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# Keep Your Head Attached: The Sperm Connecting Piece

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The sperm neck, or connecting piece, connects the head to the more distal tail regions. It is composed of two centrioles (proximal and distal) and specific pericentriolar cytoskeletal structures surrounding them: basal plate attached to the nucleus, capitulum, and striated (or segmented) columns continuing into the outer dense fibers. Together, these structures form the head-tail coupling apparatus of the spermatozoon. In mammals, the distal centriole undergoes a profound structural modification which allows efficient transmission of the tail movements to the head. Mutations in genes encoding neck cytoskeletal proteins lead to separation of the head from the tail, acephalic spermatozoa, and infertility.

*Key words:* spermatozoa, sperm neck, cytoskeleton, ultrastructure, infertility

## Introduction

The sperm cell consists of two main regions with very different structure and functions: the head, which carries the genetic material (nucleus) and the lytic compartment (acrosome), and the tail, which contains the motility apparatus (axoneme) and the power generator (mitochondria). The most proximal part of the tail, often considered a third region, is the neck, which encloses the centrioles. Because it anchors the rest of the tail and attaches it to the head, the neck is also termed connecting piece, especially when referring to its elaborate cytoskeletal structures collectively called head-tail (or head-to-tail) coupling apparatus [18]. The present review describes these structures in relation to their functions, with emphasis on recent data and concepts.

### The centrioles

During spermiogenesis, one of the centrioles, called proximal, orients itself orthogonally to the future cell axis and binds to the nucleus (**Fig. 1A, 2A**). The other centriole, called distal, is oriented along the cell axis and becomes the basal body of

the tail as the axoneme is built over it [17]. Since oocyte centrosomes disassemble at an early stage of oogenesis, the sperm cell must bring into the zygote not only its haploid set of chromosomes but also a functional centrosome as a contribution to the future organism.

In the ancestral (or primitive) spermatozoon found in animals with external fertilization, both sperm centrioles have the canonical cylindrical structure. However, in mammals and some other animals, one of the centrioles becomes structurally atypical during spermatogenesis [1]. In mammals, this is the distal centriole serving as basal body. For a long time, it was considered degenerated, and the proximal centriole was regarded as the sole ancestor of the centrioles of the embryo. However, recent data indicate that the distal centriole undergoes modification rather than degeneration during mammalian spermiogenesis: it acquires a specialized fan-like structure based on doublets rather than triplets (**Fig. 1A**), which includes assembly of specific new

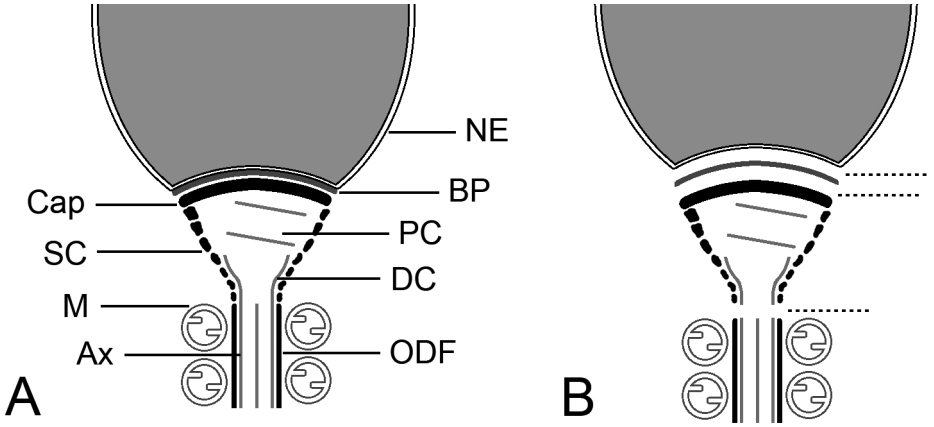


**Fig. 1.** A. Schematic drawing of the connecting piece of a non-rodent mammalian spermatozoon in longitudinal section. The cell membrane is omitted. NE, nuclear envelope; BP, basal plate; Cap, capitulum; PC, proximal centriole; DC, distal centriole; SC, striated columns; M, mitochondria; Ax, axoneme; ODF, outer dense fibers. B. The three possible positions of the head – tail separation in acephalic spermatozoa (dashed lines).

elements [2]. Moreover, it is sufficiently preserved to induce formation of a daughter centriole in the zygote [3, 5]. A notable exception are the murid rodents: in their spermiogenesis, both centrioles degenerate (**Fig. 2B**), and embryonic centrioles are formed de novo at blastocyst stage [9].

In the primitive spermatozoon, the canonical centrioles attach the tail to the head and anchor the axonemal microtubules, so that the dynein-induced sliding of microtubule doublets relative to one another is converted to flexing. Centriolar modifications that deviate from this ancestral structure are best studied in mammals and insects. While the modifications in the two groups are quite different, and the groups themselves are only distantly related, a common feature is that they both have internal fertilization. This led to the supposition that the structural changes affecting sperm centrioles are adaptations to the high biomechanical requirements of internal fertilization, which includes

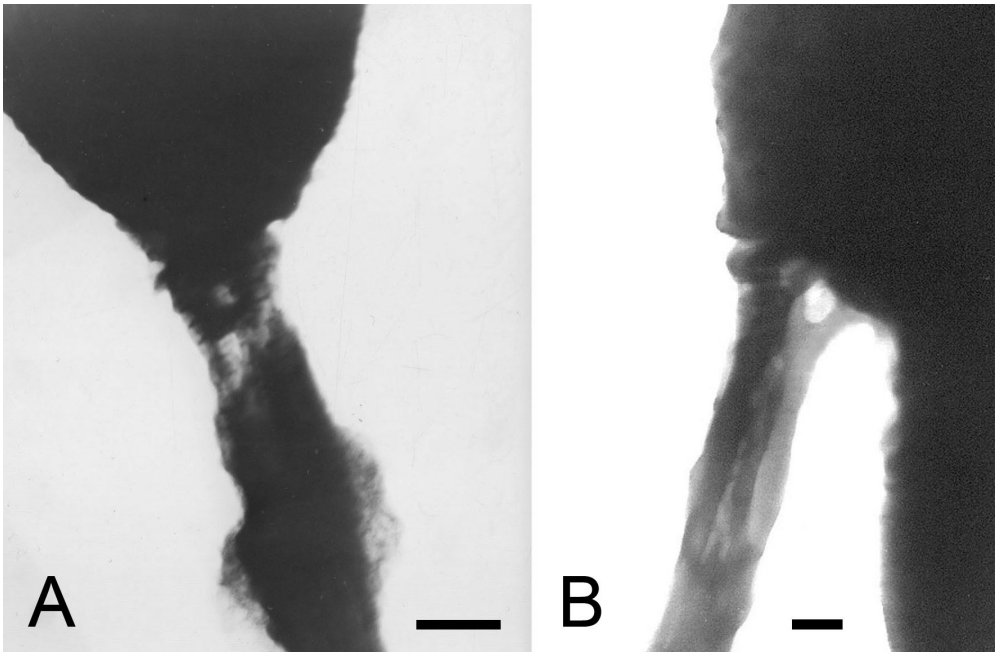


**Fig. 2.** Mammalian sperm necks observed as whole-mounts after nonionic detergent and high salt extraction. A. Human spermatozoon, with transverse view of the proximal centriole. Bar = 500 nm. B. Mouse spermatozoon, showing an empty vault where the proximal centriole has resided. Bar = 200 nm. From [13].

navigating a complex path in the female genital tract through its viscous secretions. This hypothesis was tested by comparing the structure of fish sperm centrosomes with regard to their fertilization mode, and it was found that 20.6% of internal fertilizers had evolved atypical sperm centrioles versus only 0.8% of external fertilizers [19]. A recently proposed theory postulates that the atypical structure of mammalian distal centriole makes it dynamic, so that beating of the tail generates a coordinated kinking movement of the head which contributes to progressive motility [10].

**Pericentriolar cytoskeletal structures**

While centrioles can be regarded as organizers of the sperm connecting piece, they are not the only supporting structures located there. In mammals, sperm centrioles are surrounded by an elaborate complex of cytoskeletal elements with specific structure and composition (**Fig. 1A**). It develops from dense material that accumulates around the centrioles during spermatogenesis and can be regarded as atypical pericentriolar material [10]. The most distal part of the nucleus ends with a shallow concave pit called implantation fossa which marks the beginning of the neck. The nuclear envelope in this region is narrowed, devoid of pores and with periodic thickenings between the two closely positioned nuclear membranes [4]. On the outer nuclear membrane covering the implantation fossa, an electron-dense layer called basal plate is overlaid.

A broad convex sheet named capitulum fits into the concave surface of the basal plate and binds to it through thin fibers. The other (distal) side of the capitulum is connected to the proximal centriole [17].

A characteristic complex of nine assymetric elongated supports surrounds the centrioles. Because of their cross-banded appearance under the electron microscope, they are called striated or segmented columns. Anteriorly, they fuse with the capitulum – in fact, have been described by some observers to form the capitulum by sharp bending. Posteriorly, the striated columns continue into nine thick tail filaments surrounding the axoneme and called outer dense fibers. Despite the intimate connection between the two structures in the mature spermatozoon, during spermatogenesis the outer dense fibers form much later than the striated columns and apparently independently, and fuse with them at a still later stage [17]. The segmentation of the columns is most pronounced at their proximal ends and decreases distally to the point of their fusion with the outer dense fibers [14].

Although the striated columns and their continuations – the outer dense fibers, lack motor activity, they are important for the progressive motility of the spermatozoa. The mechanical forces generated by the motor protein dynein in the axoneme are transfered from the microtubule doublets to the outer dense fibers, and from them to the striated columns [11]. This mechanism utilizing the mechanically resistant periaxonemal and pericentriolar cytoskeletal elements is better suited for mammalian spermatozoa than the ancestral arrangement relying only on the interaction between two relatively fragile structures, the axoneme and the basal body. However, the striated columns should not be regarded as solid anchors. They are in fact flexible, and adjust their positions in response to the flagellar beating. Together with the centrioles, they form a dynamic basal complex that, in addition to connection, provides movement transmission between the tail and the head [10].

**Composition and molecular defects of sperm neck pericentriolar cytoskeleton**

Despite the unique role of sperm centrioles and the atypical structure of one of them, they remain tubulin-based complexes like their counterparts in other tissues, though detailed studies of their composition will certainly reveal specific components absent in centrioles of somatic cells. The pericentriolar cytoskeletal structures are much more intriguing in this respect, because they lack known analogs in other cells and the analysis of their composition meets considerable methodological difficulties.

As in many other cases, the composition of the connecting piece and the roles of individual components have been elucidated by rare genetic disorders and experiments with knockout animals. Among the most severe sperm abnormalities are the so-called acephalic or decapitated spermatozoa, in which the absence or defect of a key neck protein leads to separation of the head from the tail and presence of headless tails and occasional tailless heads in the ejaculate [4]. The separation can occur at three different levels: between the nuclear envelope and the basal plate, between the basal plate and the capitulum (most common), and between the neck and the middle piece (**Fig. 1B**) [17].

A testis-specific protein named SPATA6 (spermatogenesis-associated protein 6) is an important component of the striated columns and the capitulum; in knockout mice, these structures fail to develop, leading to decapitation [20]. Two other proteins important for the head-to-tail attachment have been identified by the high proportion

of mutations in their genes found in patients with acephalic spermatozoa: SUN5 and PMFBP1 (polyamine modulated factor 1 binding protein 1). SUN5 is a nuclear envelope protein, and mutations in it lead to absence of implantation fossa [25]. Mutations affecting PMFBP1cause separation between the basal plate and the nuclear envelope, a defect observed also in knockout mice. Normally, the three proteins interact, with PMFBP1 sandwiched between SUN5 and SPATA6 [23]. The interaction is likely to involve other participants; another protein, named CENTLEIN, links SUN5 and PMFBP1, and knockouts for it produce acephalic spermatozoa. Knockouts for SUN5 have CENTLEIN localized on the decapitated tail, and knockouts for PMFBP1 on the detached head [21].

The connection established in spermiogenesis between the outer dense fibers and the striated columns poses the question whether they share protein components. Four major proteins have been identified in the outer dense fibers: Odf1, Odf2, Odf3 and Odf4, with the former two most prominent [22]. ODF1 (HspB10) is present in the basal plate, the capitulum, and the striated columns. Mouse knockouts for it have apparently normal spermiogenesis but their epididymal and ejaculate spermatozoa are decapitated, indicating inability of spermatozoa to withstand the mechanical forces associated with active movement [6]. The point of breakage has not yet been specified. ODF2 colocalizes and interacts with ODF1 and tubulin in sperm neck pericentriolar structures as well as the outer dense fibers. By alternative splicing, the same gene produces a universal centrosomal protein called cenexin. Homozygous knockout mouse embryos with deleted exon 9 die at preimplantation stage [16], apparently because of the lack of functional cenexin. While heterozygous knockout males in this study had normal spermatozoa and were fertile, another heterozygous knockout with deleted exons 6 and 7 displayed haploinsufficiency, with spermatozoa decapitated by separation of the neck from the middle piece [7]. A missense mutation of ODF2 was found in an infertile patient with abnormal sperm tails, but its deleterious effects were limited to the outer dense fibers [24]. There are no data about ODF3 mutations in the literature, and ODF4 homozygote knockouts are infertile due to bent sperm flagella but there is no decapitation [8].

More examples of proteins important for head-to-tail attachment are summarized in [17]. Several of them are designated by CCDC (coiled-coil domain-containing) and a number. CCDC proteins are overrepresented in spermatogenesis [15]; ODF2 and ODF3 also contain long coiled coils. This is not surprising, since the spermatozoon contains an elaborate system of specific cytoskeletal elements resembling the intermediate filaments which are based on long coiled-coil domains. When spermatozoa are subjected to a chemical dissection procedure which extracts all cytoplasmic components of somatic cells except intermediate filaments, the specific cytoskeletal structures of the sperm cells are preserved, including the striated columns and the capitulum [12]. Future studies will provide deeper knowledge about the fascinating structure of the sperm connecting piece which is instrumental for successful fertilization.

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