1 Essentials of Sperm Biology

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INTRODUCTION

That the spermatozoon has a role in reproduction has been known for centuries, but the specifics of its contribution have only come to be understood in relatively recent times. Antoni van Leeuwenhoek was the first to describe "semen animals" (spermatozoa) in an ejaculate in 1677, and in 1679, he discovered the presence of spermatozoa in the *vas deferens* and testicular tissue, leading him to conclude that sperm production was the sole purpose of the testis *(1)*. In 1683, he wrote that he was certain "that man comes not from an egg but from an animalcule in the masculine seed," and in 1685, he concluded that each spermatozoon contained both a person in miniature and a persistent and living soul. This argument was in contradiction to Harvey's and de Graaf's hypotheses that it was the egg that contained the miniature and entire human, and the semen was merely the vehicle of a stimulating spirit that started the growth of the egg into the embryo.

Acceptance of the equal role of the spermatozoon and oocyte in reproduction began in the latter part of the 19th century, and the understanding of the structure and function of spermatozoon was developed during the 20th century, following improvements in the technology of microscopy and the identification of DNA as genetic material.

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This chapter aims to provide an overview of the biology of the spermatozoon, including its role in fertilization.

SPERM PRODUCTION

Spermatozoa are highly specialized haploid cells produced in the testes of adult males. Each testis is comprised largely of convoluted seminiferous tubules, which are packed loosely into lobes separated by bands of fibrous tissue. Each seminiferous tubule is a loop, with its ends draining into the rete testis, and then into the efferent ducts and epididymis. Within each lobe, the seminiferous tubule is enclosed by loose connective tissue containing blood vessels, nerves, and lymph vessels, as well as the Leydig cells, which produce testosterone in response to luteinizing hormone.

Seminiferous tubules of adult men have a stratified epithelium of several layers and a central lumen. The basal compartment is the outermost part of the tubule contents and contains the resting spermatogonia (the basic self-renewing stem cell of the male germline) and Sertoli cells, which act to regulate the development of the germinal cells along with the maturation and release of spermatozoa into the central lumen of the seminiferous tubule. Each Sertoli cell is joined to its neighbors by tight junctions, forming the blood–testis barrier, which prevents the passage of molecules between the basal and luminal compartment of the seminiferous tubules. Another effect of the blood–testis barrier is to prevent the initiation of an immune response to spermatozoa by the man's immune system, because these antigenically "foreign" cells are not produced until puberty.

Spermatozoa are produced from their progenitor spermatogonia in a series of processes termed "spermatogenesis," "spermiogenesis," and "spermiation." Spermatogenesis begins with six cycles of mitotic proliferation of a diploid spermatogonium, resulting in the production of a clone of daughter cells—the primary spermatocytes. The primary spermatocytes then leave the basal compartment and push into the adluminal compartment of the seminiferous epithelium, where they enter the meiotic prophase. At the completion of prophase, each primary spermatocyte produces two sondary spermatocytes, each of which then divides to produce two haploid spermatids. Because all of these cell divisions are not complete, the 64 spermatids created from each spermatogonium form a syncytium; i.e., they are all interconnected by cellular bridges of cytoplasm. Spermatids are still round cells at this stage, and they differentiate into mature spermatids or testicular spermatozoa in the process of spermiogenesis.

Spermiogenesis is a complex series of maturational and transformational processes that occur in the adluminal compartment of the seminiferous tubules. In this process, the nuclear histones are replaced by protamines, which then

become crosslinked by disulphide bonds, making the chromatin highly condensed and resulting in a much smaller nucleus. The nucleus then moves into an eccentric position, closer to the cell membrane. The acrosome is generated by the Golgi complex, and this is applied to the part of the nucleus in contact with the cell membrane. One of the centrioles attaches to the opposite side of the nucleus and produces the axial filament, around which the axonemal structures develop to form the axial filament complex. Cytoplasmic reduction occurs, and the mitochondria become arranged around the proximal portion of the developing tail.

At the completion of spermiogenesis, spermatozoa are released, tail-first, into the lumen of the seminiferous tubule. The residual cytoplasm is pinched off at the neck region of the mature spermatid as it leaves the seminiferous epithelium in a process termed "spermiation." Following spermiation, the mature spermatids are transported to the rete testis and then to the epididymis where they undergo posttesticular sperm maturation, a series of morphological, biochemical, biophysical, and metabolic changes. The epididymis can be divided into three functional regions: the caput (head), corpus (body), and cauda (tail), with the general functions of sperm concentration, maturation, and storage, respectively. At the end of their transport through the epididymis, spermatozoa have acquired the ability to become motile when they come into contact with seminal fluid at ejaculation.

By definition, spermatozoa are not functionally mature until ejaculation, but there is a developing conflict between physiology and technology. The clinical procedure of injecting testicular or epididymal spermatozoa into oocytes in the process of intracytoplasmic sperm injection (ICSI) has resulted in the births of many babies worldwide in the past decade, suggesting that these immature spermatozoa are "fertile." Furthermore, recent reports of the successful use of epididymal spermatozoa for intrauterine insemination suggest that in the human, functional maturity of spermatozoa may be attained at an earlier developmental stage than in other species. However, it must be noted that for the vast majority of couples, the surgical retrieval of spermatozoa is unnecessary for fertility. Therefore, the remainder of this chapter reviews the biology of ejaculated spermatozoa.

SPERM STRUCTURE

A normal human spermatozoon is 55 to 70 μ m in length and has three main structural regions: the head, midpiece, and tail (flagellum). The principal function of the sperm head is the contribution of its haploid set of chromosomes to the oocyte at fertilization, whereas the midpiece and tail provide the motility necessary for the spermatozoon to reach the site of fertilization (Fig. 1). The sperm head contains the cell's nuclear DNA, but the chromatin is heavily con-

Fig. 1. Diagram of a human spermatozoon. The acrosome is shown covering the anterior portion of the head.

densed, and its protamines are highly crosslinked so that the sperm nucleus is stabilized and effectively inactivated until after fertilization. This makes the head of the spermatozoon inflexible, which assists in penetration of the oocyte's zona pellucida during fertilization. The anterior half to two-thirds of the sperm head is covered by the acrosome, the membrane-bound structure that originated from the Golgi complex during spermiogenesis. The acrosome contains hydrolytic enzymes that are released during the acrosome reaction. For the fertilizing spermatozoon, this occurs on or near the surface of the oocyte's zona pellucida.

The sperm midpiece contains the mitochondria that generate energy via oxidative phosphorylation; the centriole, used by the fertilized oocyte in its first cell division; and the beginnings of axoneme, the motility apparatus. The mitochondria are arranged helically around the proximal part of the axoneme, and they supply the adenosine triphosphate (ATP) necessary for flagellar motility.

The axoneme is a highly complex structure composed of microtubules and dynein that extends along most of the flagellum (Fig. 2). The central portion of the axoneme contains a pair of microtubules, which are connected to each other by linkages. These are surrounded by nine microtubule doublets, each consisting of an A subunit (a complete microtubule) and a B subunit (a C-shaped microtubule structure whose ends are attached to the A subunit). A pair of dynein arms are attached to each of the A subunits. Dynein is an ATPase, and it is thought that when the ATP generated by mitochondria reaches the dynein arms, their conformation changes, allowing them to reach out and attach to the B subunit adjacent microtubule. This attachment allows the first microtubule doublet to rachet forward relative to the second doublet. When this movement is completed, the dynein arms return to their normal conformation and release the second microtubule doublet. In turn, the dynein arms of the second microtubule doublet then reach out and attach to the B subunit of the third microtubule doublet, and the cycle continues around the nine pairs. This movement of microtubule doublets relative to each other generates and propagates the flagellar beat and confers motility.

Therefore, an absence of dynein arms in the axoneme, as occurs in Kartagener syndrome, renders the spermatozoa immotile and is a cause of infertility. However, in recent years, spermatozoa from men with Kartagener syndrome have

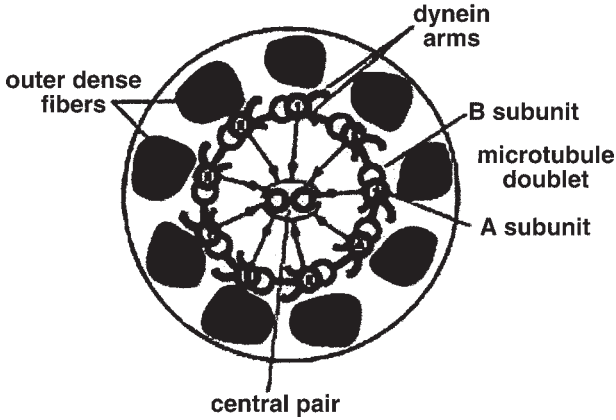

Fig. 2. Diagram of a cross-section of the sperm axoneme. (Adapted from ref. 2.)

been used successfully in ICSI, because this technique removes the requirement of sperm motility for fertilization, resulting in the births of some normal children.

A set of nine outer dense fibers is arranged outside the microtubule doublets. The exact role for these fibers is unclear, but it is thought that they act to stiffen the proximal portion of the flagellum and also cause an elastic recoil of the axoneme after microtubule doublet sliding.

SPERM FUNCTION AND PHYSIOLOGY

Following their production in the testis, spermatozoa are transported through the caput and corpus regions of the epididymis, then stored in the proximal cauda epididymis. The spermatozoa mature during epididymal transit and storage and acquire functional competence. The most obvious maturational change in spermatozoa is the acquisition of the ability to move when in contact with seminal plasma or physiological culture media—a process referred to as "activation." Other changes during epididymal maturation of spermatozoa are alterations to the plasma membrane, chromatin condensation and stabilization, and possibly some final modifications to the shape of the acrosome.

At ejaculation, sperm are transported from their storage site and are mixed with prostatic fluid and seminal vesicle fluid before passage along the penile urethra. The first fraction of the ejaculate contains most of the spermatozoa, suspended in epididymal and prostatic fluid, whereas subsequent fractions contain both prostatic and vesicular fluid. During intercourse, the spermatozoa are deposited into the vagina, near the cervical os, and must swim through the cervical mucus, traverse the uterus, enter the oviduct, and reach the oocyte in its ampullary portion for fertilization to occur.

Mammalian spermatozoa cannot fertilize oocytes immediately upon ejaculation nor upon retrieval from the epididymis. A series of metabolic and physiological changes collectively termed "capacitation" must happen before spermatozoa acquire the ability to penetrate the zona pellucida and bind to the oocyte. In nature, these changes occur during transit through the female reproductive tract, but it is possible to induce capacitation in vitro using appropriate culture media and conditions; consequently, this is an integral step for successful in vitro fertilization.

It is indicated that modifications to the sperm plasma membrane, occuring during epididymal transit, act to stabilize the spermatozoon and prevent it from capacitating in the male reproductive tract, as the completion of capacitation marks the beginning of membrane destabilization events, which eventually lead to cell death. One function of capacitation is the removal of these stabilizing residues, such as cholesterol—the removal of which renders the plasma membrane more fusogenic, being critical for successful sperm–egg interaction. It is not possible to visualize the capacitation-related changes in the sperm plasma membrane, making it impossible to assay for capacitation alone, but it may be monitored through changes in chlortetracycline-binding patterns on the sperm head or by changes in lectin-binding sites. In the human, capacitation may begin with the removal of some sperm surface components during passage through cervical mucus owing to the high-shear forces to which the spermatozoa are exposed.

The cervix acts as a barrier to sperm penetration, resulting in the less-competent spermatozoa being excluded from reaching the uterus and oviduct. Generally, motility determines whether a spermatozoon will penetrate the cervical mucus, but passage of motile spermatozoa with antibodies bound to their surface is inhibited. As described previously, motility is dependent on the function of both the sperm midpiece (for energy generation) and tail (for beat development and propagation). If there are one or more defects in the midpiece or tail, a spermatozoon's motility will be impaired. Cervical mucus is receptive to spermatozoa only in the periovulatory period, as its secretion is under endocrine control, but even at this time, spermatozoa experience high-shear forces during penetration. Therefore, for successful passage through the cervical mucus, spermatozoa must be highly progressively motile with significant lateral head movement, reflecting the amplitude of the flagellar beat. The morphology of the spermatozoon does not necessarily equate with mucus-penetrating ability, but because immotile (or poorly motile) spermatozoa cannot pass the cervix, those spermatozoa with midpiece and/or tail abnormalities are excluded. Also, considering the observation that spermatozoa with head defects are at least twice as likely to have coexisting midpiece and/or tail defects, many spermatozoa with

head abnormalities will not penetrate the cervix because of co-existing abnormal motility. However, if the only morphological abnormality of a spermatozoon is in its head shape, then the cervical mucus will not inhibit its passage.

Once past the internal os, the spermatozoa swim through the uterus and enter the oviduct via the uterotubal junction. The sperm movement pattern is likely still linear and progressive at this stage, but it is difficult to confirm this experimentally. Once spermatozoa enter the isthmus of the oviduct, it is thought that they bind to its epithelial cells, forming a "reservoir" where they are held in a quiescent "semicapacitated" state until ovulation.

Following ovulation, contractile movement of the oviduct, beating of the epithelial cilia, and sperm motility all contribute to sperm transport in the oviduct. The ampullary ciliary movements direct the fluid in the oviductal lumen from the ampulla toward the isthmus. Spermatozoa are constrained to swim against currents; hence, the directed current toward the isthmus forces the spermatozoa to swim in the opposite direction toward the ampulla, which is the site of fertilization. In accordance with careful studies only possible in experimental animals, it is postulated that small numbers of spermatozoa are released from the isthmus sequentially, resulting in relatively few spermatozoa in the ampulla at once.

Movement patterns (kinematics) of spermatozoa change concomitantly with capacitation with the development of a nondirected whiplash-style of movement referred to as "hyperactivated motility." In animal models, this hyperactivated motility has been observed in the ampulla, the site of fertilization, and has led to the development of several theories as to its physiological relevance. These include concepts of hyperactivation:

- Provides a mechanism to reduce the chance of entrapment of spermatozoa in the crypts of the oviduct.
- Maintains spermatozoa in the ampulla, thereby setting them up in a search pattern for the oocyte.
- Encourages stirring of ampullary fluid to maintain a homogeneous mixture of metabolites in the region where fertilization will occur.
- Confers upon the spermatozoon the ability to traverse the cumulus matrix, because hyperactivated spermatozoa have a tenfold higher efficiency at penetrating highly viscous media than nonhyperactivated spermatozoa.
- Provides power generation for zona pellucida penetration. (It has been estimated that the sperm–zona bonds that form in fertilization have a strength of about 4×10^{-4} μ *N*, whereas the force generated by the hyperactivated beat pattern is 2.7×10^{-2} μ *N*, two orders of greater magnitude. The force generated by nonhyperactivated spermatozoa is $<3 \times 10^{-4}$ μ *N*—not enough to disrupt the sperm–zona bond.)

It is likely that hyperactivation is an integral part of more than just one of the processes involved in sperm transport through the female reproductive tract and in sperm–egg interactions, and experimental animal studies predict that failure of hyperactivation would be associated with fertilization failure, both in vivo and in vitro.

When the spermatozoon encounters the cumulus–oocyte-complex, it traverses the cumulus matrix and binds to the zona pellucida, where it undergoes the acrosome reaction, a prerequisite for sperm–egg fusion. The acrosome reaction involves localized fusions of the plasma membrane and outer acrosomal membrane over the anterior portion of the sperm head. Vesicles composed of the plasma membrane and outer acrosomal membrane form, allowing the release of acrosomal contents, including hyaluronidase and acrosin, leaving the anterior portion of the head covered by the inner acrosomal membrane. Following completion of this reaction, the spermatozoon penetrates the zona pellucida. Although it has been taught for many years that zona penetration is a purely chemical process with the acrosomal enzymes digesting the glycoproteins of the zona pellucida, more recent work considering the role of hyperactivated motility suggests that it is more likely to be a combination of chemical softening and mechanical propulsion factors.

After successful penetration of the zona pellucida, the spermatozoon enters the perivitelline space, comes into contact with the microvilli of the oocyte plasma membrane (oolemma), and the postacrosomal region of the sperm head binds to the oolemma. Flagellar motility ceases at this time, and fusion is initiated between the oolemma and the equatorial segment of the spermatozoon. The whole spermatozoon is then engulfed by the oocyte. The nucleus of the sperm head decondenses to form the male pronucleus with a new nuclear envelope derived from components in the ooplasm. This can then fuse with the female pronucleus that was formed following the resumption of oocyte meiosis triggered by sperm-oolemma contact. The fertilized oocyte is referred to as a zygote, then subsequently as an embryo, after the first cleavage.

RELATIONSHIP BETWEEN SPERM PHYSIOLOGY AND INFERTILITY

The complexity of sperm structure and function means that it can be difficult to determine the pathophysiological reason(s) a man's infertility. If the developmental or maturational processes are perturbed, this could result in problems, such as:

- Low sperm concentration (from inefficient spermatogenesis)
- Poor sperm motility (caused by midpiece or axonemal abnormalities)
- Abnormal sperm morphology (owing to errors in spermiogenesis)

This list is clearly only illustrative—it could be extended to take in every step of each process, from the activation of spermatogonium to fertilization, and even further, because embryo development is affected by the quality of the nuclear DNA of fertilizing spermatozoon.

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