Ultrastructure of Human Spermatozoa

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3.1 Introduction

The sperm is a complex cell with a specialized function and structure compared to other cells of the body. Sperm cells are transcriptionally and translationally inactive; its DNA is tightly condensed in an almost crystalline state, packaged by protamines, and is produced in large numbers. Its objective is to deliver the intact haploid genome to the oocyte at the site of fertilization. The sperm must conserve the DNA, transport it to the site of fertilization, recognize the egg and start the process of fertilization. The human sperm migrates from the site of deposition – vagina, through the cervix into the uterus and then to the site of fertilization – ampullary region of the fallopian tube. During the travel, it completes its process of functional maturation – a process termed as *capacitation*.

To accomplish these processes, the sperm has a highly specialized structure. The structure of the sperm was first described in 1677 by Antonie van Leeuwenhoek. Advances in microscopy and the optics have immensely improved our knowledge on the description made by van Leeuwenhoek. While light microscopy can show major abnormalities in the sperm, it cannot reveal information about the submolecular structures. Electron microscopy and transmission electron microscopy have provided the much-needed details on the ultrastructure of sperm. The human spermatozoon can be grossly divided into two regions – the head and the flagellum (the tail).

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K. Gunasekaran, N. Pandiyan (eds.), *Male Infertility*,
DOI 10.1007/978-81-322-3604-7_3

3.1.1 Flagellum

The spermatozoa are typically 'stripped-down' cells with a long flagellum to propel them. It is devoid of organelles such as endoplasmic reticulum or ribosomes (Alberts et al. 2002). The tail or the flagellar region of the sperm is designed for motility of the sperm. The flagellar structure that serves as the 'tail' of the sperm is complex in higher vertebrates such as humans. The tail of the sperm can be divided into four regions (Fawcett 1975) with distinct anatomy related to their function:

- The short connecting piece
- The midpiece
- The principal piece
- The terminal piece

The structure of flagellum has:

1. The axoneme which runs throughout the length of the flagellum
2. The outer dense fibres (ODFs) which surround the axoneme in the midpiece and principal piece
3. The mitochondrial sheath (MS), which is located in the midpiece
4. The fibrous sheath (FS) which is located in the principal piece

Understanding the proteins which make up each of these flagellar structures and how these proteins interact to produce the normal flagellar beat would throw light on understanding the molecular genetics behind the reduced sperm motility in infertile animals, and humans.

3.2 Axoneme

Axoneme of the axial filament stretches across the full length of the flagellum and constitutes the motor apparatus of the sperm tail. The organization of the axoneme is similar to that of cilia and flagella of all eukaryotic cells. It has two central microtubules surrounded by nine evenly spaced microtubular doublets – the classic 9+2 pattern. The nine peripheral doublets are numbered in a clockwise direction from 1 to 9. The first doublet is situated on a place perpendicular to that of the two central microtubules. Each doublet consists of an A subunit with a complete microtubule of 26 nm in diameter and a B subunit which is an incomplete microtubule (C shaped in cross section) that is attached to the A subunit. Tubulin is the structural component of the microtubules (Farrell 1982; Curry and Watson 1995). The A tubule is made of 13 protofilaments that are aligned side by side. The B tubule is made from 10 protofilaments. Extending from A microfilament to B microfilament are 'arms' which play a crucial role in flagellar movement. The principal component of the arms is dynein (Porter and Johnson 1989; Holzbaur and Vallee 1994; Milisav 1998). Activation of the axonemal dynein ATPase results in sliding of adjacent outer

doublet microtubules and it has been proposed that these sliding results in flagellar bending (Tash and Means 1982). The doublets are connected by the protein nexin (Clermont et al. 1990). The central microtubules are interconnected by linkages and are surrounded by a pair of spiral fibres that are attached to the microtubules. The spiral fibres form the central sheath from which radial spokes go out to the A subunit of the doublet (Pederson 1970).

3.3 Connecting Piece

The connecting piece connects the flagellum and the sperm head. It is about 0.5 μ in length and consists of:

1. Capitulum
2. Segmented columns

The capitulum is a dome-shaped fibrous structure. Proteinaceous filaments run between the capitulum and the caudal surface of the nucleus. The capitulum is the site of the centrioles. It is positioned at right angles to the axis of the flagellum. Only the proximal centriole is seen in a mature spermatozoon as the distal centriole disintegrates during spermiogenesis. The centriole plays a major role in the formation of the axoneme during spermiogenesis and also a major part in fertilization and the events following fertilization. The structure of the centriole is the same as that of an axoneme but without the central pair of microtubules and has peripheral triplet in place of doublets.

The segmented columns fuse proximally into major and minor columns and attach to the capitulum. The columns attach to the outer dense fibre in the midpiece distally.

3.4 Centrosome

As early as 1887, it was postulated by Theodor Boveri that the oocyte has all elements required for embryonic development except the active division centre (Baltzer 1967). In a somatic diploid cell, the mitotic spindle is the key to the distribution of the genomic material. The spindle is derived from the centrosome. The centrosome and centriole are a part of the MTOC (microtubule-organizing centre). The centrosome consists of two centrioles and the pericentriolar material (PCM). The centriole is a pair of cylinders arranged perpendicularly, whereas the aster and the spindle fibres are derived from PCM (Palermo et al. 1994). The centriole displays the distinct 9 + 0 pattern of nine triplet microtubules. This differs from the axoneme by the absence of the central pair of microtubule. Earlier reports suggested that mammalian gametes lack centrioles. It was proven beyond doubt that centrioles are indeed part of the mitotic division in humans (Sathananthan et al. 1991) and in other species too (Guen and Crozet 1989). Sathananthan et al. described in detail the

anatomy of centrioles in human reproduction. They showed that the human oocyte does not possess any centriole and the sperm has two centrioles – proximal and distal. The proximal centriole is located in the connecting piece, next to the basal plate of the sperm head. It has a pinwheel structure of nine microtubules surrounded by electron dense material referred to as the 'black box'. The distal centriole is located perpendicular to the proximal and is aligned with the flagellum that forms the axoneme during spermiogenesis (Sathananthan et al. 1991; 1996).

The two major functions of the centrosome are (1) nucleation of microtubule and (2) mitotic spindle formation (Schatten 1994; Bornens et al. 1990). In all mammalian species, except the mice, the sperm centrosome nucleates the aster. This brings about the apposition of the female and male pronuclei. It has been demonstrated that injection of the tail alone can induce aster formation (Van Blerkom and Davis 1995). After the fusion of the gametes, the tail of the sperm is incorporated into the ooplasm. The centriole duplicates during the pronuclear stage. Centrioles have been detected up to the stage of blastocyst (Sathananthan et al. 1996). In humans, only the male gamete has an active centrosome and is the structure responsible for the first mitotic division (Palermo et al. 1994).

3.5 Outer Dense Fibre

The axoneme of the sperm is surrounded by the outer dense fibre. Each of the peripheral microtubule doublets has an outer dense fibre. They are individual fibres that are teardrop shaped, with an outer rounded edge that tapers towards the axoneme. Cranially, these fibres fuse with the connecting piece. These are suggested to facilitate sperm movement, mediated by protein phosphorylation, and serve as protector of the sperm during its passage in the male and female tracts (Tash and Means 1983). It is also suggested to act as the stiffening rods within the sperm tail.

3.6 Midpiece

The midpiece of the flagellum is about 3.5 μm in length and runs from the distal end of the connecting piece to the annulus. It is an electron dense circumferential band marking the junction between the midpiece and principal piece. A recent report by Guan and colleagues in BMC Developmental Biology 2009 described the development of the annulus, in the formation of the mature spermatozoon. Its function has not clearly been established, but it may constitute a diffusion barrier between the two compartments and/or facilitate mitochondria migration and alignment along the axoneme.

The midpiece has a mitochondrial sheath with a species-specific number of mitochondria. The mitochondria are arranged in a helical pattern around the axoneme. The human spermatozoon has a helix composed of 11–15 gyres. The mitochondrial structure in the sperm is the same as that in other cell types but has greater stability.

It is resistant to osmotic changes which might help resist stretching and compression of the mitochondria during flagellar beat. It is the site of energy production of spermatozoa and its position allows ready supply of ATP to the axoneme. The flagella activity requires energy which is obtained in the form of ATP. The ATP is supplied by the mitochondria and is hydrolyzed by ATPase in the dynein arms in the presence of magnesium.

3.7 Principal Piece

This is the longest flagellum extending from the annulus to the proximal end of the terminal piece. It is approximately 55 μm in length. It has a fibrous sheath which is the cytoskeletal structure surrounding the axoneme and the outer dense fibres. The sheath consists of two columns that is circumferentially connected by a series of closely packed filaments called ribs. In the human sperm, the ribs are 10–20 nm apart and 50 nm thick. The function of the fibrous sheath appears to be similar to that of outer dense fibres – to act as stiffening rods and provide rigid support to the flagellum and determining its planar beat (Lindemann et al. 1992). It has been seen that sperms with disorganized fibrous sheath have disrupted motility indicating its importance in motility of the sperm.

3.8 Terminal Piece

Beyond the fibrous sheath is the terminal piece of the flagellum, which is about 3 μm in length. The microtubules of the axoneme terminate in this region. The dynein arms disappear first and the A subunit takes a hollow appearance. The central pair of the microtubules terminates, after which two of the peripheral outer doublets move to the centre. The doublets separate and the open B tubule disappear. The pattern that remains is a single central tubule surrounded by a circle of single microtubule covered by plasma membrane.

3.8.1 Flagellum: Role in Infertility

The connection between male infertility and ciliopathy was first disclosed by the observation of a common ultrastruc-tural abnormality in sperm flagella and epithelial cilia in patients with Kartagener syndrome (Camner et al., 1975).

Defects in the axonemal structure of the sperm causes defects in motility, and often leads to male subfertility. These affect fertilisation. Male infertility is linked with symptoms or diseases such as Kartagener syndrome or cystic fibrosis. These result in a deficiency in the components of cilia and flagella, they are called “immotile cilia syndrome” or “primary ciliary dyskinesia,” or more recently, “ciliopathy,” which includes deficiencies in primary and sensory cilia.

A recent study by Mitchell et al in 2012, had assessed the morphology of the sperm flagellum by light microscopy and compared it to axonemal deficiencies. The study included 41 patients with known axonemal abnormalities. They could correlate flagellar abnormalities to axonemal deficiencies such as correlations between missing outer dynein arms and abnormal, short or coiled flagellum. There found a negative correlation between misassembly and spermatozoa of irregular flagella. They concluded that light microscopy analysis of flagellar abnormalities may help to identify correct diagnosis and sperm abnormalities.

The green alga Chlamydomonas has been used to generate many useful mutants for studying cilia and flagella, including mutants for the outer and inner dynein arms and other substructures (Kamiya, 2002). In animals, sperm from marine invertebrates have contributed to our understanding of the molecular architecture of flagella and the mechanism of their motility.

In humans, most such cells are multiciliated are seen in nasal and trachea epithelia , the lachrymal sac, brain ventricles (ependymal cilia), male efferent ducts and female oviducts (Fallopian tubes). The structures of the axonemes in motile multicilia have a 9 + 2 micro- tubule lattice with dynein arms, apparently the same as those in sperm flagella, although there are several reports showing minor differences in their components. The cilia is found in many parts of the male reproductive system. They are seen in epididymis, vas deferens as well. Atleast 20n genes have been identified thaqt result in ciliopathy (Inaba and Mizuno, 2016).

Knockout mice are powerful models for studying ciliopathy. However, in most cases, loss of axonemal dyneins results in embryonic lethality. This is not compatible with the symptoms of human ciliopathy, in which patients are alive and show fertility, making it possible to carry out family studies.

3.8.1.1 Centrosome

Since centrosome is critical, for the event of fertilization, the study of centrosome is relevant especially in the scenario of failed fertilization and other such clinical scenarios. There were studies by Sathananthan where he reported that incidence of centriolar abnormalities were more in immotile and non-progressively motile spermatozoa (Sathananthan et al. 1996).

Structural defects in the flagellum that have been noted are changes in the composition or number of axonemal microtubules, impairment of dynein arms. The absence of dynein arms is one of the frequent causes of sperm immobility. Disorganization of the mitochondrial sheaths or absence can impair sperm motility. A lack of ATP can also lead to sperm degeneration (Kupker et al. 1998).

3.8.1.2 Head

The function of the head of the sperm is well defined – it is to conserve the paternal DNA and deliver it to the oocyte at the time of fertilization. To achieve this, the following are indispensible:

1. The DNA should be held in a stable form.
2. The sperm should penetrate the oocyte surface.
3. Capability of membrane fusion.

The basic structure of head of the sperm is common to all mammalian species, but there can be differences in shape and size of the nucleus and the acrosome. The head of the human spermatozoa is called pleomorphic. The sperm head is about 4.5 μm in length and it is about 3.5 μm at its widest part and is slightly flattened.

3.9 Acrosome

The acrosome, which is derived from the Golgi apparatus, is a membrane-bound vesicle forming a cap-like covering on the cranial part of the nucleus. In humans, the acrosome is relatively small and covers about two third of the nucleus. This results from the topographic development over the Sertoli cell cytoplasm in the late stages of spermiogenesis, by contracting the nucleus to result in its unique shape. The acrosome has two membranes – the outer and the inner. The inner membrane is right below the plasma membrane and continues as the inner acrosome membrane which lies above the nuclear envelope. The membranes are parallel to each other and the space in between them is filled with acrosomal matrix (Huang and Yanagimachi 1985). The matrix has hydrolytic enzymes such as hyaluronidase and acrosin, a proteinase in inactive zymogen form – proacrosin and a second zymogen called sperminogen (Siegel et al. 1987). Other enzymes that are present in acrosomal matrix are acid phosphatase, phospholipases, N-acetylglucosaminidase and collagenase.

Eutherian sperms have an equatorial region which is situated at the posterior border of the acrosomal cap. The acrosomal membranes in the equatorial segment are electron dense. There is no acrosomal matrix in this segment. It is the site of accumulation of vimentin (Virtanen et al. 1984). It is hence a stable membrane structure which plays the role of stabilizing this critical region during in acrosome reaction and zona penetration. It is at the border between the anterior acrosome and the equatorial segment; thus, when the acrosome fuses with the plasma membrane, the integrity of the sperm cell is maintained.

There have been many theories of penetration of the sperm, such as enzymatic or mechanical or both. The very narrow, sharply defined penetration slit made by the sperm in the zona does not conform to the purely enzymatic penetration. Evidence has also suggested that by thrust force alone, the sperm cannot penetrate the zone. Hence, the use of both mechanisms has been proposed (Green 1988).

3.10 Perinuclear Material

The perinuclear material is situated between the acrosome and the nucleus. It is a thin layer stabilized by disulphide bonds. It acts like cement between the acrosome and nucleus. Posterior to the acrosome, the perinuclear material forms the acrosome sheath. This structure is composed of two distinct regions separated by a shallow grove. The anterior region has an electron dense material parallel to the plasma membrane, which has a series of rounded projections extending towards the plasma

membrane (Pedersen 1972b). The posterior region of the sheath has granular material with oblique cord-like structures (Koehler 1972).

3.11 Nucleus

The nucleus of the sperm has a haploid set of chromosome. It is the result of the process of spermiogenesis, the specialized process that changes the round spermatids into a mature sperm with its distinct shape. The size of the nucleus is decreased in the process by chemical and architectural modifications. The DNA in the sperm is complexed with protamines which lead to chromatin condensation. The DNA remains inactive and does not replicate until the protamines disintegrate after the entry into the oocyte (Johnson and Lalancette 2010). The protamine, which allows packaging of the sperm chromatin, neutralizes the charges of the phosphate ester backbone in the DNA. There are disulphide bonds between free thiols which give the nucleus its highly stable keratinoid nature.

Nucleus shape is species specific and is determined by the sperm genotype. Human sperms exhibit a variety of nuclear shapes. The heterogeneity in nuclear shape may be due to the difference in chromatin condensation. The posterior ring is a circumferential junction between the plasma membrane and nuclear envelope with a series of striations (Pedersen 1972b). It is a diving point between the flagellum and head. The implantation fossa of the sperm tail is situated at the base of the nucleus.

The nucleus of the sperm is extremely well packaged. The genome is repackaged into a crystalline state. During the condensation process, there is elimination of RNA, replacement of somatic histones by protamines and formation of chromatin-stabilizing disulphide bonds (Balhorn et al. 1984). Though most histones are replaced by protamines, there are some areas where the somatic-like structure is retained. In some cases, these regions are differentially marked by modified histones in a manner reminiscent of the epigenetic states observed in somatic or stem cells (Hammoud et al. 2009). This may influence the order in which genes are repackaged into a nucleosomal bound state and/or expressed following fertilization (Rousseaux et al. 2008). Recent research has led to believe that there could be more to sperm than just delivering the paternal DNA. The role of the three main structural genetic elements of the sperm nucleus, chromatin, RNA, and the nuclear matrix beyond the sperm nucleus, has been suggested by researchers which may have an impact on embryonic development.

3.12 Cytoplasmic Droplet

It is the residual cytoplasm from the process of spermiogenesis. It forms a collar shaped covering of the midpiece of the flagellum. It usually has redundant organelles such as ribosomes and mitochondria.

3.13 Plasma Membrane

The plasma membrane forms the outer boundary of all cells. It maintains the cell integrity and functions as a dynamic interface between the cell and its environment. The plasma membrane of the sperm has regional specialization with specific physical, chemical and immunological parameters. It also undergoes reorganization during its transport in the female reproductive tract during the process of capacitation.

There are five domains in the plasma membrane of the sperm, depending on the part of the sperm it is associated with. The head of the sperm has three domains covering the acrosome, equatorial region and the post acrosomal region. The flagellum has two domains covering the principal piece and the midpiece. The domains are different in their affinity for plant lectins, difference in the distribution of glycocalyx, membrane fluidity, lipid composition, intra-membranous particle distribution, binding pattern for monoclonal antibodies and membranous surface charge (Koehler 1981; Friend 1982; Villaroya and Scholler 1986 and Yanagimachi et al. 1972). The specialization allows for more efficient performance of tasks which culminates in fertilization.

3.14 Midpiece Region

The plasma membrane overlying the mitochondria contains chains of particles that follow the mitochondrial helix. In the interstices, the particles are absent and hence the plasma membrane is closely applied to the mitochondrial sheath (Philips 1970). These particles were first discovered in guinea pig by Friend and Fawcett in 1974. The main function of the plasma membrane over the mitochondrial sheath is to permit substances that are of significance in metabolism during capacitation.

3.15 Principal Piece Region

The axonemal complex is attached to the plasma membrane through a ‘zipper’, which is a longitudinal double row of staggered intra-membranous particles that overlay the dense outer fibre number 1 (Friend and Fawcett 1974). The particles of the zipper attach to the ribs of the fibrous sheath. They may have a role in controlling the axonemal functions such as the change in the flagellar beat during capacitation. The membrane of the midpiece is separated from the principal piece by the annulus.

3.16 Anterior Acrosomal Region

The plasma membrane covering the anterior acrosome has a major role in fertilization. It recognizes and binds to the zona pellucida and fuses with the outer acrosomal membrane during cortical exocytosis, during acrosome reaction. The presence

of glycocalyx is high in this region of plasma membrane. The membrane in this region exhibits fusogenic properties, which facilitates the processes in the initiation of the acrosome reaction. At the end of the acrosome reaction, the inner acrosomal membrane becomes the limiting membrane of the anterior region of the head.

3.17 Equatorial Region

This is the site of fusion of the plasma membrane with the oocyte. The plasma membrane in this region is stable. Physiological changes occur in the plasma membrane in this region around the time of acrosome reaction, without much of structural changes, as sperm oocyte fusion cannot happen without the acrosome reaction.

3.18 Post-acrosomal Region

In humans, this part consists of indistinct striations posteriorly and an anterior domain consisting of an irregular smooth ridge around the base of the acrosome (Pedersen 1972a, b).

3.18.1 Head: Role in Infertility

Pathology of acrosome has been most commonly implicated in causing subfertility in males. This could include partial or complete lack of acrosome or disorganization of the acrosome membrane. This will result in the alteration in the shape of the sperm head commonly called the round sperm head. This could also implicate a failure in the late stages of spermiogenesis. This will cause inability of the sperm to elicit an acrosome reaction, hence being unable to penetrate the zona pellucida. When all the sperms in the semen sample have a round head, it is termed globozoospermia. When there is separation of the flagellum from the head, the resulting structure is called the pin head (Zaneveld and Polakowski 1977).

The latest abnormality that has come as another armamentarium in the hands of reproductive specialists is the sperm DNA fragmentation. This is an abnormality when there are incomplete chromatin condensations displaying single-stranded DNA rather than double-stranded DNA (Johnson et al. 2011). This has been implicated in IVF failures, poor embryonic development and hence male subfertility.

Conclusion

A spermatozoon is a highly specialized cell. The importance of sperm has been never more highlighted than in the present age of advanced reproductive medicine. Though the morphological criteria have been recommended by WHO, the understanding of ultrastructure of sperm will contribute to the diagnosis and treatment conditions which contribute to subfertility.

As for correlation between ultrastructure of sperm and fertilization capacity in IVF, Alebit et al. in 1992 concluded that sperm alterations in tail do not affect the fertilizing capacity, whereas decondensation of nuclear chromatin regardless of acromosal defect was mentioned as one of the major reasons for IVF failure by Chitale and Rathur as early as 1995. In the era of ICSI, there have been no conclusive studies correlating the severity of sperm defects to success in ICSI. Yet again, the sperm remains as elusive as ever!

References

Alberts B, Johnson A, Lewis J, et al. Molecular biology of the cell. 4th ed. New York: Garland Science; 2002.

Balhorn R, Weston S, Thomas C, Wyrobek A. DNA packaging in mouse spermatids. Synthesis of protamine variants and four transition proteins. Exp Cell Res. 1984;150:298–308. doi:10.1016/0014-4827(84)90572-X.

Baltzer F. Theodor Boveri; Life and Work of a Great Biologist, 1862–1915. Univ of California Press; 1967.

Barratt CL, Kay V, Oxenham SK. The human spermatozoon – a stripped down but refined machine. J Biol. 2009;8(7):63.

Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007;22:1506–12. doi:10.1093/humrep/dem046.

Bornens M, Bailly E, Gosti F, Keryer G. The centrosome: recent advances on structure and functions. Prog Mol Subcell Biol. 1990;11:86–114.

Camner P, Mossberg B, Afzelius BA. Evidence of congenitally nonfunctioning cilia in the tracheobronchial tract in two sub-jects. Am Rev Respir Dis. 1975;112:807–9.

Clermont Y, Oko R, Hermo L. Immunocytochemical localization of proteins utilized in the formation of outer dense fibers and fibrous sheath in rat spermatids: an electron microscope study. Anat Rec. 1990;227:447–57.

Curry MR, Watson PF. Sperm structure and function. In: Gemetes- the spermatozoon. New York: Press Syndicate of the University of Cambridge; 1995. p. 45–70.

Farrell KW. Purification and reassembly of tubulin from outer doublet microtubules. Methods Cell Biol. 1982;24:61–78.

Fawcett DW. The mammalian spermatozoon. Dev Biol. 1975;44:394–436. Guan J, Kinoshita M, Yuan L. Spatiotemporal association of DNAJB13 with the annulus during mouse sperm flagellum development. BMC Dev Biol. 2009;9:23.

Friend DS. Plasma membrane diversity in a highly polarized cell. J Cell Biol. 1982;93:243–9.

Friend DS, Fawcett DW. Membrane differentiation in freeze-fracture mammalian sperm. J Cell Biol. 1974;63:641–64.

Green DPL. Sperm thrusts and the problem of penetration. Biol Rev Camb Philos Soc. 1988;63:70–105.

Guan J, Kinoshita M, Yuan L. Spatiotemporal association of DNAJB13 with the annulus during mouse sperm flagellum development. BMC Dev Biol. 2009;9:23. doi:10.1186/1471-213X-9-23.

Guen LE, Crozet N. Microtubule and centrosome distribution during sheep fertilization. Eur J Cell Biol. 1989;48:239–49.

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460:473–8. doi:10.1038/nature08162.

Holzbaur EL, Vallee RB. DYNEINS: molecular structure and cellular function. Annu Rev Cell Dev Biol. 1994;10:339–72.

Huang TTF, Yanagimachi R. Inner acrosomal membrane of mammalian spermatozoa: its properties and its possible function in fertilization. Am J Anat. 1985;174:249–68.
Johnson GD, Lalancette C. The sperm nucleus: chromatin, RNA, and the nuclear matrix. Reproduction. 2010;141(1):21–36. doi:10.1530/REP-10-0322.
Johnson GD, Lalancette C, Linnemann AK, ´de´ric Leduc F, Boissonneault G, Krawetz SA. The sperm nucleus: chromatin, RNA, and the nuclear matrix. Reproduction. 2011;141:21–36.
Kamiya R. Functional diversity of axonemal dyneins as studied in Chlamydomonas mutants. Int Rev Cytol. 2002;219:115–55.
Kazuo Inaba, Katsutoshi Mizuno. Sperm dysfunction and ciliopathy. Reproductive Medicine and Biology 2016;15:77–94.
Koehler JK. Human sperm head ultrastructure: freeze etching study. J Ultrastruct Res. 1972;39: 520–39.
Koehler JK. Lectins as probes of the spermatozoon surface. Arch Androl. 1981;6:197–217.
Kupker W, Schulze W, Diedrich K. Ultrastructure of gametes and intracytoplasmic sperm injection: the significance of sperm morphology. Hum Reprod. 1998;13 Suppl 1:99–106.
Lindemann CB, Orlando A, Kanous KS. The flagellar beat of rat sperm is organized by the interaction of two functionally distinct populations of dynein bridges with a stable central axonemal partition. J Cell Sci. 1992;102:249–60.
Milisav I. Dynein and dynein-related genes. Cell Motil Cytoskeleton. 1998;39:261–72.
Mitchell V, Sigala J, Ballot C, Jumeau F, Barbotin AL, Duhamel A, Rives N, Rigot JM, Escalier D. and Peers MC. Light microscopy morphological characteristics of the sperm flagellum may be related to axonemal abnormalities. Andrologia. 2015;47:214–220. doi: 10.1111/and.12249
Palermo G, Munne S, Cohel J. The human zygote inherits its mitotic potential from male gamete. Hum Reprod. 1994;9:1220–5.
Pedersen H. Further observations on the fins structure of the human spermatozoa. Z Zelforsch. 1972a;123:305–15.
Pedersen H. The postacrosomal region of the spermatozoa of man and Macacaarctoides. J Ultrastruct Res. 1972b;40:366–77.
Pederson H. Observations on the axial filament of the human spermatozoa. J Ultrastuct Res. 1970; 33:451–62.
Porter ME, Johnson KE. Differential localization of two isoforms of the regulatory subunit RII alpha of cAMP-dependent protein kinase in human sperm: biochemical and cytochemical study. Annu Rev Cell Dev Biol. 1989;5:119–51.
Rousseaux S, Reynoird N, Escoffier E, Thevenon J, Caron C, Khochbin S. Epigenetic reprogramming of the male genome during gametogenesis and in the zygote. Reprod Biomed Online. 2008;16:492–503. doi:10.1016/S1472-6483(10)60456-7.
Sathananthan AH, Kola I, Osborna J, Trounson A. Centrioles in the beginning of human development. Proc Natl Acad Sci U S A. 1991;88:4806–10.
Sathananthan AH, Ratnam SS, Ng SC, Tarin JJ, et al. The sperm centriole: its inheritance, replication and perpetuation in early human embryos. Hum Reprod. 1996;11:345–56.
Schatten G. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev Biol. 1994;165:299–335.
Siegel MS, Bechtold DS, Willard JC, Poakoski KL. Partial characterization and purification of human sperminogen. Biol Reprod. 1987;36:1063–8.
Tash JS, Means AR. Regulation of protein phosphorylation and motility of sperm by cyclic adenosine monophosphate and calcium. Biol Reprod. 1982;26:745–63.
Tash JS, Means AR. Cyclic adenosine 3′5′ monophosphate, calcium and protein phosphorylation in flagellar motility. Biol Reprod. 1983;28:75–104.
Van Blerkom J, Davis P. Evolution of the sperm aster microinjection of isolated human sperm centrosomes into meiotically mature human oocytes. Hum Reprod. 1995;10: 2179–82. Virtanen I, Bradley RA, Paasivuo R, Lehto VP. Distinct cytoskeletal domains revealed in sperm cells. J Cell Biol. 1984;99:1983–91.
Villaroya S, Scholler R. Regional heterogeneity of human spermatozoa detected with monoclonal antibodies. J Reprod Fertil. 1986;76:435–47.

Virtanen I, Bradley RA, Paasivuo R, Lehto V-P. Distinct cytoskeletal domains revealed in sperm cells. J Cell Biol. 1984;99:1983–91.

Yanagimachi R, Noda YD, Fujimoto M, Nicholson G. The distribution of negative surface charges on mammalian spermatozoa. Am J Anat. 1972;135:497–520.

Zaneveld LJD, Polakowski KL. Collection and physical examination of the ejaculate. In: Hafez ESE, editor. Techniques of human andrology. Amsterdam: Elsevier-North Holland; 1977. p. 147–72.