It takes two (centrioles) to tango

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Abstract

Cells that divide during embryo development require precisely two centrioles during interphase and four centrioles during mitosis. This precise number is maintained by allowing each centriole to nucleate only one centriole per cell cycle (i.e. centriole duplication). Yet, how the first cell of the embryo, the zygote, obtains two centrioles has remained a mystery in most mammals and insects. The mystery arose because the female gamete (oocyte) is thought to have no functional centrioles and the male gamete (spermatozoon) is thought to have only one functional centriole, resulting in a zygote with a single centriole. However, recent studies in fruit flies, beetles and mammals, including humans, suggest an alternative explanation: spermatozoa have a typical centriole and an atypical centriole. The sperm typical centriole has a normal structure but distinct protein composition, whereas the sperm atypical centriole is distinct in both. During fertilization, the atypical centriole is released into the zygote, nucleates a new centriole and participates in spindle pole formation. Thus, the spermatozoa's atypical centriole acts as a second centriole in the zygote. Here, we review centriole biology in general and especially in reproduction, we describe the discovery of the spermatozoon atypical centriole, and we provide an updated model for centriole inheritance during sexual reproduction. While we focus on humans and other non-rodent mammals, we also provide a broader evolutionary perspective.

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Introduction

Centrioles are subcellular organelles that are essential for many general cell processes including cell–cell communication, cell division and cell motility (Bornens 2012). Defects in their structure, number and protein composition in somatic cells can lead to devastating diseases such as cancer and microcephaly (Nigg & Holland 2018). Because of their crucial roles in these diseases, most of centriole biology focuses on characterizing them in dividing cells and targeting them for cancer treatment (Godinho & Pellman 2014, Maniswami *et al.* 2018). However, centrioles also have several important, specialized roles in differentiating cells. They help direct asymmetrical divisions to drive cell differentiation (Lerit *et al.* 2013, Chen *et al.* 2016), are essential for sensory functions such as sight (Roosing *et al.* 2014, Zach & Stohr 2014) and they are critical for lung function (Yan *et al.* 2016). These centriolar roles are well studied, but one area in need of further research is the function of centrioles during fertilization and reproduction.

It has long been accepted that centrioles are essential for fertilization, but direct experimental evidence of this is limited (Kai *et al.* 2015). Part of this limitation is due to a mystery involving the number of centrioles that the sperm contributes to the zygote. In the past, it was thought that the human sperm, the sole contributor of the centrioles, provided only one to the zygote

(Sathananthan *et al.* 1991, Kim *et al.* 2005, Ounjai *et al.* 2012, Lee *et al.* 2014, 2015, Fritz-Laylin & Fulton 2016). This idea was widely accepted and included in many reviews describing reproductive centrioles in mammals (Palermo *et al.* 1997, Nagy 2000, Sathananthan *et al.* 2001, Chatzimeletiou *et al.* 2008, Debec *et al.* 2010, Chemes 2012, Ross & Normark 2015, Patrick *et al.* 2017), but it also created an enigma surrounding where the zygote gets its second centriole in humans and other non-rodent mammals. The recent discovery of an atypical centriole in sperm attempts to resolve this mystery (Fishman *et al.* 2018).

This review centers on the formation, structure and function of the atypical centriole found in human sperm with examples from other mammals. Readers are referred to the following reviews and papers to gain information about centrioles in insects (Riparbelli *et al.* 2010, Fabian & Brill 2012, Loppin *et al.* 2015), worms (Muller-Reichert *et al.* 2010, Schwarz *et al.* 2018) and *Xenopus* (Cavazza *et al.* 2016, Sluder 2016), as well as the role of centrioles in spindle formation (Hinchcliffe 2014, Meunier & Vernos 2016), and the molecular mechanisms of centriole function in the zygote (Schatten & Sun 2011, Inoue *et al.* 2018). To help a general audience appreciate the difference between typical and atypical centrioles, the first part of the review provides a short background on centrioles and later provides a detailed description of the unique properties of sperm centrioles.

What is a typical centriole?

Centrioles (aka a basal body or kinetosome) are one of the core structures in an animal cell and were present in the ancestral eukaryotes about a billion years ago (Chapman *et al.* 2000, Hodges *et al.* 2010). Centrioles are made of many proteins that assemble their various substructures. While there is no convincing evidence of centriolar DNA or RNA that is independent from the nuclear DNA (Marshall & Rosenbaum 1999), nuclear mRNA does concentrate around the centriole (Groisman *et al.* 2000, Lecuyer *et al.* 2007). Centrioles and their derived substructures form one of the largest proteinaceous structures in the cell, with a diameter of ~200nm and length of ~500nm (about half the size of a small bacteria; Fig. 1A). They have two major functions in the cell. The first is the ancestral and evolutionary conserved function: to form cilia (aka flagella) in a process called ciliogenesis (Fig. 1B). Cilia are hair-like structures that mediate cellular movement and sense the cell environment. The second main function of a centriole evolved recently in animals: the formation of the centrosome (Bornens & Azimzadeh 2007). The centrosome is a major organizer of the microtubule cytoskeleton in animal cells. Centrosomes facilitate accurate cell division (mitosis and meiosis) and pronuclei congregation (karyogamy) and govern cell architecture and polarization (Gomes *et al.* 2005, Thery *et al.* 2006, Schatten 2012).

Centrioles have several key characteristics that allow them to execute their functions: they have an evolutionarily conserved structure and protein composition, they have dynamic accessory structures, and their number is tightly controlled (Carvalho-Santos *et al.* 2010, Hodges *et al.* 2010). Centrioles have a barrel-shaped structure and consist of a wall made of nine triplet microtubules surrounding a lumen (Winey & O'Toole 2014). The microtubules in the triplets are known as the A, B and C tubules (Fig. 1A and C). Because microtubules are polarized, with one stable end and a growing end, the centriole is also polarized. The stable end of the centriole is called the base and the end that grows the cilium, is called the tip. When the tip grows, it forms the axoneme (the cilium core), which is an extension of the centriolar microtubules (Fig. 1B). Since ciliogenesis relies on the centriolar microtubules, and the microtubules

are the most obvious substructure of the centriole, the microtubules have been deemed the defining characteristic of centrioles.

Centrioles have many substructures that associate with the microtubule wall (Fig. 1B and C; Winey & O'Toole 2014). The base of the immature centriole has a structure known as the cartwheel, which is made of a central tube with nine spokes (Guichard *et al.* 2018). The tip of the centriole lumen has a helix and columns that are attached to the centriole wall (Paintrand *et al.* 1992, Ibrahim *et al.* 2009). Around the centriole, there is a material referred to as the pericentriolar material (PCM). The PCM nucleates and anchors the astral microtubules, which aid in the formation and organization of the cytoskeleton. The PCM is built of concentric rings of distinct proteins (Fig. 1C; Mennella *et al.* 2014). In addition to the PCM, the tip of the external wall has two types of appendages: distal appendages and subdistal appendages. The subdistal appendages (aka basal foot) anchor microtubules and help position the cilium (Mazo *et al.* 2016). The distal appendages mediate vesicular traffic, which is needed to build the cilium membrane and to attach the centriole to the cell membrane; therefore, the distal appendages are essential for ciliogenesis (Graser *et al.* 2007, Sillibourne *et al.* 2013).

Centriole number is tightly controlled in dividing cells such that, during G1 phase, a standard cell has two centrioles (Fig. 1D). To maintain two centrioles, new centrioles form only when the DNA duplicates as the cell is preparing to divide, during S phase. The new centriole forms once in a cell cycle, near each preexisting centriole in a process named 'centriole duplication' (Box 1). After centriole duplication, each pair of centrioles (an old and new centriole) is recruited to one of the spindle poles during cell division, such that each daughter cell receives precisely two centrioles.

Centrioles are formed by and composed of a set of evolutionarily conserved proteins (Fig. 1E; Arquint & Nigg 2016). Centriole formation is regulated by cell cycle kinases such as PLK1 and CDK2 (Hinchcliffe *et al.* 1999, Meraldi *et al.* 1999, Shukla *et al.* 2015). Centriole nucleation is initiated by two PCM proteins that surround the preexisting centriole: SPD-2/CEP192 and Asterless/CEP152 (Blachon *et al.* 2008, Kim *et al.* 2013). These two proteins recruit Zyg-1/PLK4, the kinase that is considered the master

Box 1 Centriole duplication

Centriole duplication is the process by which a new centriole forms near a preexisting centriole. The term centriole duplication conveys the need for preexisting centriole. However, the preexisting centriole serves merely as a platform to restrict the number of new centrioles to one new centriole per one preexisting centriole (Ross & Normark 2015). The new centriole is not known to contain any material from the preexisting centriole. This distinction is important as the structure of preexisting centriole and the new centriole can be different. Therefore, a preexisting centriole can nucleate an atypical centriole as observed in insects (Blachon *et al.* 2009, Gottardo *et al.* 2015, Dallai *et al.* 2017, Fishman *et al.* 2017). Also, the atypical sperm centriole can nucleate a typical centriole in the zygote (Blachon *et al.* 2014, Uzbekov *et al.* 2018).

Figure 1 Typical centriole structure and function in a dividing cell. (A, B and C) Structure of the mother centriole (MC) (A), when it extends to form a cilium (B), and as part of a centrosome with a daughter centriole (dC) (C). (D) The centriole through the cell cycle. A cell has four centrioles in S, G2 and M phases of the cell cycle and two centrioles in G1. It takes multiple steps throughout the two cell cycles for a centriole to mature into a mother; centriole initiation begins as a procentriole (pC) that then develops into a granddaughter centriole (GdC), then to a dC and finally matures into a MC. The figure depicts the centriole's initiation and maturation with a single centriole highlighted in color in relation to the other centriole in grayscale. Red – cartwheel, Green – microtubules, Yellow – appendages. (E) Centriole initiation and maturation with specific attention to proteins and their functions.

regulator of centriole duplication (O'Connell *et al.* 2001). PLK4 phosphorylates Sas-5/Ana2/STIL that, together with SAS-6, forms the core of the centriole cartwheel (Nakazawa *et al.* 2007, Kratz *et al.* 2015, Arquint & Nigg 2016). The periphery of the cartwheel recruits Bld10/CEP135 and Sas-4/CPAP, which assist in the formation of the centriole's microtubule wall (Hung *et al.* 2000, Mottier-Pavie & Megraw 2009,

Lin *et al.* 2013, Zheng *et al.* 2014). Initially, the centriole is short (procentriole), but later, it elongates and the proteins POC1B, POC5 and CETN1/2 fill the distal lumen. Then, Ana1/CEP295 is recruited and mediates the recruitment of PCM proteins to the centriole (Salisbury *et al.* 2002, Azimzadeh *et al.* 2009, Keller *et al.* 2009, Pearson *et al.* 2009, Izquierdo *et al.* 2014, Chang *et al.* 2016, Tsuchiya *et al.* 2016)

Box 2 Spermatogenesis cell types

Spermatozoa are formed during spermatogenesis, a differentiation process that is divided into several cell types (Fig. 2A, B, C, D and E). The spermatogonia are stem cells that undergo several mitotic divisions to amplify the number of sperm in mammals (Fig. 2D). The spermatogonia then differentiate and transform into spermatocytes (Fig. 2B and C) that enter into meiosis to generate haploid cells known as spermatids. The spermatids undergo a dramatic cellular ‘metamorphosis’ from round cells to elongated and streamlined cells called spermatozoa (Fig. 2D). The testicular spermatozoa are the final differentiated cells produced by spermatogenesis, but they are not fully mature and are immotile. The testicular spermatozoa enter the epididymis, develop motility and mature as they progress through the caput (Sullivan & Mieusset 2016). Later, they proceed along the corpus and are finally stored in the cauda until transportation through the vas deferens and ejaculation. Spermatozoa continue to change after they are ejaculated as they travel along the female reproductive tract in a process called capacitation (Gervasi & Visconti 2016).

Centrioles are dynamic structures that change during the cell cycle (Bornens 2012; Fig. 1D). These changes can be divided into two types: changes that occur during centriole formation and are complete within two cell cycles and changes that occur as the mature centriole oscillates between functional states for the remainder of its life. *Centriole formation* starts in S phase as a short procentriole forms attached to the preexisting centriole wall (referred to as engaged). Then, the procentriole elongates to form a daughter centriole in G2 and forms the luminal helix and columns. Next, the daughter centriole loses its cartwheel and disengages from its mother centriole in M phase. Later, it nucleates a new procentriole in the second S phase. The centriole then recruits a large amount of PCM and functions as a centrosome during the second G2 phase, forming one of the spindle poles during its second M phase. Finally, the fully mature centriole forms its appendages and its first cilium in the second G1 phase. *After maturation*, the centriole continually oscillates between four functional states during the cell cycle: (1) forming a procentriole in S phase; (2) expanding PCM and reducing appendage size in G2 and M phases; (3) localizing to the spindle pole to orient the spindle in M phase and (4) reducing PCM size, re-growing the appendages, anchoring to the cell membrane and forming the cilium in G1 phase (Palazzo et al. 2000, Garcia & Reiter 2016).

The description above refers to the typical centriole and its behavior in a cell that continuously grows and divides, such as animal stem cells. However, centrioles can be dramatically different in one or more of their characteristics in differentiated cells. For example, many types of white blood cells do not form a cilium in G1 and in cytotoxic T lymphocytes the centriole forms a centrosome that anchors to the cell membrane but does not extend to form a cilium (Stinchcombe et al. 2015). Multi-ciliated epithelial cells produce dozens of centrioles instead of only one centriole per preexisting

centriole via a specialized structure, the deuterosome (Brooks & Wallingford 2014). In several cell types, like muscle cells and oocytes, the centrioles are thought to be eliminated during differentiation (Connolly et al. 1986, Manandhar et al. 2005, Pimenta-Marques et al. 2016). Like other differentiated cell types, sperm cells exhibit many atypical properties and are discussed in further detail below.

What are the unique characteristics of sperm centrioles?

In general, centrioles in early spermatogenesis (those of spermatogonia and spermatocytes) are similar to those found in normal cells, whereas those in late spermatogenesis (those of elongated spermatids) are different (Box 2).

Spermatogonia have two centrioles that duplicate to become four centrioles each time the cell prepares to divide (Fig. 2A). The centriole cycle of spermatogonia is like that of other stem cells, but they lack primary cilia in G1 (Nygaard et al. 2015).

Spermatocytes are formed when a spermatogonium duplicates its genome (from two sets of chromosomes, ‘2N’, to four sets of chromosomes, ‘4N’) and centrioles (from two centrioles to four), and enters meiosis. There are two types of spermatocytes, primary and secondary. Primary spermatocytes have a duplicated genome (4N) and four centrioles (Fig. 2B). Primary spermatocytes then divide by meiosis I, resulting in two secondary spermatocytes, each having two sets of chromosomes (2N) and two centrioles. In the secondary spermatocytes, these two centrioles duplicate to form four centrioles prior to the second meiotic division (Rattner 1972; Fig. 2C). Unlike in other cells, centriole formation in mammalian secondary spermatocytes takes place in the absence of genome duplication, generating

Box 3 Spermatozoon morphology

A spermatozoon is composed of a head and a tail that contains the centrioles and cilium (Fig. 2E). The tail is divided into four parts: the connecting piece (also known as the sperm neck), which houses the centrioles and specialized PCM material known as the striated columns and capitulum; the midpiece that houses part of the axoneme and the mitochondria; the principal piece, which houses the part of the axoneme without mitochondria and the terminal piece, which lacks both the axoneme and mitochondria (Werner & Simmons 2008, Lindemann & Lesich 2016, Mortimer 2018).

Figure 2 The centriole during human spermatogenesis and in the spermatozoon. The formation and function of a centriole during the differentiation of a spermatogonial stem cell (A), primary spermatocyte (B), secondary spermatocyte (C), spermatid (D), to a spermatozoon (E). 1N, 2N and 4N: number of chromosome ploidy in the nucleus; G1, S, M: cell cycle phases. The pictures are not to scale. A, annulus; Ax, axoneme; CA, centriolar adjunct; Cap, capitulum; DC, distal centriole; FS, fibrous sheath; M, mitochondrion; ODF, outer dense fibers; PC, proximal centriole; SC, striated column.

a cell that has four centrioles but only two sets of chromosomes (2N). This is an uncommon situation that is poorly understood and deserves more attention, but may resemble the centriole amplification without DNA duplication that occurs during the differentiation of multi-ciliated cells (Loukil *et al.* 2017). Interestingly, the centrioles of mammalian spermatocytes do not form a cilium, but the spermatocyte centriole in insects forms a cilium that is present throughout meiosis I and meiosis II (Riparbelli *et al.* 2012, Basiri *et al.* 2014).

Haploid spermatids are formed when the secondary spermatocytes divide by meiosis II. Spermatids undergo dramatic morphological changes throughout their development to produce spermatozoa (Box 3) (Fig. 2D); spermatids and spermatozoa do not undergo any further divisions. Early spermatids (round spermatids) consist of one set of chromosomes (1N) and a centrosome with two centrioles surrounded by PCM. In spermatids, the mother centriole is renamed the distal centriole (DC) because it occupies distal location relative to the nucleus. The daughter centriole is named as proximal centriole (PC) because it resides proximal to the nucleus.

Mammalian centrioles in early spermatid stages are similar to typical G1 centrioles. The early spermatid centrioles attach to the cell membrane and form a cilium (Fig. 2D). However, in later stages, centriole behavior deviates in five important ways:

- (i) *centriole attachment to the nucleus* – The two early spermatid centrioles migrate from the cell periphery to the interior where they attach to the nucleus via a specialized structure named the Head-Tail Coupling Apparatus (Liska *et al.* 2009). This type of centriolar attachment is unique to sperm cells and is poorly understood. Maintaining the centrosome near the nucleus is important in several cellular contexts and is mediated by the LINC complexes formed by SUN and KASH proteins (Starr & Fridolfsson 2010, Kracklauer *et al.* 2013). Recently, SUN proteins were found to anchor the tail to the head in humans and *Drosophila* (Gob *et al.* 2010, Kracklauer *et al.* 2010, Shang *et al.* 2017).
- (ii) *transition zone migration to form a cytosolic cilium* – In most cells, the cilium is separated from the cytosol and the mitochondria by a gate called the transition zone, which is found at the centriole-cilium junction (Malicki & Avidor-Reiss 2014). However, in mammals, the part of the axoneme that is close to the centriole is exposed to the cytoplasm and associated with mitochondria, forming the midpiece of the sperm tail. This exposure of the axoneme to the cytosol is unique to sperm cells and forms a specialized type of cilium named the cytosolic cilia (Avidor-Reiss & Leroux 2015). This process is mediated by the migration of the transition zone (also known as the annulus) along the axoneme (Toure *et al.* 2011, Basiri *et al.* 2014, Avidor-Reiss *et al.* 2017). The migration of the transition zone that forms the midpiece requires more research. Interestingly, the midpiece size seems to change specifically with the level of sperm competition; for instance, the midpiece is shorter in deer mice, which are monogamous and have little sperm competition (Fisher *et al.* 2016). In established lines of house mice, sperm with a larger midpiece have a faster swimming velocity (Firman & Simmons 2010). These differences in midpiece size highlight the important role sexual evolutionary pressure plays in shaping the spermatozoon (Gomendio & Roldan 1991, Lupold & Pitnick 2018).
- (iii) *centriolar adjunct formation* – During spermiogenesis, the PC microtubules elongate to form an extension called the centriolar adjunct. The adjunct, while visually similar to the axoneme is unexpected because the PC is the analog of the immature daughter centriole; this function is usually restricted to the elder, mature, mother centriole. The adjunct resembles a cytoplasmic cilium in that it is exposed to the cytoplasm, and not encapsulated by the cell membrane. The adjunct is transient in most mammals, including *Rhesus*, but is present in ejaculated human spermatozoa (Manandhar & Schatten 2000). The presence of the adjunct in the spermatozoa was proposed to be one of three human spermatozoa characteristics that point to its relative immaturity (Zamboni & Stefanini 1971). The centriolar adjunct in mammals should not be confused with the insect sperm's centriole adjunct, which is a specialized PCM that associates with the DC (Dallai *et al.* 2016).
- (iv) *manchette formation* – The manchette is a transient microtubule-based structure that surrounds the spermatid and helps in reshaping the head during spermiogenesis. The mechanism of manchette microtubule nucleation is unclear; one model is that the sperm centrioles or centriole adjunct are mediating manchette nucleation (for review, see O'Donnell & O'Bryan 2014, Lehti & Sironen 2016). This model has been supported by the finding that the daughter centriolar protein CNTROB resides in the capitulum, the PCM structure near the PC and is also essential to maintain the head/tail attachment (Liska *et al.* 2009).
- (v) *centriole remodeling (aka centrosome reduction)* – The fifth deviation is that the centrosome and its centriole are remodeled (or reduced) to have atypical structure and composition (Sathananthan *et al.* 1991, Fishman *et al.* 2018). This is discussed in detailed below.

What are centrosome reduction and centrosome remodeling?

Historically the term 'reduction' is used to describe the decrease in cell components in the gametes prior

to fertilization. This reduction forms gametes that complement each other, such that their fusion will result in a single cell that has appropriate quantities of organelles, chromosomes and cytoplasm. For example, during the formation of the spermatid and oocyte, the nuclear content of each cell is reduced to one set of chromosomes (1N) so that upon fertilization, the resulting zygote has two sets of chromosomes (2N). A similar reduction is expected for centrioles because a somatic dividing cell must have precisely two centrioles. However, the reduction pattern observed in centrioles is different from that in the genome. The oocyte loses both its centrioles so that the oocyte's centriolar contribution will be compatible with that of the sperm and will result in a zygote with exactly two centrioles (Connolly *et al.* 1986, Schatten 1994, Manandhar *et al.* 2005, Pimenta-Marques *et al.* 2016).

Another form of centrosome reduction takes place in the sperm. Originally, it was thought that non-rodent mammalian sperm loses one of its centrioles as a part of the centrosome reduction process (Sathananthan *et al.* 1991). This idea was based on the observation that the sperm loses PCM and centriolar proteins as a part of centrosome reduction, and it was concluded that the sperm was left without a recognizable PCM or centriole. However, it was recently discovered that the sperm maintains both of its centrioles, although one of them is remodeled into an atypical structure and composition. During this remodeling process, specialized sperm PCM structures replace the typical PCM (Fig. 2D; Fawcett & Phillips 1969). The specialized PCM structures are called the striated columns and the capitulum; they are thought to have a structural role in connecting the sperm head to the tail. Their function is poorly understood, but it is thought that they are involved in sperm tail beating (Iwashita & Oura 1980, Ounjai *et al.* 2012).

Complimentary to the sperm, the oocyte lacks centrioles, but has PCM proteins diffused in the cytosol (Severson *et al.* 2016). Once the sperm and the oocyte fuse during fertilization, the sperm centrioles recruit maternal PCM proteins to form the zygote centrosomes. This recruitment is referred to as centrosome reconstitution (Schatten *et al.* 1986).

Because the sperm's centrosome is not lost, but rather remodeled, the term centrosome remodeling has been adopted to encompass centrosome reduction, as well as structural changes, protein enrichment and redistribution in the centrioles, striated columns and capitulum. During centrosome remodeling, many PCM proteins are reduced and the PC loses some proteins typically found in a centriole while maintaining its overall barrel-shaped structure. The DC undergoes several changes; it loses proteins typically found in a centriole, but some centriolar proteins are also enriched. The PCM and centriolar proteins that remain are redistributed within the DC, striated columns, and capitulum. Additionally,

the DC loses its barrel shape and becomes splayed and flattened.

Altogether, in mammals, the early spermatid has two typical centrioles (the DC and PC) (Fig. 2D). During spermatid differentiation, the striated columns and capitulum replace the typical PCM, the DC's structure and composition change and the PC's protein composition is modified. This change generates a spermatozoon where all the centrosome components (PC, DC and PCM) are modified at least in one way, leading to the hypothesis that the whole sperm centrosome is remodeled but not eliminated (Fig. 2E).

What is the structure and protein composition of the remodeled DC, PC and PCM?

In human spermatozoa, the protein composition of the remodeled sperm centrosome is distinct from that of a typical centrosome (Fig. 3). The remodeling process includes the loss and enrichment of some centrosomal proteins, the appearance of sperm-specific proteins, the formation of protein bars in the DC, and the redistribution of centrosomal proteins between the PC, DC and PCM. The PCM transforms into the specialized structures, the capitulum and striated columns. The capitulum and striated columns contain sperm-specific proteins such as SPATA6 (Yuan *et al.* 2015), SPAG4 (Shao *et al.* 1999) and speriolin (Goto *et al.* 2010). The typical PCM proteins γ -tubulin, PCNT, CEP152 and CEP192 are missing, and the striated columns and capitulum include the centriole proteins RTTN and CEP295. The PC retains its typical centriole structure and some centriolar proteins, such as CEP135, CEP120 and CEP76. However, the PC is missing the centriole cartwheel protein SAS-6, as well as centriole wall proteins CNTROB and RTTN.

The DC develops an atypical centriole structure and has a subset of centriole proteins. The centriole microtubules are doublets instead of triplets. The doublets are disconnected, possibly due to the loss of the protein linker between adjacent microtubules. The disconnected doublet-microtubules splay out, forming an oblong cone. Inside the cone, bars of proteins are wedged along the microtubules. The DC is missing the centriole wall proteins CEP135, CEP120, RTTN and CEP295, centriolar tip proteins CP110 and CEP76 and the appendage proteins CEP89 and CEP164. Instead, the DC has rods made of centriolar lumen proteins CETN1/2, POC5 and POC1B. The bars appear to be capped at their base by the PCM protein CEP63. The centriolar protein CPAP associates with the DC. Interestingly, the transition zone protein CEP290 appears to form a ring in the junction between the DC and the axoneme. How the typical centriole changes into an atypical centriole is unknown, and identifying the molecular mechanism underlying this change is an important future direction.

Figure 3 Map of centrosome proteins in the spermatozoa. Proteins are grouped based on their location in a typical centriole (linker, cartwheel, centriole wall, etc.). Centriole protein names are color coded based on their location in the sperm remodeled centriole (capitulum, striated columns, PC, distal centriole (DC) and axoneme). Purple italic font marks proteins that were investigated, but are absent from the remodeled centriole. Gray font marks proteins that were not tested in the spermatozoon. Bi-color proteins are present in two locations corresponding to the color schema in the sperm centriole drawing.

How and when does the DC remodeling take place in various mammals?

The DC is remodeled in all mammals studied to date, suggesting that it is an evolutionarily conserved process that varies only in degrees of completion (Box 4) (Fig. 4; Fawcett & Phillips 1969, Gordon 1972, Manandhar *et al.* 1998). The DC has unique properties in various groups and appears to progressively change during evolution to make the DC more atypical in some groups. In amniotes like snakes, the DC becomes atypical by having a central pair of microtubules in the

lumen of the barrel-shaped DC, much like the central pair of an axoneme. In bovine, the DC's microtubules splay and large DC rods are present (1.5-to -2-fold larger in length and width than human spermatozoa's DC, based on super resolution microscopy). In humans, the DC rods are smaller, and in mice, the DC rods and microtubules appear to be absent (Manandhar *et al.* 1998, Fishman *et al.* 2018) and our unpublished data). More information on the evolution of sperm centrioles can be found in (Avidor-Reiss 2018). Below, we will focus on the changes in centriole microtubules and the appearance of bars/rods in the mammalian DC.

Box 4 Models of centriole inheritance during animal sexual reproduction

Unlike a general cell that has two functional centrioles, the oocyte has inactivated centrioles or no centrioles (Pimenta-Marques *et al.* 2016, Verlhac 2016). Therefore, it is thought that the embryo's first centrioles are paternal, originating from the sperm centrioles (Fig. 5). Indeed, spermatids and spermatozoa of many animal groups (e.g., frogs, *C. elegans* and sea urchin) have two typical centrioles in the spermatozoa (PC and DC) (Fig. 5A; Houliston & Elinson 1991). In the zygotes of these animals, each of the two typical sperm centrioles duplicate to form new typical centrioles (the zygotic daughter centriole); each of the centriole pairs form one of the spindle poles. However, the spermatozoa of most mammals, including humans (Fig. 5B) and insects (Fig. 5C) have only one centriole with a typical structure (although with atypical protein composition) and the other centriole has an atypical structure and an atypical protein composition (Khire *et al.* 2016, Fishman *et al.* 2018). The atypical centriole originates as a typical centriole in spermatids and only becomes atypical after a remodeling process (Fig. 5B). The mammalian and insect DC remains attached to the axoneme throughout zygotic development (Riparbelli & Callaini 2010, Fishman *et al.* 2018). Both centrioles form a zygotic daughter centriole and one of the spindle poles. Fly spermatozoa also have one typical (the DC) and one atypical centriole called the proximal centriole-like (PCL) structure, which lacks microtubules altogether but is still functional. However, unlike the mammalian atypical centriole, the PCL is formed in the spermatid and is, from conception, atypical (Fig. 5C). The PCL structure is further modified by centrosome remodeling, and the PCL loses its wall. In rodents (e.g., mice and rat), the centrioles are thought to be absent in the spermatozoa (Fig. 5D; Manandhar *et al.* 1999, Manandhar *et al.* 2005, Simerly *et al.* 2016). Therefore, mice zygotes are thought to have no centrioles. It was proposed that centrioles form in the mouse embryo *de novo* at the 32/64-cell stage (Courtois *et al.* 2012), but *de novo* centriole formation is error prone and therefore unlikely (Wang *et al.* 2015). However, it is possible that the centrioles of murine spermatozoa are even more atypical than those of other mammals, potentially explaining why they are yet to be detected.

The DC microtubules

In mammals, the dogma was that centrosome reduction degenerates the DC, leaving a nonfunctional remnant in the spermatozoa near the PC. Consistent with this idea, most conventional electron microscopy studies found that the expected location of the DC lacks centriolar microtubules, has remnant microtubules or contains only the axoneme's central pair (Manandhar & Schatten 2000). In non-rodent mammals, Manandhar and Schatten found that epididymal and ejaculated spermatozoa had only a PC with a typical barrel-shaped structure with nine triplet microtubules. In contrast, two studies report that DC microtubules are present, albeit with highly modified organization, in the spermatozoa of humans, monkeys and rabbits (Zamboni & Stefanini 1971, Fishman *et al.* 2018). Together, these studies find that (1) the axoneme central microtubule pair extends through the remodeled DC, (2) the triplet microtubules become doublets, (3) the link found between the microtubule triplets that holds them tightly together is absent, and the microtubule doublets splay out and (4) the 8–9 doublets adopt a flattened cone shape. The presence of DC microtubules is verified by immunostaining microscopy against tubulin and acetylated tubulin in humans, suggesting that the microtubules are acetylated. Similar immunostaining is observed in other mammals such as bovine, monkeys, pigs and rabbits (our unpublished data), suggesting atypical DC microtubule organization is a general property of mammals.

The DC bars

Centrosome remodeling results in the appearance of electron-dense bars in the lumen of the DC visible using electron microscopy and rod-shaped aggregates of centrosomal proteins visible using immunofluorescent microscopy. These bars and rods are unique to the elongated spermatids and spermatozoa and are not found in other centrioles. While it is a logical expectation that the electron-dense bars and centriolar protein rods are the same structure, this needs verification. Below, we describe the distinct observations of the DC's bars and rods in various mammalian orders.

Primates/human

In human spermatozoon, high-pressure freezing, freeze substitution electron microscopy detects the DC bars (Fishman *et al.* 2018). Three centriolar proteins (CETN1/2, POC1B and POC5) exhibit a rod shape in the DC by super resolution microscopy. Both the human DC bars and rods are smaller than those observed in bovine (Fig. 4).

Laurasiatheria –ungulates (hoofed animals)

There are two ultrastructural studies describing the presence of two electron-dense bars in the mature spermatozoa of bovine and boar. One study used classical, chemically fixed, electron microscopy in boar spermatozoa (Nicander & Bane 1962). The other study uses electron cryotomography to generate a 3D structure

Figure 4 Various animal species exhibit some or all steps in DC remodeling. (A) Phylogenetic tree depicting the relative evolutionary position of snake, bovine, human and mouse. (B) Stages in DC remodeling during spermiogenesis of hamster. The centriole is penetrated by the central pair (CP), and then has splayed microtubules, then robust rods appear, then they reduce in size and finally, the microtubules and rods disappear. Whether there is a very atypical DC structure or no DC at all at the end of the remodeling is unknown. (C) The associated stages of DC remodeling in spermiogenesis of four animal species. The DC is progressively more modified in snake, bovine, human and mouse. The DC of snake spermatozoon is penetrated by the CP (Bii). The DC of bovine spermatozoa is penetrated by the CP, and also has splayed microtubules and robust rods (Biii). The DC of human spermatozoon is penetrated by the center pair, has splayed microtubules and the rods are present, but are of reduced size (Biv). The mouse appears to have no DC (Bv).

of the connecting piece in bovine sperm (Ounjai *et al.* 2012). Super resolution microscopy of the centriolar protein POC5 exhibits a rod-like shape in the DC with two major rods and one minor rod (Fishman *et al.* 2018). These studies suggest that the DC rods in ungulate spermatozoa are more robust than in humans (Fig. 4).

Rodents

In rodents, electron-dense DC bars are observed transiently during spermiogenesis, but not in spermatozoa (Fig. 4). The observation of the DC bars during spermiogenesis, in parallel to the other changes of the DC, is interesting as it provides insight into the process of DC rod formation. In guinea pigs, chinchilla and Chinese hamsters, the DC undergoes a dynamic multi-step remodeling process (Fawcett & Phillips 1969, Gordon 1972; Fig. 4B). Initially, like in typical cilia, the DC is connected to an axoneme, which is made of nine axonemal doublets, the two central microtubules (Fig. 4Bi). Then, the two central microtubules of the axoneme extend into the centriole lumen (Fig. 4Bii), the DC doublet-microtubules start to spread out, electron-dense bars form in the DC lumen near the two central microtubules, the DC's nine triplet microtubules become doublets and separate from each other slightly (Fig. 4Biii). The DC doublet-microtubules continue to spread out and the bars are reduced in size. Finally, in the testicular spermatozoon, the DC bars and microtubules disappear.

Gordon (1972) found that the electron-dense bars are surrounded by electron light material, referred to as 'internal cylinders.' This study used an uncommon staining protocol that includes exposure to ethanolic phosphotungstic acid after the glutaraldehyde fixation and acetylation, which may have allowed for this observation. Altogether, it appears that the electron-dense bars in the DC lumen are present transiently during mid-spermiogenesis. It is important to test if these bars have similar composition to the rods observed in other mammals, as this would support using mice spermiogenesis as a model for DC remodeling.

Basal tetrapod

Insight from a phylogenetic study of tetrapods may shed light on the origin of the DC bars. It is thought that the DC of the ancestral tetrapod had several novel traits (Healy & Jamieson 1994, Jamieson 2014). The DC was very long (several micrometers), extending all along the sperm midpiece. Each of the nine DC microtubule groups was made of two complete microtubules (the A and B microtubules) and an incomplete C-microtubule. The DC had the two central microtubules normally found only in the axoneme's center, which is an atypical property for centrioles. The two central microtubules associate with electron-dense material; this electron-dense material may be the ancestor of the DC bars. Since the electron-dense

material associates with the two central microtubules, it is possible that the two central microtubules have a role in the formation of the bar structure. Therefore, studying basal tetrapod DC formation may provide critical insight to the evolution of DC remodeling.

Is the mouse a good model for human centriole inheritance and function?

One perplexing aspect of the recent discovery of the human atypical DC is its apparent disagreement with the current literature in mice, rats and other rodents. Rodents appear to lack sperm centrioles due to centrosome reduction (Schatten *et al.* 1985, Manandhar *et al.* 1998). Furthermore, the centrosomes and the two large asters that are present in the zygote of humans and other non-rodent mammals are not present in the mouse zygote. Instead, like in the oocyte, the mouse zygote forms many mini asters that participate in the formation of an acentriolar spindle (Schatten *et al.* 1985, Calarco 2000, Coelho *et al.* 2013, Clift & Schuh 2015). This spindle is unlike the pointy spindle of non-rodent mammals in that it is barrel-shaped, with flat poles. These flat spindle poles recruit centrosomal proteins despite their lack of typical centrioles (Coelho *et al.* 2013). Furthermore, while the DC-attached base of the human and bovine sperm tail localizes to a spindle pole, the mouse's sperm tail does not localize to the spindle pole (Simerly *et al.* 1995b). Therefore, if mouse sperm contains an atypical centriole, it does not form a dominant sperm aster and is likely discontinuous with the axoneme, or it must be cleaved prior to the first mitosis, as was proposed in *Xenopus* (Fechter *et al.* 1996). Instead of centrioles, the microtubule bridges resulting from embryonic divisions seem to be the dominant microtubule-organizing centers (Zenker *et al.* 2017). Despite that, the mouse sperm tail is maintained up to the 16- to 32-cell stage (Simerly *et al.* 1993). Typical centrioles that act as microtubule-organizing centers appear in mice only at the blastula stage in an undescribed, presumably *de novo*, mechanism (Gueth-Hallonet *et al.* 1993, Coelho *et al.* 2013, Bangs *et al.* 2015).

While the data supporting typical centrioles appearing suddenly in rodent blastulas is significant, this idea that the centriole forms *de novo* is predominantly based on negative results and the *de novo* mechanism of centriole formation in the embryo is unknown. Also, studies on *de novo* centriole formation in tissue culture showed uncontrolled centriole number, which results in abnormal cell division (La Terra *et al.* 2005, Wang *et al.* 2015). Therefore, it is not clear how the correct number of centrioles is formed during rodent embryogenesis. Additionally, experiments in which a mouse sperm was injected into a cat egg resulted in a dominant sperm aster, as would be expected if the mouse sperm contained functional centrioles

(Comizzoli *et al.* 2006, Jin *et al.* 2012). This finding suggests that centriole's ability to form a centrosome and an aster in the zygote is determined by a maternal program, independent of the competency of the centrioles to organize microtubules. This idea is supported by experiments in which a cat sperm was injected into a mouse egg (Xu *et al.* 2011). In this study, the spindle appears barrel shaped (see Fig. 3 in that paper) regardless of the species of sperm injected, suggesting that the mouse egg's maternal program suppresses the formation of the sperm aster, independent of the presence of sperm centrioles. Together, these two cross-species fertilization studies lay the framework to suggest that in mice, the oocyte program that silences the centriole during meiosis continues to function in the early zygote. Indeed, it was proposed that mice show a gradual transition from the meiotic barrel-shaped spindle (observed in the oocyte) to a mitotic pointy spindle (observed in somatic cells) over several cell divisions (Courtois *et al.* 2012, Zenker *et al.* 2017). Perhaps then, the extension of the oocyte's centriole independence provided evolutionary flexibility, which ultimately resulted in an extensive centrosome reduction program in rodent sperm.

Alternatively, one can speculate that mouse sperm contains a highly remodeled, unrecognizable centriole precursor. A similar idea was proposed in the amoeba *Naegleria gruberi*. In this single cell protist, centrioles were also thought to form *de novo* (Fritz-Laylin & Fulton 2016), but recently, a new model was suggested based on an atypical centriole precursor (Lee *et al.* 2014, 2015). *Naegleria* has two forms: an amoeba and flagellate. The amoeba form undergoes mitosis and lacks recognizable centrioles. The flagellate is a differentiated and non-dividing form that has two centrioles (basal bodies) that appear during differentiation. These studies from the Lee lab suggest that the centrioles form from a preexisting centriole precursor, which bears no resemblance to a typical centriole. This precursor duplicates in the amoeba, thus explaining how a precise number of centrioles can be inherited without the preexisting typical centrioles. This model of inheritance in *Naegleria* is currently controversial, but it provides an alternative framework to study centriole inheritance in a system that lacks recognizable centrioles, such as mice.

In conclusion, the *de novo* model is currently widely accepted for rodent centriole inheritance, but it is worth noting that alternative explanations exist. The idea that rodent sperm may lack centrioles challenges the idea that centrioles are essential for microtubule organization during fertilization and early development. However, the role and implications of the human atypical centriole in maintaining centriole number during embryogenesis should not be overlooked since it is possible that rodents also possess atypical centrioles that maintain a precise centriole number.

What is the function of the remodeled centriole?

To form a developmentally competent zygote, a sperm must swim faster than other male's sperm and fertilize the oocyte, and, together with the oocyte, form a functional cell. Therefore, a logical hypothesis is that the remodeled sperm centrosome may have a role in one or more of these functions. In the spermatozoa, it was proposed that the remodeled DC functions as a 'transitional connecting centriole,' establishing continuity between the axoneme and the PC (Zamboni & Stefanini 1971). In this model, the PC is the 'basal body of the flagellum and the center of the sperm motility'. However, this model does not clarify the role of DC remodeling and how the remodeling helps in motility.

An insight into the function of the remodeled centriole may be gained from tracking the global change in sperm organization during evolution. It appears that flagellum symmetry correlates with fertilization types. In animals that use external fertilization, the flagellum has mostly radial symmetry (nine-fold symmetry). The only structure that does not have nine-fold symmetry is the central pair of microtubules found in the axoneme's central apparatus. This central pair is fixed in place relative to the axoneme and is important to generate planar movement (Ishijima *et al.* 1988, Nicastro *et al.* 2005, Gadelha *et al.* 2006). However, in animals with internal fertilization, the flagellum has increased bilateral symmetry (two-fold symmetry) (Lindemann & Lesich 2016). In addition to the presence of a central pair, the flagellum contains accessory structures, such as, fibrous sheath, outer dense fibers (ODF) and striated columns, which have bilateral symmetry. For example, the ODF exhibits bilateral symmetry because although there are nine lobes of electron-dense material, the third and eighth lobes are larger than the others. As a result, the overall structure of the mammalian connecting piece appears flattened within the plane of the flagellar beat (Ounjai *et al.* 2012). The accessory structures are believed to be passive elastic elements that influence the degree of flexibility, the plane of flagellar motion and the shape of the flagellar beat (Eddy *et al.* 2003, Lindemann & Lesich 2016). Importantly, the remodeled DC also appears to exhibit bilateral symmetry. This bilateral symmetry throughout the sperm tail, and particularly in the DC, may enhance the sperm's planar movement that may be suitable for navigating a complex environment such as the female reproductive tract.

In the zygote, the remodeled DC functions as a centriole, recruiting maternal PCM proteins, reconstituting a centrosome that forms an aster, and participating in spindle pole formation (Fig. 5). Also, the remodeled DC serves as a platform for the formation of a new centriole. These are normal centriolar functions, despite the DC's atypical appearance. But, the advantage of having an atypical centriole in the zygote is not clear. Studies in the fly suggest that centrosome remodeling is

Figure 5 Models of centriole inheritance in various animal species. Model of sperm centriole inheritance in (A) frogs, (B) human, (C) flies and (D) mice. Ax, axoneme; Ca, centriole adjunct; DC, distal centriole; PC, proximal centriole; PCL, proximal centriole-like structure; ZdC, zygotic daughter centriole.

essential for centrosome reconstitution, aster formation, normal fertility and normal embryo development, but did not point out the specific benefit (Khire *et al.* 2015). In mammals, one potential role of the atypical centriole is providing a signal for differential fates of the zygote's daughter cells; however, this has not yet been tested.

Therefore, an important future direction is to understand the role of the centriole remodeling in the zygote.

The first function of the sperm centrioles in the zygote is to reconstitute the centrosome that produces the large sperm aster. Astral microtubules are nucleated by γ -tubulin complexes (Kollman *et al.* 2011). Therefore, it is interesting

that the sperm centrioles lose their γ -tubulin complexes during centrosome reduction. One explanation may be that the biology of the spermatozoon and the zygote are so distinct that to better execute their specialized functions, they use different isoforms of the same protein. One way to replace the sperm-specific isoform with a zygote-specific isoform is by removing it during sperm centrosome remodeling and recruiting the proper isoform from the egg cytoplasm. In support of this idea, it was reported that during spermatogenesis, the fly expresses only the γ -tubulin isoform Tub23C. Then, the egg switches to expressing the Tub37CD isoform that continues to be present in early embryogenesis (Wilson *et al.* 1997).

In addition to the differences in the number, structure and composition of the gamete centrioles, the zygote deviates from classic centriole biology. Shortly after the sperm fuses with the egg (syngamy) (Fig. 6A), the sperm

centrioles recruit maternal PCM proteins to reconstitute a centrosome (Fig. 6B) before the centrioles duplicate (Fig. 6C), unlike in a classic cell cycle when the PCM is recruited to the centriole after procentriole formation, as part of centrosome maturation (Schatten 1994). The reconstituted centrosome that is found near the male pronucleus forms a large aster, known as the sperm aster (Longo & Anderson 1968); this is different from the classic cell cycle in which the mother centriole forms a cilium at this stage (Sanchez & Dynlacht 2016). In parallel to PCM recruitment and sperm aster formation, the egg that is arrested in metaphase of meiosis II, restarts meiosis and generates a new polar body and the female pronucleus. Having an acentriolar spindle dividing the female chromosomes while the centriole forms the sperm aster is a situation unique to the zygote and is not found in the normal cell cycle. Once the

Figure 6 The centrioles in the zygote. When the sperm fuses with the oocyte, the oocyte is arrested at meiosis II (A). Upon entry, the oocyte finishes the second meiotic division and extrudes the second polar body, while the sperm centrosome (DC and PC) is reconstituted and forms a large aster (B). Then, the maternal pronucleus migrates toward the paternal pronucleus and the centrioles begin duplication (producing two zygotic daughter centrioles), presumably while the DNA is duplicating (C). Next, the zygote centrosomes separate from each other while the pronuclei begin to merge (D). Then the zygote undergoes mitosis, much like a normal dividing cell (E), and this division results in two blastomeres (F), which will not form cilia like a normal cell in G0/G1. These two blastomeres will alternate between M and S phases; they will not form cilia or exhibit cytoplasmic growth until the blastocyst stage (Artus *et al.* 2006). The timing of the events in approximate hours post insemination and the similar phase (stage) in regular cell cycles are indicated and timing approximations are based on (Mio & Maeda 2008). The exact timing of centriole duplication is unknown; this drawing is based on parsimony and assumptions based on normal centriole duplication. 1N, 2N, 4N, chromosome ploidy; DC, distal centriole; PB, polar body; PC, proximal centriole; ZdC, zygotic daughter centriole.

sperm aster and female pronucleus are formed, the sperm aster facilitates the migration of the female and male pronuclei toward each other to allow karyogamy. This migration is thought to be mediated by cytoplasmic dynein motors that are attached to the pronuclei (Payne *et al.* 2003, Meyerzon *et al.* 2009, Tanimoto *et al.* 2016, Tanimoto *et al.* 2018).

Later in zygote development, around the time the two pronuclei are in apposition, the zygote enters an S phase-like state where chromosomes duplicate (Fig. 6C). At that time, each of the two sperm centrioles nucleate a new procentriole. Around that time, the two pairs of centrioles separate, each forming a centrosome with an aster. This separation is like a 'G2' phase in a dividing somatic cell (Fig. 6D).

Next, similar to 'M' phase, each centrosome localizes to a spindle pole in non-rodent mammals (Fig. 6E). One interesting difference from dividing somatic cells is that at metaphase, the asters in the poles are very small and they grow large during anaphase and telophase (Navara *et al.* 1994, Simerly *et al.* 1995a, Kim *et al.* 1996, Wu *et al.* 1996). The difference in aster size at different stages of human zygotic mitosis may be explained by a recent paper that suggested that mouse zygote mitosis starts with two spindles (Reichmann *et al.* 2018). It was found that mouse zygotes have both a male and a female spindle that function separately up until metaphase. During anaphase, the two spindles align, forming one spindle, where the paternal and maternal chromosomes are found in distinct groups (Fig. 5D). However, since the mouse's barrel-shaped spindle is so visually different than the pointy spindle of humans, it is possible that this two-spindle arrangement is unique to rodents. It has been proposed that the lack of recognizable centrioles in mice may be implicated in this two-spindle zygotic division, and therefore, it would be important to test if a two align spindles occurs in non-rodents zygotes that possess sperm centrioles (Zielinska & Schuh 2018). Interestingly, insects also keep the paternal and maternal genetic material separate during zygotic metaphase (Tram *et al.* 2002, Snook *et al.* 2011).

Finally, once the zygote divides, it gives rise to two daughter cells (blastomeres), which resume the canonical cell division cycle (Fig. 6F). Altogether, the zygote has many unique centriolar characteristics. It leaves an open question as to if atypical centrioles are an adaptation needed for the unique characteristics of the zygote or if they are an outcome of evolutionary pressure to make more efficient motile spermatozoa.

Concluding remarks

The discovery of an atypical centriole in the sperm of human and other non-rodent mammals begins to resolve a debate on centrioles in reproductive biology that has spanned decades. However, how rodents get their first embryonic centriole is still an enigma. This finding

necessitates new experimental and clinical adaptations. Basic researchers should address how atypical centrioles form and function, how the function of the two centrioles is coordinated and how both eventually form a typical centrosome. Evidence in animal studies suggests that the atypical centriole may be important for sperm motility and early embryonic development, but direct findings in humans are limited.

The discovery of the atypical centriole has several potential clinical implications:

- (i) A defect in the centrosome remodeling process could potentially be a new cause of reproductive diseases such as idiopathic infertility, early pregnancy loss and embryo development defects. Therefore, studies of the correlation between atypical centriole abnormalities and these conditions are needed.
- (ii) Some currently used treatments or procedures of male infertility (e.g., intracytoplasmic sperm injection (ICSI)) may, unknowingly, adversely affect the atypical sperm centriole. Additionally, these treatments and procedures may be insufficient to overcome defects in the atypical centriole. Therefore, studying the atypical centriole during ICSI and developing a test to diagnose abnormalities in the atypical centriole might improve ICSI success rates.
- (iii) Interfering with atypical centriole formation could potentially be a target for male contraception. Therefore, identifying sperm-specific regulators of atypical centriole formation would be significant.

With all these potential roles of the atypical centriole and its implications in existing therapies, an important future research direction is to better understand the fundamental cellular processes that form the sperm's atypical centriole, as well as sperm centriole-associated reproductive diseases.

Due to the nature of this work, collaborative projects between clinicians and basic researchers are imperative to determining the impact of the atypical DC on fertility and reproduction, and also to capitalize on any findings to improve fertilization, pregnancy, healthy births and contraception. The discovery of a new sperm atypical centriole opens the door to new research directions and holds potential to lead to exciting innovative approaches in reproductive biomedicine.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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