

## Chapter 2

# Sperm Centrioles and Their Dual Role in Flagellogenesis and Cell Cycle of the Zygote

## Structure, Function, and Pathology

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**Abstract** This chapter examines the current knowledge on the role of the spermatid centrosome. The dual role of the centrosome as a spermatid basal body that generates the sperm flagellum and as the nucleation site for sperm aster formation in the zygote is mirrored in different sperm pathologies in infertile men that are reviewed in detail. Information is discussed on different sperm centriolar and centrosomal anomalies that are involved in failed fertilizations or abnormal development of the embryo. Particular attention is paid to specific centrosomal anomalies of genetic origin that cause dysfunction of the sperm centrosome with abnormal assembly of the sperm aster and failed pronuclear apposition and cleavage of the zygote. The studies highlight the key role played by sperm centrosomes in flagellogenesis and early zygote development and encourage further investigation on the physiopathology of sperm centrosome-related fertility failures to fully expose the basic mechanisms involved.

### 2.1 Centrosomes, Centrioles, and Basal Bodies

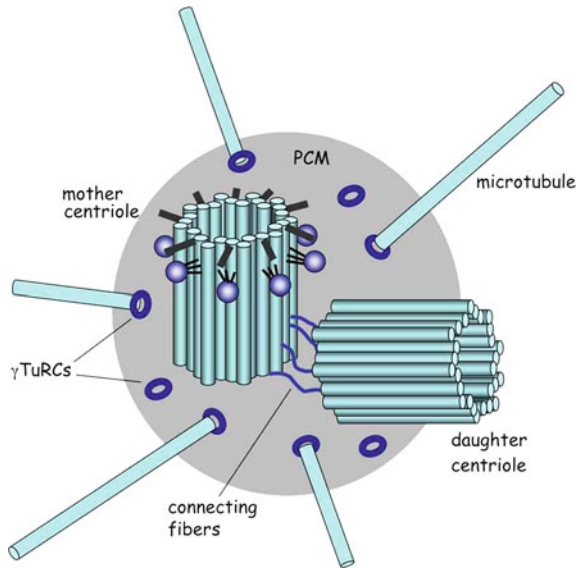
Centrosomes are ubiquitous organelles found in eukaryotic cells. They are composed of a pair of barrel-shaped centrioles, hollow cylindrical structures with their walls composed of nine triplet microtubules in a “pinwheel” arrangement. The two centrioles, perpendicular to each other (diplosomes), are surrounded by a dense fibro-granular “cloud” of pericentriolar material (PCM) that constitutes the

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**Fig. 2.1** Schematic representation of the centrosome. A pair of barrel-shaped centrioles formed by nine triplet microtubules (diplosome) is surrounded by the PCM where  $\gamma$ -tubulin ring complexes ( $\gamma$ TuRCs) serve as nucleation sites for microtubules. Distal and subdistal appendages can be observed at the *upper* end of the mother centriole. Reproduced with permission from J. Lüders

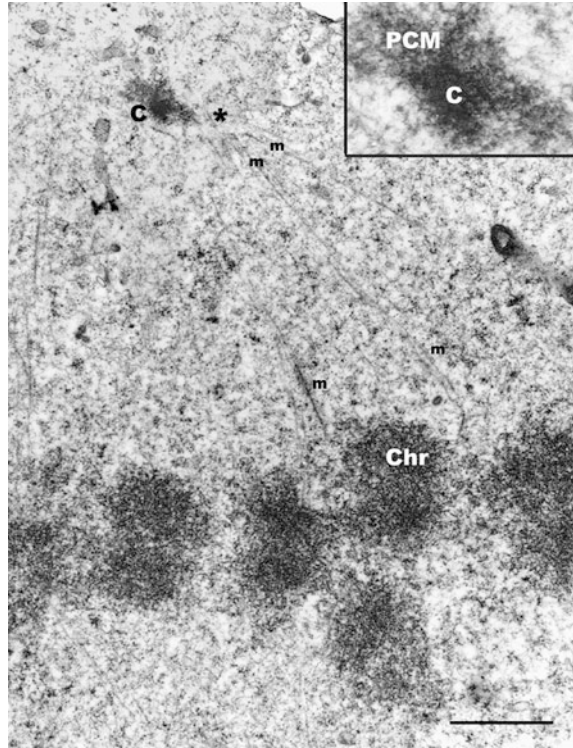


microtubule organizing center (MOCT) of the cell (Fig. 2.1). The PCM is organized as a framework that supports microtubular motor proteins like kinesins and dynein, coiled coil proteins, centrin, pericentrin, speriolin, Cdc20 (spindle checkpoint protein), and NuMA (Nuclear Mitotic Apparatus protein) among about 100 other proteins (reviewed by Schatten and Sun 2009). Centrosomes are involved in numerous cell functions, among them translocation of signal transduction molecules, movement of cell organelles along microtubules and organization of the cytoskeleton, mitotic spindle, and zygote sperm aster. Microtubules do not originate from centrioles themselves but from the  $\gamma$ -tubulin ring complex, a collection of annular structures contained in discrete densities of the PCM (Figs. 2.1, 2.2). The  $\gamma$ -tubulin ring complex serves as nucleation site for tubulin, the main component of microtubules, polarized structures with a minus end anchored to the PCM and a distal plus end where microtubules elongate by polymerization of  $\alpha$ - and  $\beta$ -tubulin heterodimers.

Centrioles and basal bodies are structurally similar and functionally interconvertible. In dividing cells, centrosomes organize the mitotic spindle for chromosome alignment, duplication, and partition between daughter cells (Fig. 2.2). During generation of cilia and flagella, centrosomes migrate to the cell periphery where distal centrioles dock to the cell membrane to become basal bodies from which ciliary and flagellar axonemes originate. When these cells enter mitosis, basal bodies move back to the cytoplasm and reconstitute centrosomes. This alternating dual role is essential to understand the functioning of spermatids and spermatozoa.

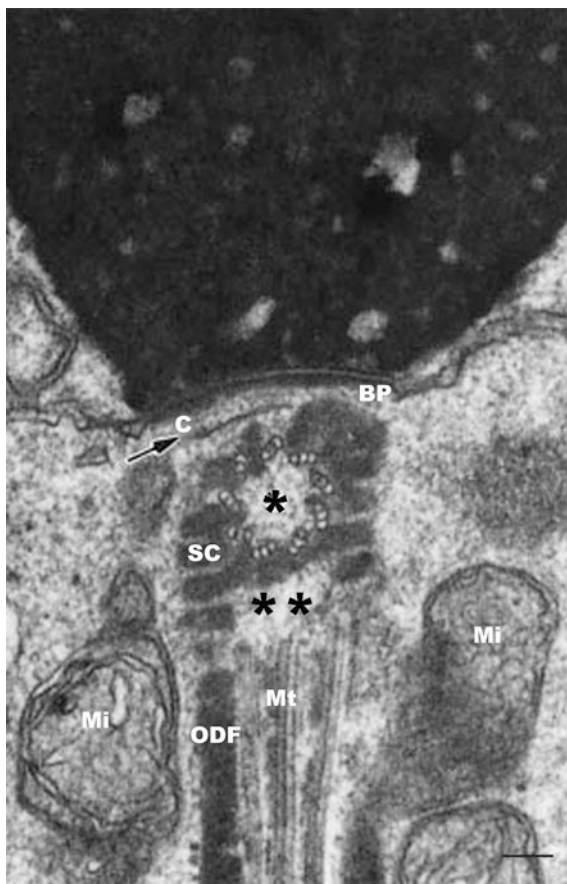
Mammalian spermatozoa are the end product of spermiogenesis, a complex differentiation process in which organelles of round spermatids undergo a series of

**Fig. 2.2** Mitosis of a guinea pig spermatogonium. At one pole of the mitotic spindle a centriole (*C*) is surrounded by dense PCM (inset detail). Note that the proximal ends of spindle microtubules (*m*) converge toward nucleation sites on the area surrounding the centriole (\*). The distal ends of spindle microtubules (*m*) are anchored to metaphase chromosomes (*Chr*). Bar represents 3  $\mu$ m (This figure belongs to the author and is originally reproduced in the present text)



modifications that result in the elaborated structure of mature spermatozoa. The Golgi complex develops into the sperm acrosome and mitochondria organize around the sperm axoneme giving rise to the midpiece (mitochondrial sheath). Spermatids derive from meiosis II of secondary spermatocytes, the last cells to divide in spermatogenesis. Since spermatids will not enter a new mitotic cycle, their centrosomes undergo a functional shift to basal bodies that serve as templates for the assembly of axoneme doublet microtubules by direct tubulin nucleation on subunits a and b of distal centriolar triplets. As flagellar axonemes grow, basal bodies migrate to the cell periphery where distal centrioles dock perpendicular to the plasma membrane as the axoneme sprouts toward extracellular spaces (Fig. 2.4a). In successive steps the basal body-flagellar complex invaginates and attaches to the nuclear envelope at the concave implantation fossa (Fawcett 1981; Holstein and Roosen Runge 1981). ODF2 (a protein component of sperm outer dense fibers) is involved in the initial docking of centrioles to membranes (reviewed by Hoyer-Fender 2010). As spermatid nuclei elongate, acrosomes occupy their cranial pole while basal bodies take up the caudal pole. This topographical arrangement is critical for normal sperm development since it defines the bipolarity of spermatid nuclei and the alignment of heads, midpieces, and tails along the sperm longitudinal axis. As we will discuss later, alterations in this

**Fig. 2.3** Human sperm connecting piece. The proximal centriole (\*), sectioned at right angle, is enclosed laterally by segmented columns (SC) and cranially by the capitulum (arrow, C) which is lodged in the implantation fossa at the caudal pole of the sperm head. A dense basal plate (BP) lines the outer leaflet of the nuclear envelope at the implantation fossa. Distal ends of SC are continuous with outer dense fibers (ODF) of the sperm axoneme. Axonemal microtubules (Mt) end cranially in a rarefied area formerly occupied by the distal centriole (\*\*). Mitochondria (Mi). Bar represents 0.1  $\mu\text{m}$  (Figure 3 was originally published by Chemes et al. (1999) and reproduced, modified from the original, with permission from the publisher)



migration–attachment of basal bodies–flagella will result in misalignments of the tail and serious structural and functional sperm anomalies.

The growth of the sperm axoneme is accompanied by complex modifications in the dense PCM. In its place, new proteins organize in nine longitudinal segmented columns (SC) and the capitulum (C) of the connecting piece (Fig. 2.3) (Fawcett 1981). SC and C constitute a dense shield that lodges and encloses both centrioles. The SC are nine cylindrical structures with periodic densities that fuse cranially to form the capitulum, a curved plate-like disk that links connecting pieces to sperm heads by its association to basal plates, dense structures that line the outer nuclear membrane at the implantation fossa. At their caudal end each SC is continuous with one of the nine outer dense fibers (ODF) that associate to peripheral microtubular doublets of the growing axoneme. In many mammals, including humans, the distal centriole vanishes after giving rise to the sperm axoneme, leaving few remnants in mature spermatozoa.

A phosphorylated protein complex has been reported that localizes to ODFs, SC, and C. This complex may regulate sperm centrosomal function through ODF dephosphorylation and connecting piece disassembly since it has been reported that dephosphorylation of sperm midpiece antigens initiate aster formation in rabbit zygotes (Pinto-Correia et al. 1994; Long et al. 1997; Schalles et al. 1998). Long et al. (1997), Rawe et al. (2008) and Hoyer-Fender (2010) have noted that the sperm basal body-centriole must first disengage from the connecting piece to be able to organize the zygote centrosome by recruiting oocyte-derived PCM. Proteasomes localized to the sperm neck are probably necessary for normal centriolar release (Wójcik et al. 2000; Wójcik and DeMartino 2003; Rawe et al. 2008). The organization of centrioles (basal bodies) docking to cell membranes and giving rise to microtubular axonemes is an evolutionary conserved mechanism common to ciliated and flagellated cells. Vashishtha et al. (1996) have studied in *Chlamydomonas* the role of KHP1, a kinesin-homologous protein that localizes to basal bodies and centrioles and possibly acts as a transporter of protein components to their distal site of assembly in axonemes or aster microtubules. Prior to mitosis, flagella are resorbed and basal bodies duplicate to become centrosomes that occupy *Chlamydomonas* spindle poles from where aster microtubules radiate. These observations point to the dual function of basal bodies/centrioles in flagellar assembly and mitotic spindle formation. Similar phenomena occur after fertilization in humans: flagella detach from sperm heads, and basal bodies (proximal centrioles) recruit PCM to become the zygote MTOC, from which the sperm aster and mitotic spindle will assemble. Sutovsky et al. (1996) have reported that after sperm incorporation into oocytes connecting pieces break down and microtubules first associate with proximal centrioles to form sperm asters that direct pronuclear migration and fusion. During this process, capitulum and SC move away and disintegrate in the cytoplasm. After syngamy, sperm centrioles form the zygote centrosome that subsequently duplicates and migrates to both poles of the cell to assemble the mitotic spindle as the embryo enters its first cell cycle.

The need for a functional centriolar complement was demonstrated by Palermo et al. (1997) and Colombero et al. (1999) who showed that injection of separated sperm components (head only, separated head and tail, isolated tail) is followed by oocyte activation and bipronuclear formation, but ultimately results in abnormal centrosomal function and embryonic mosaicism. They concluded that the integrity of the sperm head-neck region is essential for human early embryogenesis. Experimental evidence presented by Comizzoli et al. (2006) points to the importance of complete centriolar maturation, since aster formation was reduced after injection of testicular immature spermatozoa when compared to that obtained with fully mature ejaculated sperm. Recent investigations have shown that the pericentrosomal area is enriched in proteasomes and may function as a proteolytic center of the cell. Under conditions of cell stress or proteasome inhibition increased numbers of proteasomes and ubiquitinated proteins concentrate around the centrosome forming “aggresomes” (Wójcik 1997a, b; Fabunmi et al. 2000; Wójcik and DeMartino 2003; Rawe et al. 2008). These evidences support an active proteasome involvement in centrosomal function during early zygote

development. Alterations in these mechanisms are essential events in the physiopathology of some sperm-related fertilization failures.

## 2.2 Sperm Pathologies in Infertile Men with Special Reference to Those Related to Centrioles and Centrosomes

Teratozoospermia has been reported as an important cause of male infertility. Two main forms of sperm anomalies can be identified in teratozoospermia (Chemes 2000). The first and more frequent variety consists of heterogeneous combinations of sperm anomalies randomly distributed in different patients. These alterations are referred to as *non-specific* or *non-systematic sperm anomalies*. They are usually secondary to andrological conditions of diverse etiologies that affect the testis or the seminal pathway. No genetic component is present. The second variety is characterized by a specific phenotype that affects most spermatozoa in all patients suffering from the same condition. These alterations may be called *systematic anomalies* because the sperm phenotype involves specific organelles and repeats in most spermatozoa. Systematic alterations show family clustering and have proven or suspected genetic origin. To this variety belong *acephalic spermatozoa* (Perotti et al. 1981; Chemes et al. 1987b, 1999), *round head acrosomeless spermatozoa* (Holstein et al. 1973; Nistal et al. 1978), the *miniacrosome sperm defect* (Baccetti et al. 1991), Dysplasia of the Fibrous Sheath (DFS or *stump tail defect*, Chemes et al. 1987a, 1998), and the dynein-deficient axonemes of Primary Ciliary Diskinesia (PCD, Afzelius et al. 1975). Each of these phenotypes is the consequence of distinctive pathologic mechanisms involving different sperm organelles.

Headless sperm flagella, loose heads, and abnormal head–tail alignment are the distinguishing features of a human syndrome of genetic origin characterized by abnormalities in sperm centrioles and the head–neck junction. Later, we will review what is currently known on this interesting sperm pathology.

Various kinds of defects in centrosomes and cilia have been reported in patients suffering from “ciliopathies”, a group of disorders of ciliated cells caused by mutations in different genes (Tammachote et al. 2009). These comprise syndromes affecting CNS, eyes, kidneys, biliary ducts, respiratory tract, etc. Among them, lack of dynein arms or other axonemal components is the structural basis of immotility in respiratory cilia and sperm flagella in patients with PCD (Afzelius et al. 1975; reviewed by Chemes and Rawe 2003).

As pointed out by Schatten and Sun (2009), even though genetic components most likely play a role in centrosome pathologies, these can also have acquired origins, including exposure to a variety of environmental factors or toxic compounds that can disrupt centrosomal function.

Examining the ultrastructure of zygotes and aster development after fertilizations with abnormal spermatozoa, Sathananthan (1994) and Van Blerkom (1996)

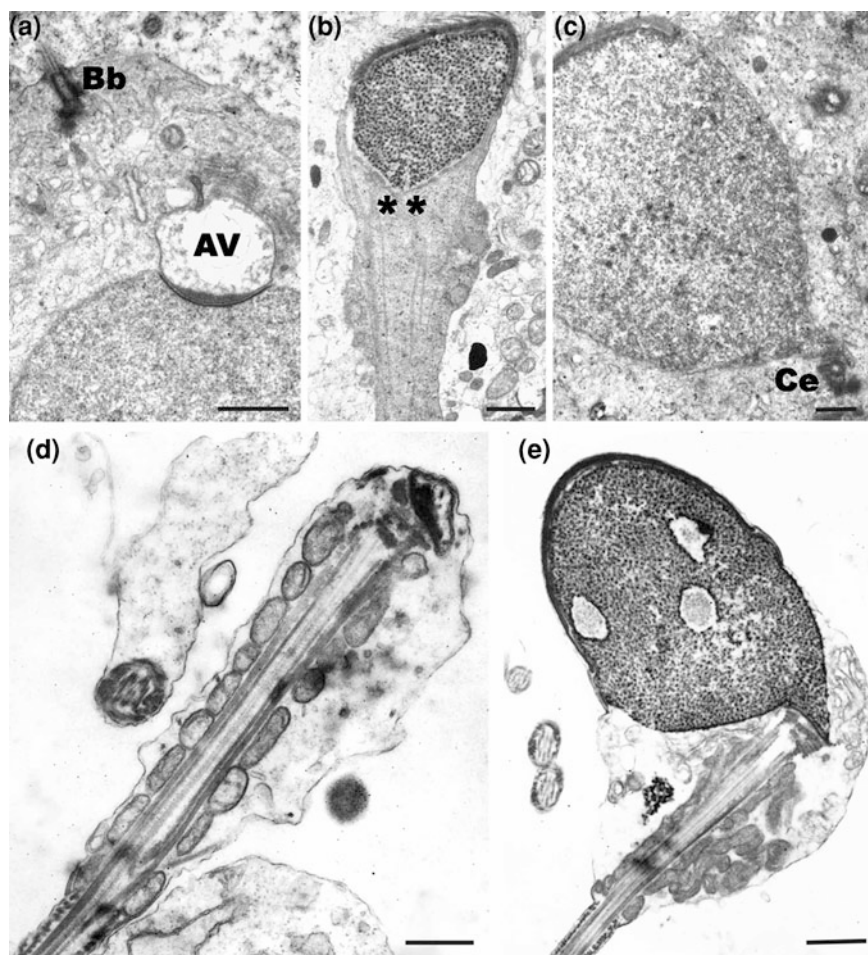


reported sperm centrosome dysfunctions as a cause of infertility or abortive embryonic development. More recently, Sathananthan et al. (2001) identified structural alterations in sperm centrioles of infertile men, including disorganization or loss of centriolar triplets, loss or abnormal positioning of proximal centrioles, and intrusion of mitochondria within centrioles. Hewitson et al. (1997) and Rawe et al. (2008) have also suggested that centrosomal anomalies are responsible for defective sperm aster formation or microtubule elongation in human post-ICSI fertilization failures.

Injection of isolated sperm tails (containing the proximal centriole) into oocytes results in the formation of sperm asters (Van Blerkom and Davis 1995). In later studies it was demonstrated that the use of heterologous ICSI systems (human–bovine, human–rabbit) provide objective information on the capacity of spermatozoa to elicit normal aster development and constitute a novel tool to examine sperm centrosomal function of infertile men (Terada et al. 2002, 2004; Yoshimoto-Kakoi et al. 2008). Using this technique sperm centrosomal failures were reported in teratozoospermia and globozoospermia (Nakamura et al. 2002; Terada et al. 2010), the rate of sperm aster formation from infertile men was found to be lower than that from fertile individuals (Rawe et al. 2002), and Hinduja et al. (2010) communicated that centrosome proteins centrin,  $\alpha$  and  $\gamma$ -tubulin, were reduced in oligoasthenozoospermic patients. As a consequence of these observations efforts to develop in vitro methods to restore defective sperm centrosomal function in humans are underway (Nakamura et al. 2005; Terada et al. 2010).

In summary, centriolar and centrosomal abnormalities are involved in failed fertilizations or abnormal development of the embryo. However, all these reports derive from experimental observations or laboratory studies after post-ICSI fertilization failures and do not identify diagnostic categories of clinical value in human infertility. We and others have described a human syndrome of genetic origin in infertile men with systematic teratozoospermia.

In 1987 we published a paper entitled *Lack of a Head in Human Spermatozoa from Sterile Patients: a Syndrome Associated with Impaired Fertilization* (Chemes et al. 1987b). Three adult males were reported who suffered from primary sterility and presented a characteristic sperm defect that repeated in all semen samples examined. Most spermatozoa (75–100 %) presented with minute “heads”, no larger than 1  $\mu\text{m}$  in diameter and negative for the Feulgen reaction, which indicated lack of sperm heads. Two main abnormal configurations could be observed. Some forms had cephalic ends with minute spherical thickenings containing sperm centrioles and connecting pieces followed by normally structured midpieces and flagella (Fig. 2.4d). The other type was similar but without midpieces. In a second publication, we documented the findings in 10 patients, the largest series published to date (Chemes et al. 1999). A third abnormal variety could be observed in which heads were present but abnormally attached to midpieces, with no linear alignment with the sperm axis (Figs. 2.4c, e). The angles between heads and tails were up to 180°. Normally formed spermatozoa amounted to no more than 1 %. Immature spermatids in semen showed their flagellum-middle piece complexes abnormally related or completely divorced from nuclei.



**Fig. 2.4** Ultrastructure of neck attachment misalignments and acephalic spermatozoa. **a** At an early stage of spermiogenesis this spermatid still shows a normally positioned basal body (*Bb*) anchored to the cell membrane. Acrosomic vesicle (*AV*). **b** This elongating spermatid lacks a nuclear implantation fossa (\*\*), and the flagellar anlage is not attached to the nucleus. **c** Another spermatid showing centrioles (*Ce*) with abnormal implantation into the nucleus. **d** An acephalic spermatozoon. There is no head. Centrioles, mitochondrial sheath, and flagellum are normal. **e** Spermatid with abnormal angle between head and midpiece. Flagellar attachment is similar to that depicted in panel **c**. Bars represent 1  $\mu\text{m}$  (Figure 4 was originally published by Chemes et al. (1999) and Chemes and Rawe (2010) and reproduced, modified from the original, with permission from the publishers)

Various earlier publications had reported single patients with similar sperm phenotypes and identified them as “microcephalic”, “pin-head”, or “decapitated” spermatozoa (Zaneveld and Polakoski 1977; Nistal et al. 1978; LeLannou 1979; Perotti and Gioria 1981; Perotti et al. 1981; Baccetti et al. 1984). We introduced the



term “acephalic spermatozoa” (Chemes et al. 1999) and, in agreement with Perotti and Gioria (1981), proposed that abnormal head-midpiece alignments originated in the testis because centrioles failed to attach normally to spermatid nuclei. This failure could also result from a nuclear defect that interferes with the formation of the implantation fossa, normal lodging site for the sperm proximal centriole. Nuclei and flagella develop independently and become separated within the seminiferous tubules or in the seminal pathway as a consequence of increased instability of the head-midpiece junction. This interpretation is supported by observations that the separation or abnormal relations between heads and tails increase due to mechanical stress in centrifugation or sperm *in vitro* manipulation (Chemes et al. 1999; Kamal et al. 1999). The admixture of acephalic spermatozoa and abnormal head-middle piece connections expresses different degrees of abnormalities of the head-neck junction with acephalic forms representing the most extreme situation. In most cases the sperm neck was the preferred region where cleavage between heads and midpieces took place. In occasional reports separation resulted from dissociation between proximal and distal centrioles (Holstein et al. 1986) or due to other sperm defects at more distal locations. The study of a testicular biopsy in one of our patients confirmed that alterations started very early during testicular spermiogenesis with abnormal relations between spermatid nuclei and tails (Fig. 2.4) that resulted in abnormal lateral implantations or completely independent development. When present, heads implanted at abnormal angles on the middle piece. The caudal nuclear pole of elongating spermatids appeared as a protruding area without an implantation fossa to lodge the proximal centriole.

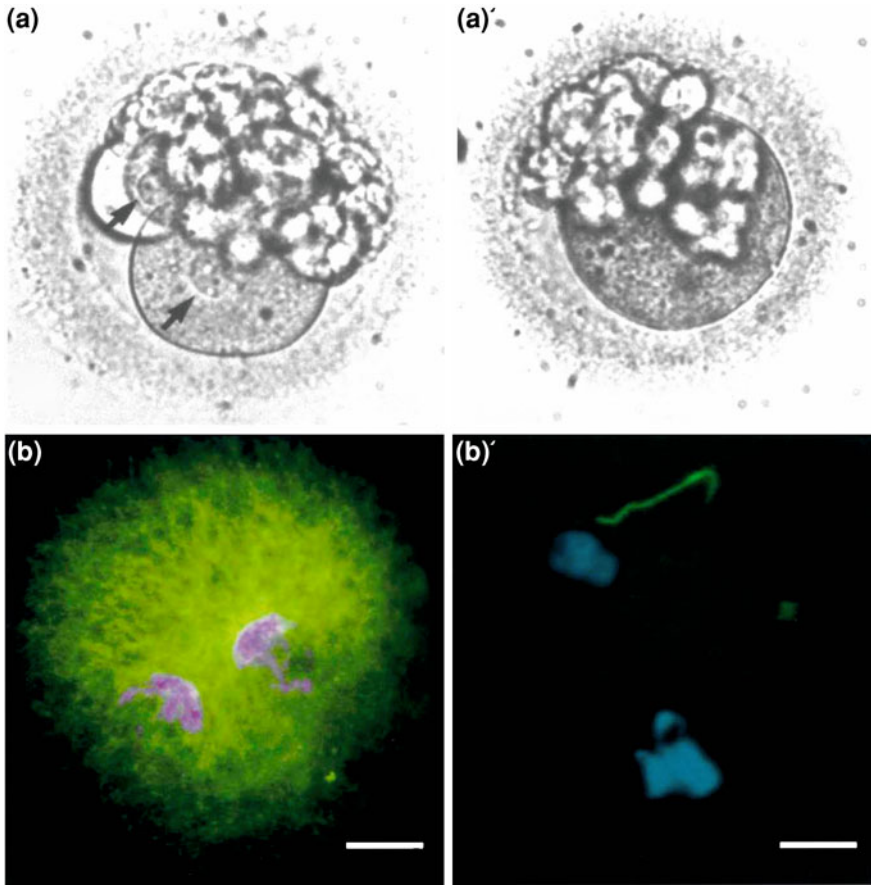
We had previously shown (Chemes et al. 1978) that in early human spermiogenesis the spermatid nucleus differentiates a cranial pole where the Golgi complex attaches to form the acrosome. Shortly after, the centriole-flagellum complex approaches the opposite pole of the nucleus and attaches to it. Acephalic spermatozoa derive from the failure of this caudal migration, while some acrosomeless spermatozoa result from the lack of proper attachment of the Golgi complex to the cranial pole of the spermatid nucleus (Zamboni 1992). The unusual case described by Aughey and Orr (1978), with round acrosomeless heads and acephalic spermatozoa in the same patient indicate that these two abnormal mechanisms have combined, suggesting that there are different pathologies derived from an abnormal differentiation of the bipolar nature of spermatid nuclei. In very recent studies (Alvarez Sedo et al. 2012) we have found that failures of proper Golgi attachment to nuclei are indeed accompanied by frequent failures in head-tail connections.

In one of the reported patients, that had around 1 % normal spermatozoa in his ejaculate, it was possible to follow the evolution of seminal profiles over an extended period, before, during, and after pharmacologic suppression of spermatogenesis. Testosterone propionate treatment was instituted to achieve oligo-azoospermia in an attempt to promote expansion of the clone of normal spermatozoa during spermatogenic recovery that follows testosterone administration. However, sperm morphology did not change along the course of spermatogenic regression-recovery, the percentage of normal spermatozoa remained very low, and about 99 % of all newly formed spermatozoa were again acephalic.

The uniform pathologic phenotype, its origin as a consequence of a systematic alteration during spermiogenesis, the fact that seminal characteristics remain constant along clinical evolution and even after pharmacologic induction of germ cell depletion-repopulation and the familial incidence reported in men and bulls (Bloom and Birch 1970; Baccetti et al. 1989, Chemes et al. 1999) indicate that this distinctive phenotype is a centrosome-related primary sperm defect that results from an abnormal spermatogenic programming of genetic origin. Very recently, Liska et al. (2009) and Kierszenbaum et al. (2011) reported mutations in Centrobilin and IFT88, two sperm proteins that localize to spermatid centrioles and manchettes. Both phenotypes show separation of centrioles from their normal nuclear attachment site, disruption of head–tail coupling, and spermatid decapitation. No communication of similar mutations in humans is available to date.

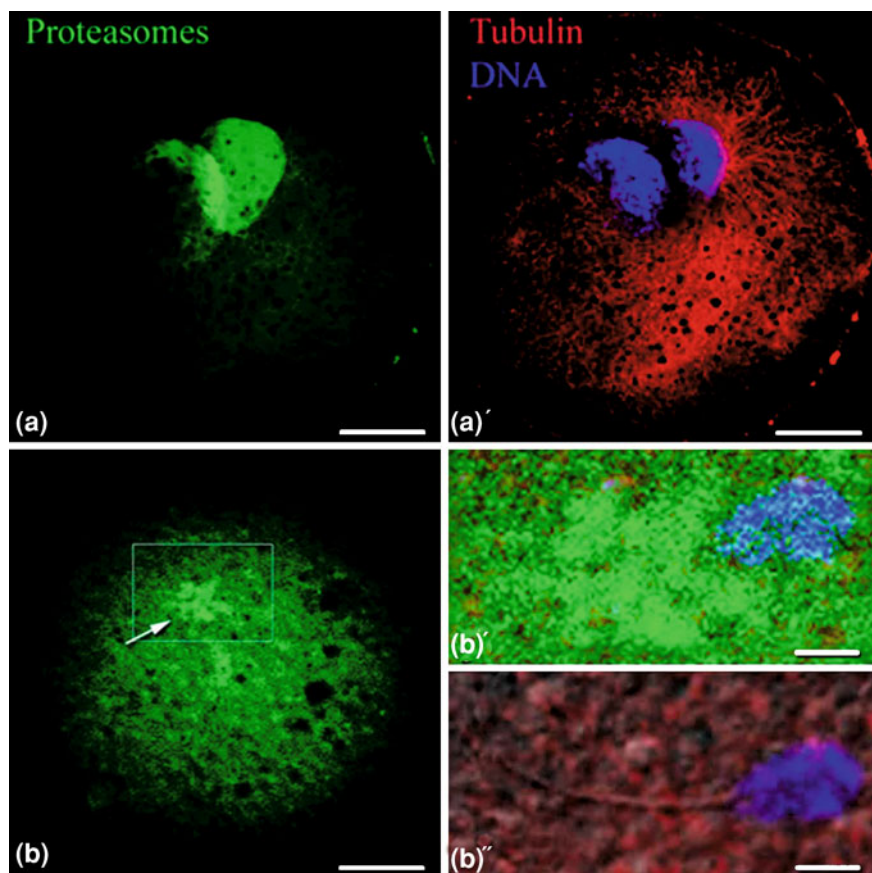
All reported patients suffered from long standing primary sterility. In some cases acephalic forms predominate, which makes impossible any attempt at assisted reproduction (LeLannou 1979; Perotti et al. 1981; Holstein et al. 1986; Chemes et al. 1987b, 1999; Baccetti et al. 1989; Toyama et al. 2000). However, in other patients there were good numbers of nucleated forms with alterations in the head–midpiece alignment. This opened the way to consider their use in oocyte microinjections. In the first reported attempt, nucleated spermatozoa were microinjected into four good quality metaphase II oocytes (Chemes et al. 1999). All of them fertilized and formed pronuclei, but zygotes remained at the pronuclear stage and degenerated before syngamy and cleavage (Fig. 2.5). Comparable results were communicated by Saias Magnan et al. (1999) and Rawe et al. (2002), but this last report also documented high  $\beta$ hCG plasma levels followed by preclinical abortions when microinjected spermatozoa were rigorously selected avoiding anomalies of the head–neck junction. When these abnormal spermatozoa were used in a heterologous bovine–human ICSI system, sperm asters either failed to form or had an arrested development (Fig. 2.5, Rawe et al. 2002). The first births in this condition were reported by Porcu et al. (2003) and, more recently, two successful ICSI attempts in one of our patients were followed by pregnancies and births of healthy children (Coco et al., manuscript in preparation). These dissimilar results indicate variations in the degree of abnormalities of the head–neck junction, some of them compatible with normal centrosomal function.

Various observations have demonstrated the ultrastructural integrity of proximal centrioles in spermatozoa with defects of the head–tail attachment (Baccetti et al. 1989; Chemes et al. 1999). In the search for the nature of this centrosome abnormality we realized that there was dissociation between the function of both centrioles. While distal centrioles successfully completed development of flagellar axonemes, proximal centrioles were unable to attach normally to spermatid nuclei and failed to reconstitute zygotic centrosomes. This type of functional dissociation between both centrioles has its counterpart in PCD (Primary Ciliary Diskinesia or Immotile Cilia Syndrome) where the function of proximal centrioles is preserved (immotile PCD spermatozoa fertilize oocytes when microinjected) while distal centrioles generate abnormal sperm axonemes. This double function and dissociated pathology is an interesting dualistic model underscoring a high degree of autonomy between proximal and distal centrioles.



**Fig. 2.5** **a, a'** Spontaneous post-ICSI fragmented zygotes after failure of syngamy that followed microinjection of spermatozoa with neck anomalies. In **A** two pronuclei are clearly seen (*arrows*). **b** heterologous ICSI. Normal sperm aster formation (*tubulin green fluorescence*) after injection of a normal human spermatozoon into a bovine oocyte. **b'** When a spermatozoon with neck anomalies is microinjected the sperm aster fails to form. The sperm flagellum (*tubulin green fluorescence*) is still associated with the male pronucleus. **a, a'**, original magnification X400; **b, b'**, bars represent 25  $\mu\text{m}$  (These figures were originally produced by Chemes et al. (1999) and Rawe et al. (2002) and are reproduced, modified from the original, with permission from the publishers)

*What impaired mechanisms can account for centrosomal dysfunction?* The proximal centriole must disengage from the connecting piece to be able to reconstitute the zygote centrosome. Long et al. (1997) characterized a phosphorylated protein complex from sperm ODF and connecting pieces that may be involved in the regulation of sperm centrosomal activity after connecting piece disassembly, and Pinto Correia et al. (1994) reported that dephosphorylation of sperm connecting piece antigens is required for initiation of aster formation in rabbit oocytes. Centriole release after fertilization



**Fig. 2.6** Proteasomes and fertilization. **a, a'** Zygote obtained after in vitro fertilization of bovine oocytes in control conditions. **a** Proteasomes (*green fluorescence*) are concentrated on both pronuclei. **a'** Complete development of sperm aster (*tubulin red fluorescence*) and fully apposed pronuclei (*blue fluorescence*). **b** Bovine oocyte after IVF in the presence of E446 anti proteasome antibodies delivered by the Chariot reagent (Rawe et al. 2008). An intense labeling of proteasomes (*green fluorescence*) is seen on the cytoplasm of the zygote and concentrated on the sperm connecting piece (*arrow, boxed area*). **b'**: Detail at higher magnification. Strong proteasome concentration (*green fluorescence*) covers the connecting piece close to sperm nucleus (*blue fluorescence*). **b''** Same area as **b'**. Failure of microtubule polymerization and sperm aster formation (*tubulin red fluorescence*). The sperm connecting piece and flagellum are clearly seen and the nucleus (*blue fluorescence*) has not decondensed. Bars represent 20  $\mu\text{m}$  (**a, a', b**) and 5  $\mu\text{m}$  (**b', b''**) (Figure 2.6 was originally produced by Rawe et al. (2008) and modified by Chemes and Rawe (2010), and is reproduced with permission from the publishers)

may involve various mechanisms including ubiquitin-mediated proteolysis of selected targets by 26S proteasomes recently localized near the centrosome in the neck region of human spermatozoa (Wójcik et al. 2000; Rawe et al. 2008). A reduction below 20 and 40 % of control values was found in the activities of proteasome enzymes

Chymotrypsin and Peptidylglutamyl peptidase indicating that proteasomes of spermatozoa with neck abnormalities were endowed with deficient proteolytic machineries (Morales et al. 2004; Rawe et al. 2008). The important role of these organelles is also supported by Platts et al. (2007) who reported that the major cellular system negatively disrupted in teratozoospermia was the ubiquitin–proteasome pathway. We hypothesized that the failure of centriolar release after sperm penetration was due to insufficiency of proteasome-dependent proteolytic disassembly of the sperm connecting piece. In bovine IVF experiments with pharmacologic and immunologic neutralization of proteasomes, aster development and pronuclear apposition were markedly inhibited (Fig. 2.6), (Rawe et al. 2008). Proteasomes and polyubiquitinated proteins were recruited around the sperm connecting piece. These conglomerates (“aggresomes”, Johnston et al. 1998, see Fig 2.6b, b’) may represent failed attempts to overcome proteasome insufficiency when their capacity to degrade ubiquitinated proteins is exceeded. These findings point to the male complement of proteasomes as probably involved in the release of a functional centriole after proteolytic degradation of the sperm connecting piece. Similar features have been reported by Rawe et al. (2008) in zygotes from couples with spontaneous post-ICSI fertilization failure.

The assembly of such a complex structure as the sperm neck, with centrioles encased by a shield of dense proteins organized in the connecting piece and its sequential disassembly into the zygote are processes for which pathways still have to be successfully worked out. The studies summarized in this chapter highlight the central role played by the sperm neck ubiquitin–proteasome system in early zygote development and encourage further investigation on the physiopathology of sperm-related fertility failures to fully expose the basic mechanisms involved.

**Acknowledgments** This chapter was the result of extensive review of the literature on sperm centrosomes and centrosome-related sperm pathologies, including previous publications of our group, in particular Chemes et al. 1978, 1987b, 1999; Rawe et al. 2002, 2008.

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The Centrosome  
Cell and Molecular Mechanisms of Functions and  
Dysfunctions in Disease  
Schatten, H. (Ed.)  
2012, XIV, 398 p., Hardcover  
ISBN: 978-1-62703-034-2  
A product of Humana Press