrounded by dense pericentriolar material (PCM) (Figs. 1 and 2). It is located in the sperm neck just beneath the basal plate, hidden in a "black box" consisting of the capitulum (roof) below the basal plate, flanked laterally by nine segmented columns that interface with outer dense fibers of the midpiece. Beneath the proximal is the remnant of the distal centriole aligned at right angles to the PC and represented by a few MTs, cut longitudinally, and flanked against the dense fibers in line with the outer doublets of MT's of the axoneme. The central doublet of MTs traverses through the distal centriole region to terminate in PCM. Material similar to PCM may also be located within the centriole. In longitudinal sections the PC is seen to terminate in a centriolar adjunct composed of MTs that extend through a lateral aperture of the black box into the cytoplasm of the neck region (13,14).

In most somatic cells, the centrosome is typically composed of two centrioles surrounded by PCM aligned at right angles to one another. It is a self-reproducing organelle, which organizes mitotic spindles (see review in Refs. 15–18). The centriole per se has been a central enigma in cell biology (15), because its role in cell division has been controversial (see functions of centrioles).

THE MATERNAL CENTROSOME

The mature human oocyte does not have either centrioles or functional centrosomes associated with its meiotic spindle, arrested at metaphase II (5,6). The spindle is anastral, barrel-shaped, and aligned more or less at right angles to the oocyte surface. Neither spindle poles show dense centrosomal material, in contrast to a distinct band that is easily demonstrable by TEM in mouse oocytes, which have a functional maternal centrosome (19–21). The spindle MTs end abruptly at the poles, and the pole directed outward is closely bound to the egg cortex. The absence of centrioles in the egg was also reported in the mouse (20–22), cow (23), and sheep (24,25).

THE ZYGOTE CENTROSOME

The embryo inherits its centrosome (centriole) from the sperm cell in humans (1,5), sheep (24,25), and cows (23) documented by TEM. Parallel studies by FM using antitubulin and anticentrosome antibodies coupled with DNA staining have confirmed the paternal inheritance of centrosomes in these species: humans (2,12), cows (26,27), and also marsupials (28) and rhesus monkeys (29). So there is little doubt now that embryos of most mammals inherit their mitotic potential from the male gamete, like most other animals. It is now thought that there is a blending of paternal and maternal centrosomes in the zygote centrosome and that the paternal centrosome is dominant in the human (2,5,6).

Monospermic, dispermic, and polyspermic human ova reveal sperm centrioles closely associated with expanding sperm heads at every stage of sperm incorporation, right up to the formation of the male pronucleus (Figs. 3 and 4). Sperm midpieces and tails, mitochondria, and other remnants of the "black box" are often found in close proximity to centrioles in serial sections, confirming their paternal origin without doubt (Figs. 3–5). Well-organized sperm asters seen by FM (2,12), however, are not clearly visible in the early stages of sperm chromatin decondensation, although a few MTs are evident in their vicinity (5). This is because we examine thin sections by TEM, and not whole embryos. Furthermore, MT-stabilizing and -enhancing substances (2) were not used during the processing of embryos for TEM (5,6).

Both TEM and FM studies indicate that the sperm centrosome initially organizes a sperm aster (12), after the centriole duplicates at the pronuclear stage (5,6)

Fig. 1. Proximal sperm centriole in cross section showing nine triplets of microtubules within the "black box" situated in the sperm neck region. m, mitochondria; mp, midpiece; n, nucleus. Original magnification, $\times 55,000$. (Reproduced from Ref. 5; @97%)

Fig. 2. Proximal centriole in longitudinal section with centriolar adjunct on left. Dense material is surrounding the centriole (PCM) and within the centriole. Remnants of the distal centriole is shown by the arrowhead. Original magnification, $\times 55,000$. (Reproduced from Ref. 5; @97%)

Fig. 3. Incorporated, expanding sperm head in ooplasm, still attached to midpiece (3 hr after insemination). Note the centriole in the neck region beneath the basal plate (arrow). m, sperm mitochondria; o, ooplasm. Original magnification, $\times 11,000$. (Reproduced from Ref. 45; @97%)

Fig. 4. Developing male pronucleus in ooplasm (3 hr after insemination). The centriolar complex is attached to the pronucleus (arrow); m, sperm mitochondria. Original magnification, $\times 14,000$. (Reproduced from Ref. 10; @97%)

Fig. 5. Duplicating centriole associated with pronuclei (not shown) in a 3PN dispermic embryo. The diplosomes are aligned almost at right angles to one another and surrounded by fine granular material (PCM). m, sperm mitochondria. Original magnification, $\times 34,000$. (Reproduced from Ref. 5.)

Fig. 6. Sperm aster at one spindle pole (prometaphase) showing duplicated centrioles (arrowhead). The parent is cut in transverse section, while the daughter is in longitudinal section. Original magnification, $\times 12,750$.

Fig. 7. Note the dense PCM around the parent and less PCM along the daughter centriole. Astral MTs originate from specks of dense PCM (arrowheads). Original magnification, $\times 170,000$. (Reproduced from Ref. 5.)

(Figs. 5–7). Evidently, centriolar duplication occurs soon after the sperm centriole is released from the black box. The sperm aster then enlarges, splits, and organizes the first mitotic bipolar spindle at syngamy (bipolarization) (Figs. 8 and 9). Bipolarization is the essence of mitosis and ensures that one cell divides into two equal daughter cells during its division (Fig. 11) (17). Dispermic tripronuclear ova may produce tripolar spindles with centrioles at one or two poles (1,5) or have two sperm asters associated with each male pronucleus (12). Tripolar spindles eventually form three cells, instead of two (5,6). These results further confirm the role of the male centrosome in initiating mitosis.

REPLICATION AND PERPETUATION OF THE SPERM CENTROSOME IN EMBRYOS

These events have been documented in early human embryos in an extensive study of centrioles by TEM (5,6). A similar study of bovine embryos has revealed a similar pattern of centriolar behavior showing that the cow is a more appropriate model than the mouse for research in mammalian fertilization and early embryogenesis (10,23,30). Thus it is becoming increasingly apparent that the descendants of the sperm centrosome (centriole) are the ancestors of centrosomes in fetal and adult somatic cells that play a principal role in mitotic cell division (5,6,10).

In human embryos, centrioles were detected at all stages of preimplantation development from the pronuclear stage to the hatching blastocyst, after both monospermic and dispermic fertilization (5,6,30) (Figs. 10 and 12–15). Most spindles are bipolar, while tripolar spindles are rare, occasionally found in embryos at syngamy derived from dispermic ova. Dispermic spindles may also be disorganized in their organization of MT's. The incidence of mitotic cells in cleaving embryos is usually very low, there being one or two cells in each early embryo (one- to eight-cell stage) and a few more in later stages (morula to blastocyst) assessed in serial sections.

In interphase embryos centrioles are usually located close to nuclei (Figs. 12 and 14) and are often associated with Golgi complexes (Fig. 15). Occasionally they are attached to the nuclear envelope and may show a few MTs radiating from PCM during interphase. The centrosome was regarded as the "cyto-centrum" or cell center (3,4), and now we know that it organizes the cytoskeleton of the cell. Of course, Boveri did not have modern microscopes such as the TEM or FM to visualize centrioles and MTs, and this

makes his theory of paternal inheritance of centrosomes all the more brilliant.

Centrioles duplicate at interphase (Figs. 4 and 11) and are precisely located at spindle poles during mitosis (Figs. 8 and 10). The first duplication of the sperm centrosome occurs at the pronuclear stage (Fig. 5) and the diplosomes so formed organize the sperm aster (Fig. 7). Typically each diplosome consists of two centrioles aligned at right angles to one another and may be located at either pole. Often only one centriole can be detected at each pole and the other appears in serial sections or may not be detected at all, due to technical difficulties encountered in TEM (6). In most instances centrioles are sectioned obliquely but can be detected with some experience, because they have a halo of dense PCM and the triplet MT structure (Figs. 9 and 12). The PCM is finely granular and osmiophilic and resembles centrosomal material described in the sea urchin by TEM (17,31). A similar pattern of centriolar behavior was seen in bovine embryogenesis (30).

FUNCTIONS OF THE SPERM CENTRIOLE

The role of centrioles in general has been controversial, because the dense centrosome material (PCM) is now regarded as the principal microtubule-organizing center (MTOC) (16–18), nucleating MTs of asters and spindles in both embryos and somatic cells. The centriole forms the heart or core of the centrosome and gives it an unmistakable identity. Centrioles are clearly absent in plant cells and in mammalian eggs, which still divide either mitotically or meiotically. Both centrioles and centrosomes are self-reproducing entities and need to duplicate at each cell cycle in synchrony with the chromosome cycle (17). The impression gained through our human studies is that the sperm centriole has an important function in that it initiates the cell division process in the embryo, a fundamental requirement for embryogenesis. It not only transmits the sperm centrosome, which has hardly any PCM, but also appears to serve as a nucleus for further attraction and nucleation of dense centrosomal material when the sperm aster is formed soon after fertilization (5,6). This material could be partly of maternal origin, because the oocyte is now activated and proceeds to cleave. The dormant, nonfunctional maternal centrosome of the egg is also probably activated and the zygote centrosome seems to be a blending of both paternal and maternal centrosomes, with the centriole playing a central role. There is some molecular evidence that this might be the case in human and bovine

Fig. 8. One-cell embryo at syngamy (dispermy). Bipolar spindle showing single centriole (arrow) and chromosomes arranged on the equator at metaphase. Original magnification, $\times 5100$. (Reproduced @ 97%)

Fig. 9. Distinct centriole at the spindle pole at a higher magnification. Note the characteristic pinwheel arrangement of microtubule triplets. Original magnification, $\times 255,000$. (Reproduced @ 97%)

Fig. 10. Bipolar spindle (half) in a four-cell blastomere showing a centriole (arrowhead), prominently located at one pole. Note the halo of dense PCM. Original magnification, $\times 8500$. (Reproduced from Ref. 5 @ 97%)

Fig. 11. Diagram of sperm centriolar inheritance in the human based on TEM. (A) Sperm proximal centriole in centrosome in sperm neck. (B) Incorporated sperm in ooplasm showing developing sperm aster. (C) Duplicating sperm centriole associated with the male pronucleus. Note the close association between male and female pronuclei. (D) Sperm aster (centrosome with duplicated centriole) organizing the first mitotic spindle at prometaphase. Pronuclear envelopes are disorganizing and the chromosomes are condensed. (E) The first bipolar mitotic spindle at syngamy showing duplicated centrioles (diplosomes) at either pole. Note the increase in pericentriolar material around centrioles and chromosomes at the equator (metaphase). (Reproduced from Ref. 5.)

embryos (2,5,30,32). It is imperative that we clearly define both paternal and maternal components of the zygote centrosome in situ by developing the technology such as immunogold labeling to identify these components by TEM. There is no doubt that the sperm centrosome is the more dominant of the two centrosomes in the human zygote and is the principal organizer of the zygote mitotic apparatus. This conclusion is also supported by FM studies of tubulin (MTOC) in the human (12,33,34). The nonfunctional maternal centrosome can also be activated during parthenogenesis and eggs cleave to about the eight-cell stage after Ca^{2+} ionophore activation (35). Recently sperm asters have been induced by injecting isolated sperm neck

and midpieces into the human oocyte, visualized by confocal laser microscopy (33). This further proves that the sperm centrosome is capable of producing mitotic figures in early embryos, without any sperm nuclear interaction.

It has been speculated that there needs to be only one functional centrosome to ensure normal fertilization and embryo development (36). Introduction of more than one centrosome into the oocyte, such as in dispermic penetration, leads to mosaicism following the formation of abnormal spindles. Aneuploidy and other nuclear aberrations are also prevalent in human embryos (see reviews in Refs. 37 and 38). Centrosomal defects could also induce chromosomal aberra-

Fig. 12. Typical centriole in an eight-cell blastomere in perfect cross section, located close to its nucleus (n). Note the characteristic microtubule triplet structure surrounded by dense PCM. Dense material is also found within the centriole. Original magnification $\times 255,000$. (Reproduced from Ref. 5 @97%)

Fig. 13. Centriole in an eight-cell embryo blastomere at anaphase. Note MTs associated with dense PCM. Original magnification $\times 51,000$. (Reproduced from Ref. 5@97%)

Fig. 14. Trophoblast cell of a hatching blastocyst with a centriole (arrowhead) close to its nucleus. z, zona pellucida. Original magnification, $\times 5100$.

Fig. 15. The centriole (arrowhead) is associated with a Golgi complex (g). n, nucleus. Original magnification, $\times 34,000$. (Reproduced @ 97%)

tions in embryos, which are morphologically expressed as nuclear defects in blastomeres. Such defects include incomplete incorporation of chromosomes, formation of micronuclei, multiple nuclei, and abnormal spindles (20,38). These are clear indications of abnormal mitoses during embryonic cleavage.

SPERM CENTROSOME AND INFERTILITY: CLINICAL IMPLICATIONS

In 1991, we postulated a hypothesis that if a defective male centrosome (centriole) is inherited by a human oocyte, it might lead to abnormal cleavage and compromise embryonic development (7,8). This theory was further expounded in a review of sperm function (9). The hypothesis was based on defects observed of the PC in the neckpiece of sperm from motile, poorly motile, and immotile sperm samples (10,39). Centriolar defects observed in sperm with severe asthenospermia include loss of MTs in triplets, loss of triplets (half centrioles), disorganized triplets with vacuoles, and granular aggregates within the triplets, and loss of centrioles. These defects may also be present in normal sperm samples, but to a lesser extent. A variety of axonemal defects has been reported by Hancock and de Kretser (40) in asthenospermic sperm samples, which may be related to centriolar defects—an investigation worth pursuing. However, centriolar structure is more difficult to evaluate in the sperm neck or within the ooplasm after incorporation, where they are more often sectioned obliquely. Morphological assessment of the PCM is far more difficult within the “black box,” although this too should be considered in the overall evaluation of the sperm centrosome. There is further evidence from subsequent studies by FM that the sperm centrosome could produce defective sperm asters and MT configurations and cause mitotic arrest at syngamy in failed fertilization oocytes (12,41). Hence the sperm centrosome could block fertilization at any stage of mitotic activity after its incorporation to syngamy. There is also some evidence in bovine embryos that sperm aster size differs with sperm quality, affecting fertilization and live birth rates (42).

The functional sperm PC is believed to be the kinetic center of sperm motility (43), while the distal centriole which gave rise to the sperm tail during spermiogenesis persists as a remnant in ejaculated sperm (13,14). Sperm motility is essential to bring the sperm to the egg surface for gamete fusion both in vivo and in vitro. This clearly is the function of the sperm flagellum and midpiece. The sperm have to

move through several natural barriers such as the cervical mucus, uterus, oviduct, and egg vestments, where poor-quality sperm can be weeded out at each stage and would never reach the egg-surface. This is clearly the mechanism by which nature selects the most motile sperm for fertilization excluding immotile sperm with possible centrosomal defects. Sperm motility, however, is not necessary for sperm–egg fusion (44,45). Once the sperm is incorporated into the ooplasm, the sperm centrosome evidently takes over, nucleating MTs into asters and bipolar spindles to bring maternal and paternal chromosomes together for mitosis (1,5,6,12). Any defects of the sperm centrosome at this stage could be perpetuated in later embryos and lead to abnormal or irregular cleavage, which is quite common in embryos cultured in vitro after IVF or sperm injection (20).

CENTROSOMES AND SPERM INJECTION: FUTURE DIRECTIONS

Intracytoplasmic sperm injection (ICSI), which has been very successful in assisted fertilization, could be used to test our hypothesis on sperm centrosomal function especially when sperm with slow, sluggish, or no forward motility is used for ICSI. Usually a motile sperm is selected for ICSI, and it is imperative that the sperm neck be intact because the centriole is located there. This technique has no need for sperm penetration of egg vestments, as during IVF. There are now indications that fertilization and pregnancy rates are lower when sperm with zero motility are used for ICSI (46). Further research should be directed toward assessing failed fertilization, mitotic arrest at every stage of embryo development, and irregular cleavage at least up to the eight-cell stage, when poorly motile sperm with no forward progression are used for ICSI.

CONCLUSIONS

The phenomenon of oocyte activation has taken a new meaning and needs to be redefined in the light of recent revelations on the activities of the sperm centrosome (centriole) in human embryos. Oocyte activation in the true sense of the concept should now precisely mean initiation of mitotic cleavage after the entry of the sperm centrosome into the egg at fertilization. Evidently there is a reorganization of the whole oocyte cytoskeleton after the entry of the sperm centrosome to establish bipolarization, a prerequisite to normal cleavage. Hence activation now has a deeper

meaning than mere onset of embryonic cleavage, because we know the real mechanics of initiation of somatic cell division in the human zygote. The other aspects of oocyte activation as we know it are the extrusion of the second polar body to complete maturation, the cortical reaction involving release of cortical granules, pronuclear formation, and the bringing-together of paternal and maternal chromosomes at syngamy to establish the embryonic genome (20). Indeed, TEM and FM have helped us understand some of the intricacies of early human development.

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