Sperm Biology from Production to Ejaculation

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Sperm Biology from Production to Ejaculation

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Damayanthi Durairajanayagam, Anil K. Rengan, Rakesh K. Sharma and Ashok Agarwal

Introduction to the Male Reproductive System

The male reproductive system is a complex and intricate system that produces spermatozoa or sex cells to carry the genetic material of the male. The components of the male reproductive system include the hypothalamic–pituitary–gonadal (HPG) axis, and both the external and internal sexual organs. The male reproductive system forms during the early stages of embryonic development, becomes fertile during puberty and maintains the masculinity of the adult male. The external genitalia include the scrotum, testes, and penis whereas the internal genitalia include the epididymis, seminal ducts, spermatic cords, seminal vesicles, ejaculatory ducts, bulbourethral or Cowper's glands, and the prostate gland. The testes produce the male gametes (spermatozoa). The excurrent duct system matures, stores, and transports the gametes to the penis for expulsion, and the accessory glands produce and modify the contents of the semen.

The Scrotum and the Regulation of Testicular Temperature

The testes are the only organs in the human body located externally. Each testis is individually housed in a sac-like structure called the scrotum. The temperature of the underlying testes is reflected by the temperature of the scrotum. The process of spermatogenesis is optimal at temperatures 2–4 °C lower than that of core body temperature [1]. In order to maintain a hypothermic testis, the scrotum has several integral properties that facilitate the dissipation of heat. These include thin scrotal skin, minimal subcutaneous fat, sparse distribution of hair, and a large number of sweat glands. In addition, the scrotal skin hangs loose and wrinkled with a large, total surface area that adjusts according to the ambient temperature.

The cremaster and dartos muscles in the testis also help to regulate testicular temperature. The cremaster muscle is a thin layer of skeletal muscle that surrounds each testis and spermatic cord. When this muscle contracts, the testes rise closer to the abdomen, keeping them warm when ambient temperature is low. The dartos muscle is a thin layer of smooth muscle fiber beneath the scrotal skin. When contracted, the dartos muscle causes the exposed scrotal skin surface area to decrease and heat to be conserved. Conversely, when both these muscles are in a relaxed state, the testis hangs further from the abdomen, enveloped by the scrotal skin. This aids in keeping the temperature of the testes lower than that of the core body. Furthermore, rising external temperatures activate the cutaneous receptors on the scrotal skin to initiate sweat secretion and active heat loss through the evaporation of sweat [2].

The Testes

The human testes are a pair of ovoid (ellipsoid) structures measuring approximately 4.5–5 cm in length by 2.5–4 cm in width and about 15–25 mL in volume (Fig. 5.1). The tunica albuginea, the outer capsule of the testes, is composed of a thick and flexible (though not stretchable) fibrous layer of connective tissue [3]. The parenchyma of the testis is divided by the septa (connective tissue) into 250–300 conical lobules. Each of these lobules consists of masses of highly convoluted seminiferous tubules. Both ends of the seminiferous tubules connect at the hilus to form the rete testis [4]. The seminiferous tubules secrete fluid that flows into the rete testis to be collected and delivered to the excurrent ductal system of the epididymis [5].

Each testis is composed of two distinct compartments: (1) the tubular compartment that contains the seminiferous tubules and (2) the intertubular compartment that lies

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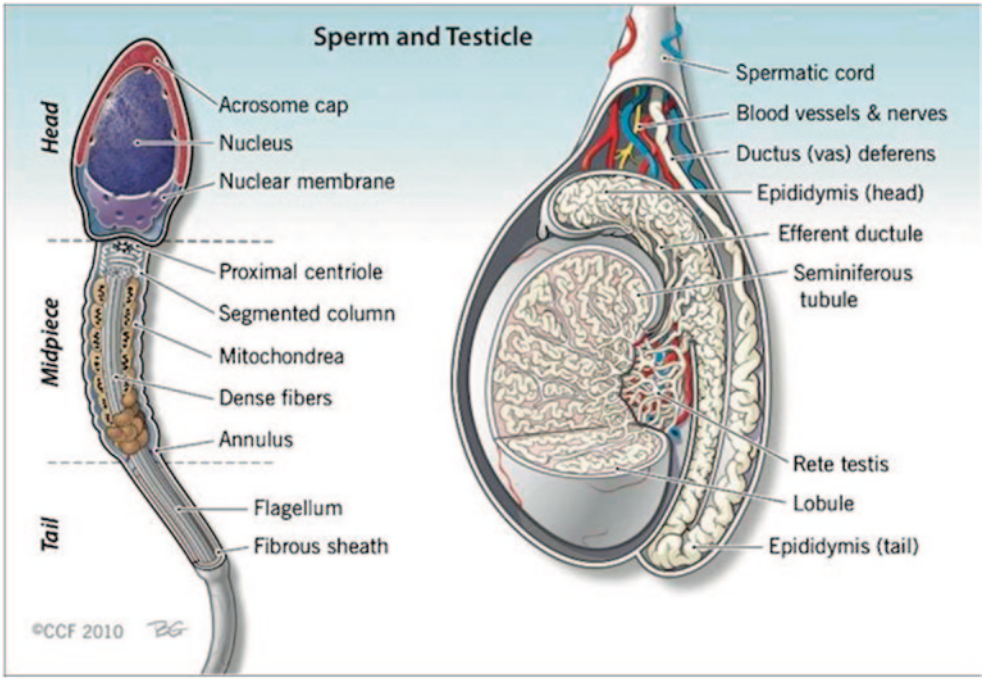

Fig. 5.1 The human spermatozoa, testis, and epididymis. To the *left* is a mature human spermatozoon showing the components that make up the *head*, midpiece and *tail* sections. To the *right* is a view of the human testis and the seminiferous tubules, as well as the epididymis, showing the corpus (*head*) and caudal (*tail*) sections. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010–2013. All rights reserved.)

between the seminiferous tubules and contains the interstitial tissue. Each of these compartments is anatomically separate but remains closely linked together. Within the seminiferous tubules are the spermatogonial germ and Sertoli cells. The Sertoli cells provide a hormonally active environment for the evolution of primitive germ cells into mature male gametes or spermatozoa.

The bulk (90%) of the testicular volume is made up of the seminiferous tubules and the germ cells that lie within the invaginations of the Sertoli cells, which make up the germinal epithelium. The seminiferous tubules also consist of peritubular tissue or lamina propria [6]. The peritubular tissue contains myofibroblasts that cause peristaltic contractions of the seminiferous tubules. This movement helps to transport the developing, immotile germ cells to the rete testis [7]. The intertubular spaces within the lobules contain clusters of Leydig or interstitial cells that make up the endocrine portion of the testis. The interstitial tissue consists primarily of blood and lymph vessels, nerve and collagenous fibers, macrophages, and a variety of connective tissue cells. The spermatogenic process is dependent on intra- and extratesticular hormonal regulatory processes, the functions of the intertubular microvasculature, Leydig cells, and other cellular components in the interstitium (intertubular space) [8].

The testis is responsible for synthesizing (steroidogenesis) and secreting androgens (i.e., testosterone), which is directly interrelated to its second function, producing spermatozoa (spermatogenesis). These functions are under hormonal control via the pituitary gonadotropins—luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

Hormonal Control of Spermatogenesis (Extrinsic Influences)

The hormonal regulation of spermatogenesis is under the control of the hypothalamus–pituitary–gonadal (HPG) axis. This axis begins as the higher center sends signals to the hypothalamus, which acts as the integrating center. The hypothalamus releases gonadotropin releasing hormone (GnRH) in discrete pulses that peak every 1.5 h. GnRH acts on the anterior pituitary to stimulate gonadotropin production (LH and FSH). A continuous production of GnRH will cause gonadotrophin desensitization, which will diminish LH and FSH release. LH is released in a similar pulsatile pattern to that of GnRH while FSH release is influenced by inhibin. LH and FSH act on the testes to produce testosterone and inhibin, respectively. LH acts on the Leydig cells in the testes to stimulate testosterone production through the conversion of cholesterol. When testosterone levels accumulate, it exerts a negative feedback effect at the pituitary (short loop) to suppress the release of LH and at the hypothalamus (long loop), which ultimately suppresses GnRH production and thereby regulates testosterone levels. FSH acts on the Sertoli cells to stimulate inhibin and androgen-binding protein (ABP) secretion. Accumulating inhibin levels exert a negative feedback effect at the pituitary to suppress FSH release, thereby regulating inhibin levels.

FSH is required at the onset of puberty to initiate spermatogenesis as its action on Sertoli cells is necessary for germ cell maturation. Testosterone is essential for maintaining the spermatogenic process. Its actions are facilitated by the Sertoli cells. Spermatocytes have ABP receptors but not

androgen receptors whereas the Sertoli cells have androgen receptors. The binding of ABP to testosterone may assist testosterone movement toward the lumen of the seminiferous tubule onwards to the epididymis. FSH also induces the conversion of testosterone to 5α-dihydrotestosterone (5α-DHT) and 17β-estradiol. 5α-DHT is more active than testosterone and along with 17β-estradiol, is involved in the development and function of the penis, scrotum, accessory sex glands, secondary sex characteristics, libido and potency.

Leydig Cells

Leydig cells are embedded in groups that surround the connective tissue between seminiferous tubules in the testicle. These endocrine cells are the principal source of testosterone, the production of which is stimulated by LH (Table 5.1). In adults, testosterone in circulation is kept within the physiological range of 300–1200 ng/dL while intratesticular levels of testosterone are far higher. In the testes, testosterone levels are highest at the basement membrane of the seminiferous tubules.

Testosterone

Testosterone, the major male androgen in circulation and in the Leydig cells, is responsible for primary and secondary sex characteristics. It is synthesized from cholesterol in the Leydig cells. Primary sex characteristics are structures responsible for promoting the development, preservation, and delivery of sperm cells while secondary sex characteristics are structures and behavioral features that externally differentiate men from women.

Sertoli Cells

Sertoli cells, also known as sustentacular or nurse cells, are highly specialized cells that regulate the development of spermatogonia into spermatozoa (Table 5.1). They originate from the tubular basement membrane and extend up toward the lumen of the seminiferous tubules. The basement membrane acts as a barrier that prevents large molecules in the interstitial fluid from entering the tubule but allows the entry of testosterone. Sertoli cells provide sustenance for developing spermatogonia and are involved in germ cell phagocytosis. The formation of lipid droplets in Sertoli cells is associated with this phagocytosis [9]. The number of lipid droplets found in Sertoli cells increases as the testes advance in age [10]. They also produce and secrete anti-Müllerian hormone (AMH), inhibin, activin, growth factors, enzymes, and ABP. AMH is involved in embryonic development and contributes to the regression of Müllerian ducts. Inhibin, another hormone, helps to regulate FSH secretion from the anterior pituitary. When FSH binds to high-affinity FSH receptors on the Sertoli cells, ABP is secreted (by Sertoli cells) into the lumen of the seminiferous tubule, where it binds to testosterone (secreted by Leydig cells). This causes testosterone to become less lipophilic and more concentrated within the luminal fluid.

Neighboring Sertoli cells have membrane specializations at the basolateral side that forms a band, sealing the cells together and forming a tight junction. The blood–testis barrier prevents molecules in the blood from moving past the tight junctions toward the lumen of the seminiferous tubules. This ensures that the germ cells in the later stages of development remain inaccessible to any harmful molecules in circulation.

The Blood–Testis Barrier

In the mammalian testes, the blood–testis barrier is composed of specialized junctions that are tightly bound between adjacent Sertoli cells in the epithelium of the seminiferous tubule. This barrier is also known as the Sertoli cell seminiferous epithelium barrier. The strong intercellular junctional complexes that link two adjacent Sertoli cells in the tubule form an additional barrier between the tubular lumen and the interstitial fluid outside the tubule. This divides the seminiferous tubule space into two parts: the basal (basement membrane) compartment that is in contact with blood and lymph vessels and the adluminal (lumen) compartment that is isolated from these fluids. The blood and lymph vessels and

Table 5.1 Functions of the Leydig and Sertoli cells

Functions of the Leydig cells	Functions of the Sertoli cells
Initiation and maintenance of spermatogenesis	Maintains the integrity of seminiferous tubules epithelium
Activation of the hypothalamus–pituitary–gonadal axis	Secretion of hormones—inhibin and androgen-binding protein (ABP)
Production of testosterone—manifestation of male secondary sex characteristics	Secretes tubular fluid into the tubular lumen for transport of sperm within the duct
Differentiation of male genital organs	Delivery of nutrients to germ cells
Masculinization of the brain and sexual behavior	Steroidogenesis and steroid metabolism
–	Aids in process of phagocytosis and elimination of cytoplasm
–	Regulates the spermatogenic cycle
–	Acts as a hormonal target for LH, FSH, and testosterone

nerves are located in the interstitium between the tubules and do not penetrate the seminiferous tubules [11]. The Sertoli cells are surrounded by closely aligned myoid or peritubular cells. These arrangements collectively form the blood–testis barrier, which provides an immunologically privileged site for spermatogenesis to thrive.

The fluid found in the tubular compartment of the testes differs from that in found in the interstitium as the former contains low concentrations of glucose and high concentrations of potassium ions and steroid hormones. The tight junctions of the blood–testis barrier break and reform around the migrating cells to ensure that the barrier remains intact.

Intrinsic Regulation

The process of spermatogenesis is also regulated independently from within the testis. The Leydig cells secrete (1) testosterone, (2) neuroendocrine substances that serve as neurotransmitters, and (3) growth factors for neighboring Leydig cells, blood vessels, lamina propria of the seminiferous tubules, and Sertoli cells [12–14]. Leydig cells also contribute toward the nutrition of the Sertoli cells and help to regulate blood flow in the intertubular microvasculature [3]. The cells of the peritubular tissue influence myofibroblast contractility and regulate spermatozoa transportation via peristaltic movements of the seminiferous tubules. The Sertoli cells deliver different growth factors, and various germ cells participate in the development and regulation of other germ cells.

Spermatogenesis

Spermatogenesis is an extremely intricate process of cell differentiation, starting with germ cell (spermatogonia) development and culminating in the production of highly specialized spermatozoa. This process produces the genetic material required for species replication. Spermatogenesis occurs in the lumen of the seminiferous tubules. It was classically believed that human spermatogenesis takes about 64 days in the testis (from spermatogonium to spermatid) with an additional 10–14 days in the epididymis for maturation of spermatozoa. Thus, the entire process took about 70 ± 4 days to complete [15]. However, a more recent report suggests that the entire process from production to ejaculation of spermatozoa is completed within a shorter period: an average of 64 ± 8 days (with a range of 42–76 days) [16]. Spermatogenesis begins at puberty and occurs continually throughout the entire male adult life span in contrast to oogenesis, which is finite in women. The baseline number of precursor cells in the testes is regulated by FSH. Early in embryonic development, the gonocytes, which precede the formation of spermatogonial germ cells, undergo active mitotic replication [17].

Spermatogenesis involves a series of cellular events that begin in the basal compartment and end in the apical compartment. The basal and the luminal compartments are kept separate by tight junctions. In the seminiferous tubules, the developing cells are arranged in a highly ordered sequence from the basement membrane toward the lumen (Fig. 5.2). Spermatogonia are positioned directly on the basement membrane. Primary spermatocytes, secondary spermatocytes, and spermatids lie closest to the lumen. Spermatogonia and primary spermatocytes are found in the basal compartment whereas secondary spermatocytes and spermatids are found in the adluminal compartment.

During spermatogenesis, two events occur in the basal compartment outside the blood–testis barrier: (1) the renewal and proliferation of spermatogonia via mitosis and differentiation and (2) the cell cycle progression from type B spermatogonia to preleptotene spermatocytes. The following three events occur in the adluminal or apical compartment behind the blood–testis barrier: (1) the cell cycle progression from zygotene to pachytene and then to diplotene spermatocytes, followed by meiosis I and meiosis II; (2) spermiogenesis, during which the round spermatids develop into elongated spermatids and eventually spermatozoa; and finally (3) spermiation, which involves spermatozoa maturation and subsequent release into the lumen (Table 5.2).

The following is an overview of the spermatogenic events. First, the primary spermatocytes undergo two meiotic divisions. The first division gives rise to two haploid secondary spermatocytes, which is followed by the second division, which gives rise to four haploid spermatids (1n, 23 chromosomes). Two of these spermatids carry the X maternal chromosome while the other two spermatids carry the Y paternal chromosome. Each spermatid will subsequently undergo spermiogenesis, a metamorphosis into spermatozoa. The spermatozoa are then released into the lumen of the seminiferous tubule (Fig. 5.3).

Spermatogoniogenesis

Spermatogonia are a population of long-living primordial germ cells that undergo mitosis to provide a renewing stem cell population and meiosis for spermatozoa production. Germ cells are named according to their morphological appearance and can be categorized into two classes: Type A and Type B. In humans, Type A cells, the most rudimentary of cells, can be further classified as "pale Type A (A_p)" and "dark Type A (A_d)" spermatogonia. A_p spermatogonia can divide mitotically into more A_p cells or Type B spermatogonia. Type A spermatogonia comprise the stem cell pool whereas Type B spermatogonia continue to develop into spermatids.

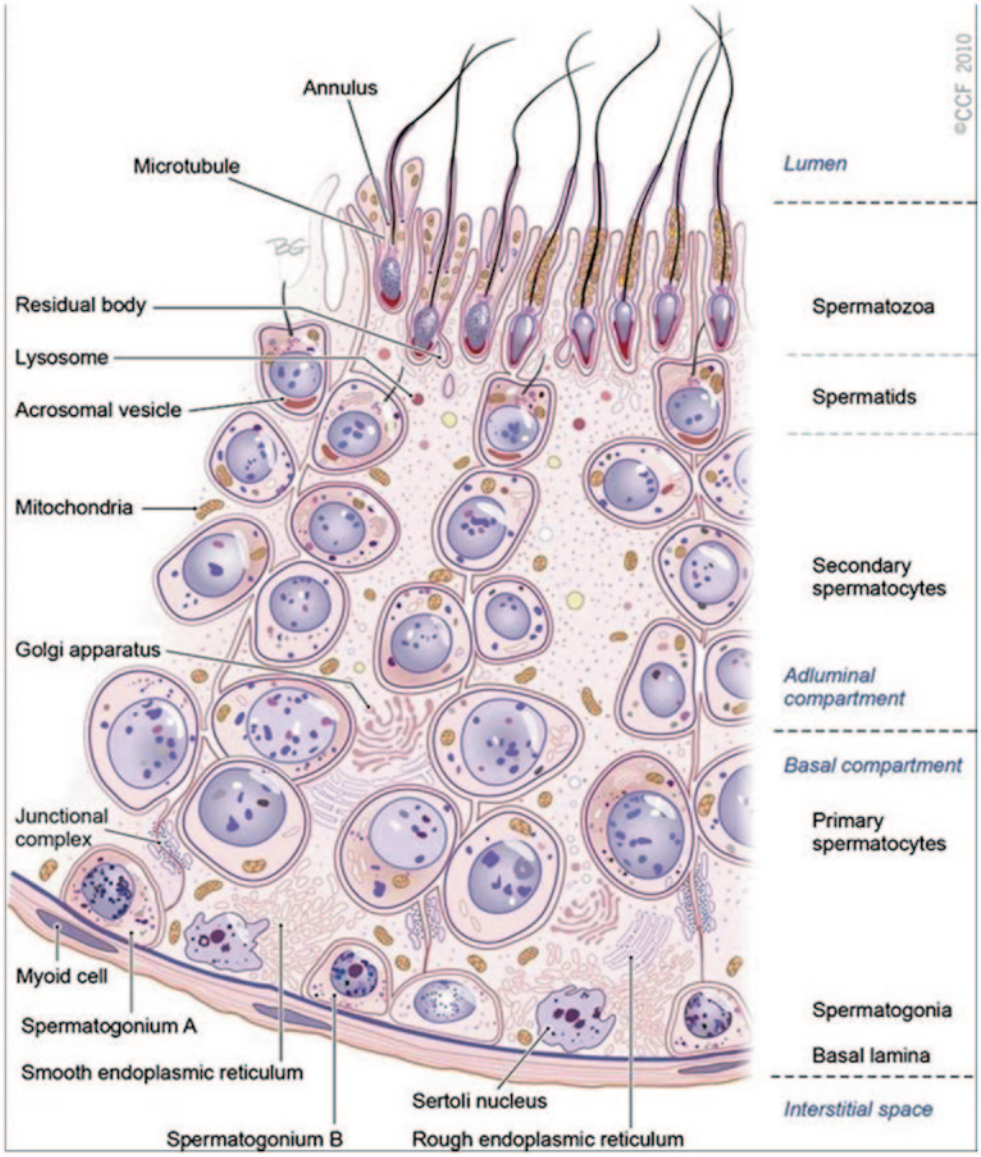

Fig. 5.2 Seminiferous tubule. A cross section of the germinal epithelium in the seminiferous tubule. The germinal epithelium is divided by the Sertoli cell into two compartments, i.e., the basal and adluminal compartments. Fully formed spermatozoa are released into the lumen. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010–2013. All rights reserved.)

Table 5.2 Terminology in spermatogenesis

Process	Description
Spermatogoniogenesis	Process of producing spermatogonia through multiple mitoses to amass a large population of stem cells, most of which undergo meiosis to produce spermatozoa
Spermatogenesis	Process of differentiation of a spermatogonium into a spermatid Purpose: to produce (via mitosis and meiosis) the necessary genetic material for species replication
Spermatocytogenesis	Process of producing spermatocytes that occurs in the basal compartment of the seminiferous tubules
Spermiogenesis	A complex metamorphosis that transforms round spermatids (from the final division of meiosis) into a complex structure spermatozoon
Spermiation	Process whereby a mature spermatid frees itself from the Sertoli cell and enters the tubular lumen

A_p spermatogonia remain attached to the basal membrane and continue to replenish its numbers, allowing the spermatogenic process to persist despite the aging process. Spermatogonia continuously increase in number via successive, but usually incomplete, mitosis. On the other hand, A_d cells seldom divide, potentially serving as a dormant reserve or nonproliferative stem cells that give rise to A_p spermatogonia [15].

Type B spermatogonia have more chromatin within the inner nuclear envelope than to the intermediate or type A

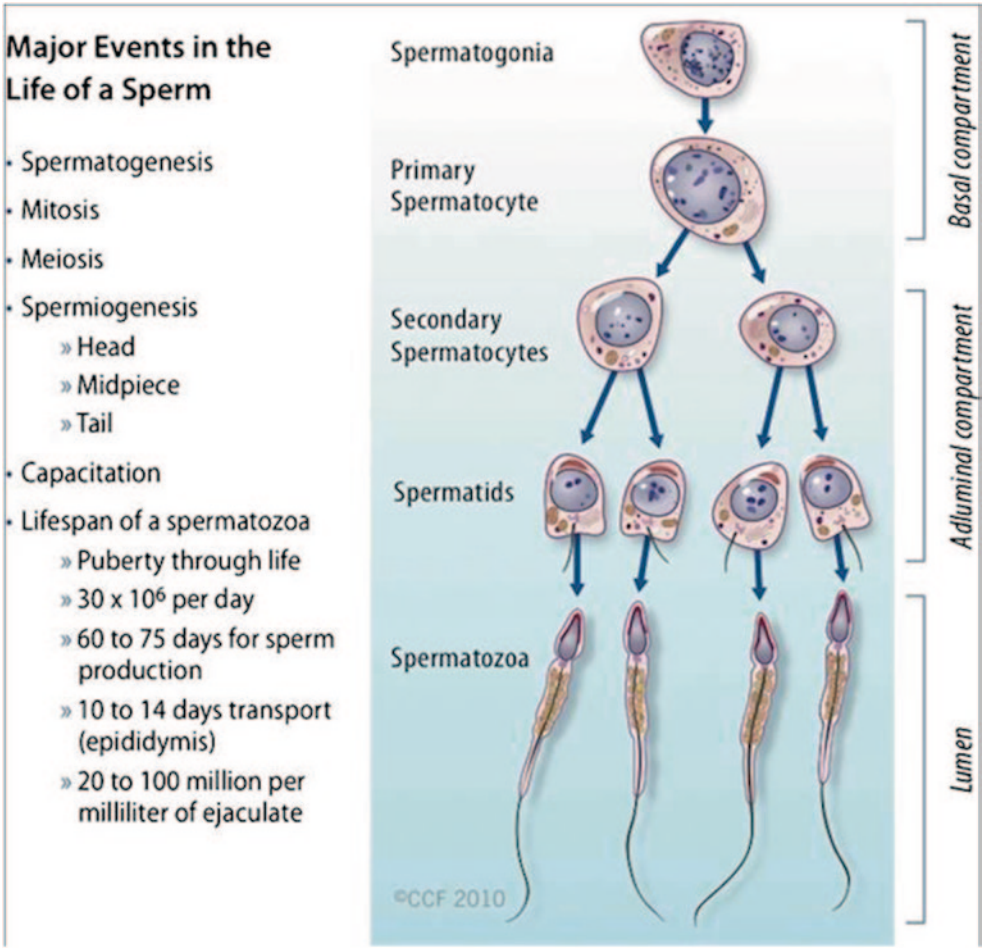

Fig. 5.3 Spermatogenesis. Major events in the life of a sperm involving spermatogenesis, spermiogenesis, and spermiation. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010–2013. All rights reserved.)

spermatogonia. Type B spermatogonia divide mitotically to produce primary spermatocytes, operating as differential precursors to the preleptotene spermatocytes. Spermatogonia remain joined by intercellular bridges but dissolve in the advanced phases of spermatid development. The synchrony of germ cell maturation is thus maintained [18], which is likely to aid in its biochemical interactions.

Spermatocytogenesis

Spermatocytogenesis involves the formation of spermatocytes and takes place in the basal compartment of the seminiferous tubule. The process begins with the primary spermatocytes undergoing meiosis I to form secondary spermatocytes. The prophase of the first meiotic division is very long and thus, the primary spermatocyte has the longest lifespan. Secondary spermatocytes then undergo the meiosis II to produce spermatids. Secondary spermatocytes have a comparably shorter lifespan of 1.1–1.7 days.

Spermatogenesis, from spermatogonium division to spermatozoa release into the tubule, takes about 64 days to complete. Sperm released into the lumen of the seminiferous tubules are immature and incapable of moving on their own. They are pushed through the lumen both by other developing sperm cells moving toward the lumen and by the bulk flow of fluid secreted by Sertoli cells. Sperm cells entering the epididymis complete maturation after 10–14 days of transit, aided by protein secretions from epididymal cells.

Disruption of Spermatogenesis

Type A spermatogonia are necessary for spermatogenesis, and in cases of reduced spermatogenesis, it is likely that A_d spermatogonia are absent [8]. When Type A or Type B spermatogonia are absent and the germinal epithelium is made up only of Sertoli cells, then spermatogenesis will not occur. This "Sertoli Cell Only Syndrome" may be congenital (absence of spermatogonia from birth) or acquired (spermatogonia destroyed by exposure to radiation, etc.). Spermatogenic arrest at the spermatogonial stage occurs when A_p spermatogonia fail to develop into Type B spermatogonia [19].

Mitosis (Cytodifferentiation of Spermatids)

Mitosis involves nuclear division and separation of duplicated chromosomes to form two daughter cells with genetic content exactly identical to its parent cell (diploid, $n=46$). Mitosis is vital for proliferation and maintenance of spermatogonial cells. Meiosis involves an intricate series of events that encompass the duplication of chromosomes, nuclear envelope breakdown, and equal division of chromosomes and cytoplasm that leads to the formation of two daughter cells. Specific regulatory proteins interact on DNA loop domains during cellular replication [20, 21]. The germ cells involved in the mitotic phase are the Type A spermatogonia, which first form the Type B spermatogonia and later the primary spermatocytes. Through a series of mitotic divisions, developing germ cells, which are interconnected by intracellular bridges, produce primary spermatocytes—the largest germ cell of the germinal epithelium. The baseline number of spermatogonia is established after puberty. Mitosis then supplies the precursor cells and initiates the differentiation and maturation processes.

Meiosis

Meiosis is a complex process during which chromosomal exchange of genetic material occurs to form four daughter cells with half the number of chromosomes (haploid, $n=23$) compared to their parent cells. The purpose of meiosis is to ensure genetic diversity. The germ cells involved in the meiotic phase are the primary spermatocytes, secondary spermatocytes, and spermatids. Meiosis occurs twice in succession as meiosis I and meiosis II; each meiotic process consists of prophase, metaphase, anaphase, and telophase. Prophase itself is made up of four stages: leptotene, zygotene, pachytene, and diplotene. Leptotene takes place in the basal compartment while the remaining three take place in the adluminal compartment. Meiosis I is the reducing division in which the number of chromosomes are halved (i.e., the replicated chromosomes in one cell is split between two diploid cells). Meiosis II is the division in which there is no DNA replication and the sister chromatids are split, resulting in four halpoid cells.

The meiotic process is regulated by its own specific mechanisms [22]. In the seminiferous tubules, meiosis begins with the detachment of Type B spermatogonia from the basement membrane to form preleptotene primary spermatocytes. In theory, each primary spermatocyte yields four spermatids, but the actual yield is lower as some of these germ cells are lost in the process. After meiosis I, each daughter cell (secondary spermatocyte) contains one half of the homologous chromosome pair. The secondary spermatocytes then quickly undergo meiosis II, during which time the chromatids separate at the centromere, yielding early round spermatids with haploid chromosomes "22X" or "22Y." During the entire meiotic phase, homologous chromosomes pair up, cross over, and exchange genetical material to form an entirely new genome. Defects during meiosis include apoptotic spermatocytes and spermatogenic arrest of primary spermatocytes. These germ cells bordering the seminiferous tubules cease to develop further and disintegrate [8].

Spermiogenesis

In spermiogenesis, haploid spermatids undergo complete differentiation or morphogenesis to form highly specialized spermatozoa with fully compacted chromatin. These morphological changes begin after meioses I and II. In humans, there are eight different stages ($S_{a\text{-}1}$, $S_{a\text{-}2}$, $S_{b\text{-}1}$, $S_{b\text{-}2}$, $S_{c\text{-}1}$,$S_{c\text{-}2}$, $S_{d\text{-}1}$,and $S_{d\text{-}2}$) involved in the maturation of spermatids to spermatozoa. Each stage is identifiable by the maturing cell's morphological characteristics. In the postmeiotic phase, there is progressive condensation of the nuclear chromatin (to about 1/10 the volume of an immature spermatid) with the inactivation of the genome. In addition, the Golgi apparatus forms the acrosome cap, and the flagellum structures begin to develop [8]. Histones—alkaline proteins that condense the DNA—are converted into transitional proteins, and protamines are converted into well-developed disulfide bonds. Defects during spermiogenesis include acrosomal and flagellar defects, absence of the acrosome or the midpeice of the flagellum, and impaired nuclear condensation in malformed spermatids [8].

Nuclear Development

The nucleus and its contents undergo several changes during spermatogenesis. During the first eight steps of spermiogenesis [23], the nucleus elongates and flattens, giving the head its characteristic oval shape. This nuclear compaction is believed to facilitate oocyte penetration and help to optimize spermatozoa swimming capacity [24]. This nuclear compaction includes chromatin remodeling. During the last postmeiotic phase of spermiogenesis, histone molecules, around which DNA is organized, are converted to translational proteins that are then converted to protamines [25]. Protamines contain large amounts of cysteine, which aids in disulfide bond formation as the sperm cells mature in the epididymis [26–28]. Protamines in the chromatin of the spermatozoa are replaced by histones from the oocyte within 2–4 h of fertilization.

Spermiation

During spermiation, the mature sperm cell releases itself from the Sertoli cell and moves into the lumen of the seminiferous tubule [28]. Spermatids originating from the same spermatogonia remain attached to each other by bridges, facilitating the transfer of cytoplasmic products. Spermiation may also involve the movement of spermatids as they progress toward the lumen of the seminiferous tubules [28]. Mature spermatids close their intracellular bridges and disconnect from the germinal epithelium, becoming free cells (spermatozoa). At this stage, portions of the sperm cell cytoplasm, known as the cytoplasmic droplet, are eliminated. However, the cytoplasmic droplet may remain in immature spermatozoa during the process of spermiation, becoming "excess residual cytoplasm" [29].

The Cycle or Wave of Seminiferous Epithelium

Spermatogenesis involves the division of primitive spermatogonial cells into germ cell types through the process of meiosis. At any given time, groups of cells in different developmental phases are present within the germinal epithelium of the seminiferous tubule. Germ cells are localized in spatial units known as stages, designated by Roman numerals. Each stage is distinguished by (1) acrosome development, (2) meiotic phase, (3) nucleus shape, and (4) spermatozoa release into the lumen of the seminiferous tubule [30] (Fig. 5.4). The same typical aspects of germ cell epithelium appear every 16 days [8]. The time it takes for Type A spermatogonial to divide is shorter than that required for the entire process of spermatogenesis. The development of Type A spermatogonia into mature spermatids followed by the delivery of mature spermatozoa through the epididymal duct system takes anywhere between 42 and 76 days [16].

Rete Testis and Epididymis

Spermatozoa in the lumen of the seminiferous tubules leave the testis through the rete testis and several vasa (ductuli) efferentia. The ductuli combine to form a single, highly convoluted duct at the head of the epididymis. The epididymis, located along the dorsolateral edge of each testis, allows for post-testicular maturation and storage of spermatozoa during their passage from the testis to the vas deferens. It is divided into three unique segments: the caput epididymis (head) for spermatozoa concentration, corpus epididymis (body) for spermatozoa maturation, and cauda epididymis (tail) for spermatozoa storage. As they pass through the epididymis, spermatozoa attain their full maturity, fertilizing ability, and motility, although they typically do not move under their own control until after ejaculation. The epididymal epithelium is sensitive to androgen stimulation and possesses both absorptive and secretory abilities. As they journey through the epididymis, spermatozoa undergo changes in membrane protein composition, phospholipid and fatty acid content, net surface charge, immunoreactivity, and adenylate cyclase activity. At the caput, a significant amount of the fluid that carries spermatozoa from the seminiferous tubules is reabsorbed, greatly increasing spermatozoa concentration. Spermatozoa remain motionless in the male genital tract and are transported by the flow of fluid in the testes, and thereafter by contraction of the organs.

Spermatozoa mature outside the testes, leaving those within the testes with poor motility and fertilization ability. The cauda epididymis stores mature spermatozoa, permitting repetitive, rich ejaculations. Sperm cell storage capacity diminishes distally. At the cauda epididymis, spermatozoa lose fertilizing potential first, motility second, and vitality last. These cells, along with nearly half of all spermatozoa released from the testes, will disintegrate and undergo reabsorption by the epididymal epithelium. This includes older gametes that must be eliminated from the male reproductive tract regularly to ensure high quality of the ejaculate.

Vas Deferens

The vas deferens is a muscular tube adjacent to the prostate that extends from the epididymis, passing through the inguinal canal into the peritoneal cavity and opening into the urethra. Near the prostate end, the vas deferens enlarges and forms a gland called the ampulla. This portion, along with excretory canals of the seminal vesicles, forms the ejaculatory ducts and joins the urethra. The ampulla is where the majority of sperm cells are stored for ejaculation. Few spermatozoa find their way from the caudal epididymis into the seminal vesicle where they will then degenerate. These cells are generally found in the terminal portion of the ejaculate.

Accessory Sex Glands

The seminal vesicles, prostate gland, and Cowper's (bulbourethral) gland are collectively known as the accessory sex glands. These glands secrete fluids that act as the medium for sperm transport and sustenance (Table 5.3). These secretions make up the seminal plasma of the ejaculate. The seminal vesicles join the ampullary portion of the vas deferens and produce fructose and coagulating proteins. The prostate gland is located at the junction of the vas deferens and the urethra. Fluid produced by the prostate contains zinc, citric acid, and acid phosphatase, which give semen its typical odor. In addition, the prostate secretes enzymes that liquefy

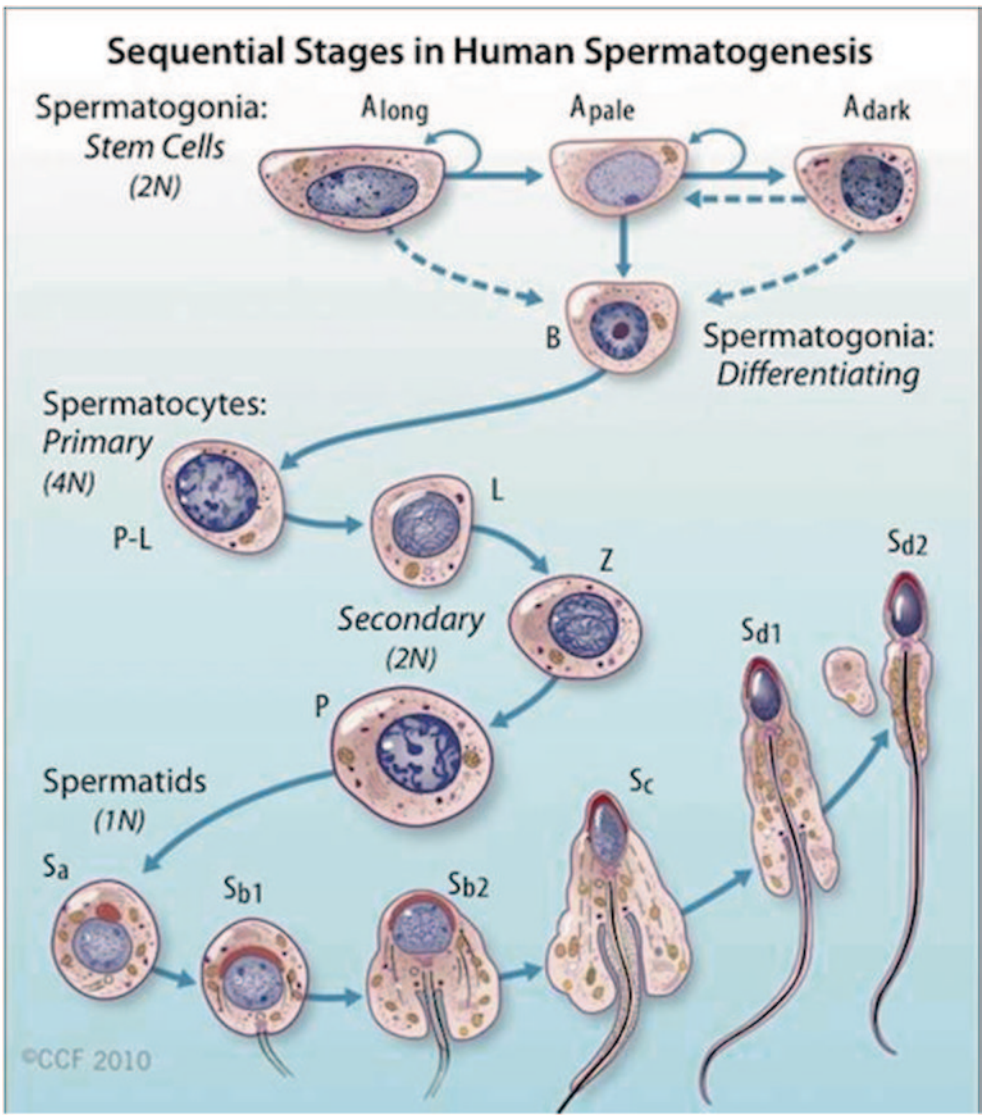

Fig. 5.4 Stages in spermatogenesis. The sequential stages of differentiation during spermatogenesis: from a diploid germ cell into a fully functional spermatozoon. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010–2013. All rights reserved.)

Table 5.3 Composition of semen

Component	Function	Source
Sperm	Carries the paternal genetic material	Seminiferous tubules
Mucus	Acts as a lubricant	Bulbourethral glands
Water	Provides a liquid medium	All accessory glands
Buffers	Neutralizes the acidic environment of the vagina	Prostate, bulbourethral glands
Nutrients		
L-carnitine	Nourishes the spermatozoa	Epididymis
Fructose Vitamin C		Seminal vesicles
Citric acid		Prostate
Enzymes	Forms coagulum in vagina, then liquefies	Seminal vesicles and prostate
Prostaglandins	Smooth muscle contraction; aids sperm transport within both the male and female reproductive tract	Seminal vesicles

the seminal coagulum. The Cowper's gland is situated distal to the prostate gland and empties into the bulbous urethra. Fluid from the Cowper's gland lubricates the urethra prior to ejaculation.

Structure of Spermatozoon

A morphologically normal sperm cell is about 45–50 μm in length and consists of a head and tail.

Head

According to Kruger's strict criteria [31], a morphologically normal head should be smooth and symmetrically oval in shape with a broad base and tapering apex. The sperm head measures between 4.0–5.5 μm in length and 2.5–3.5 μm in width, with a length-to-width ratio of between 1.50 and 1.70 [32, 33]. The head is the most important part of the mature male gamete as it contains a nucleus, which is composed of packed chromosomal paternal genetic material (mostly DNA) containing 23 chromosomes. The nucleus comprises about 65% of the head, but like most somatic cells, lacks a large cytoplasm to match [34].

Acrosome Region

The head also contains a well-defined acrosome region, a cap-like covering of the anterior two thirds of the head (40–70% of the apex) [33]. The acrosome is represented by the Golgi complex [35, 36]. The acrosome contains a number of hydrolytic enzymes, such as hyaluronidase and acrosin, which are required for fertilization [34]. During fertilization, the acrosomal membrane fuses with the oocyte plasma membrane oocyte at numerous sites. This is followed by the acrosome reaction, an event characterized by acrosomal enzyme release from the head tip.

Among the common abnormalities of the sperm head are defective shape or size and the presence of numerous vacuoles (>20%) within the head surface. Shape defects include large, small, tapering, pyriform, amorphous, double heads, and various other combinations [33].

Neck

The neck is formed by the fragile junction between the head and tail portion.

Tail

The tail measures 40–50 μm in length (nearly ten times the length of the head) and provides motility for the cell. The sperm cell's entire motility apparatus is contained in the tail, propelling the sperm body via waves generated in the neck region that pass along distally in a whiplash manner.

The tail can be divided into the midpiece (anterior portion), principal piece, and endpiece (posterior portion). Ideally, the midpiece supports the head at exactly the center position. It should be slender as well (maximum width of 1 μm), yet thicker than the rest of the tail and between 7.0 and 8.0 μm in length. The tail diameter should be between 0.4 and 0.5 μm, measuring about 50 μm in length. The tail should have a well-defined endpiece, without any coiling or abnormal bending (over 90°). The midpiece consists of tightly packed mitochondria surrounded by a sheath. The mitochondria in the midpiece supply energy in the form of ATP for tail movement. The principal piece is the longest part of the tail and comprises most of the propellant machinery. Motility plays a very important role in sperm transport through the cervix; the sperm cells need to maintain motility despite being suspended in fluid secreted by the female reproductive organs. Moreover, motility is required to avoid phagocytosis by polymorphonucleocytes found in female body fluid.

Common abnormalities of the neck and midpiece region are the absence of the regions themselves, thickened neck, distended or irregular/bent midpiece, abnormally thin midpiece (no mitochondrial sheath), or a combination of these abnormalities [33]. The presence of excess residual cytoplasm (i.e., a cytoplasmic droplet greater than one third the area of normal sperm head) at the posterior portion of the midpiece is another common abnormality. The cytoplasmic droplet is released during ejaculation as long as the sperm has sufficiently matured in the epididymis. Common tail defects include short or multiple hairpin broken tails, irregular widths, coiled tails with terminal droplets, or a combination of these defects [33].

Erection and Emission

An erection is caused by sexually related psychic and/or physical stimulation. Before an erection occurs, visual, auditory, olfactory, and tactile stimulation triggers acetylcholine release by the parasympathetic nervous system. Acetylcholine causes vasodilation of the pudendal arteries, which leads to increased blood flow to the corpus cavernosum and corpus spongiosum of the penis. As the venous outflow is compressed, the penis becomes engorged with blood and grows more turgid, leading to an erection. Penile erection is required for penetration into the vagina for sperm deposition. Erectile dysfunction is the repeated inability to achieve or maintain an erection rigid enough for sexual intercourse.

Semen, the mixture of sperm and fluids, is expelled via a neuromuscular reflex in two sequential phases: emission and ejaculation. At the start of emission, a series of coordinated sequential contractions begins in the testis efferent ducts, the cauda epididymis and the convoluted portion of the vas deferens. The contractions advance in an assimilated manner, propelling the sperm from the cauda epididymis forward into the prostatic urethra. Here, the prostatic fluid, the sperm-rich fraction from the ampulla, and the fluid from the seminal vesicle are deposited into the prostatic urethra. This action propels sperm from the efferent ducts, through the ejaculatory ducts, and into the urethra. The filling of the urethra with

sperm initiates sensory signals that travel to the sacrospinal region of cord. The internal urethral sphincter is closed by sympathetic discharge to prevent retrograde ejaculation into the urinary bladder. During the emission phase, when spermatozoa pass into the urethra, sympathetic stimulations release adrenaline and initiate contraction of the smooth muscles surrounding the ampulla, deferens ducts, and the cauda epididymis.

Ejaculation

Ejaculation is initiated after emission, and the process expels semen from the penile urethra. It includes external sphincter relaxation and rhythmic prostate contractions. The bulbospongiosus muscle propels the semen in an antigrade manner out of the external urethral meatus. Sperm that is not ejaculated will gradually die and undergoes cytolysis. Ejaculation involves both the sympathetic and parasympathetic nervous systems. Parasympathetic fibers initiate the contraction of the bulbospongiosus muscle, which leads to forcible expulsion of the semen from the urethra. Ascending impulses contribute simultaneously toward the sensation of orgasm.

The ejaculate, or semen, is freshly produced at the time of ejaculation. Ejaculation normally occurs in a definite sequence. First, a small amount of Cowper's gland fluid is extruded followed by prostatic fluid and the sperm-rich fraction from the ampulla, and finally secretions from the seminal vesicle (Table 5.3). These secretions form the seminal coagulum, a gel-like substance that normally liquefies in about 20 min. Sperm cells are trapped within the gel matrix of the coagulum and remain immotile until activation upon liquefaction.

Once the ejaculate is expelled, detumescence of the penis begins under sympathetic control. Noradrenaline is secreted, causing dilation of the penile vasculature and penile flaccidity.

Capacitation and Acrosome Reaction

The spermatozoa undergoes several chemical changes during capacitation, which occurs in the cervix, uterine cavity, and the fallopian tubes during the estrogenic phase. Capacitation allows the acrosome reaction to occur as the sperm and oocyte come into contact with one another. As with epididymal maturation, capacitation is also required before fertilization can occur. Capacitation takes place after ejaculation into the female reproductive tract. During capacitation, spermatozoa undergo a sequence of biochemical changes that ultimately enable them to fertilize an ovum. The sperm plasmalemma is reorganized to support the subsequent acrosome reaction; seminal plasma factors are removed and modifications are made to the sperm membrane, sterols, lipids, glycoproteins, outer acrosomal membrane, and surface charge. The concentration of intracellular free Ca^{2+} increases as well [37]. In particular, it is the removal of cholesterol from the surface membrane that allows for the acrosome reaction to occur [38].

The acrosome reaction is a form of exocytosis that gives spermatozoa the ability to advance through the zona pellucida and prepares them for fusion with the ovum membrane. This process helps to dispel the contents of the acrosome, including surface antigens and enzymes, for successful fertilization. D-mannose binding lectins on the sperm surface, for example, have been shown to help bind spermatozoa to the zona pellucida [39, 40]. The acrosomal enzymes digest the outer acrosomal membrane and the plasma membrane to which it is attached. At this point, the head is covered only by the inner acrosomal membrane. The posterior region of the head is enclosed by a single membrane known as the postnuclear cap. The acrosome and postnuclear cap overlap to form the equatorial segment, which does not take part in the acrosome reaction. The spermatozoon progresses forward and rapidly penetrate the three layers of the oocyte, moving through the cumulus oophorus, corona radiate, and zona pellucida, respectively. Once inside the perivitelline space, the cortical reaction is induced, triggering the completion of meiosis II in the oocyte. Next, the spermatozoon attaches to the vitelline membrane at the postnuclear cap area and fuses with the oocyte membrane. Consequently, its tail breaks off at the midpiece, detaching from the head, and is followed by axoneme and head decondensation to free the male chromatids. Epididymal maturation, capacitation, and the acrosome reaction induce cellular and chromatin modifications in germ cells for their transformation into fully functional spermatozoa (Fig. 5.5).

Spermatogenic Efficiency

In humans, it takes a spermatogonium approximately 64 days to differentiate into four mature spermatids and into mature spermatozoa [41]. The daily production rate of spermatozoa is 3–4 million per gram of testicular tissue [42], which is meager in comparison to that of laboratory animals. More than 75 % of the developed sperm cells perish due to apoptosis or degeneration, and more than 12.5 % of the remaining cells are abnormal. In the end, the spermatogenetic potential for reproduction amounts to approximately 12 % [13]. Daily sperm production gradually decreases with advancing age. This reduction could be attributed to the loss of Sertoli cells, increase in germ cell degeneration during prophase of meiosis, loss of primary spermatocytes, and the loss of Leydig cells, non-Leydig interstitial cells, and myoid cells [30].

Initial information regarding the success of spermatogenesis is obtained by evaluating ejaculate under light

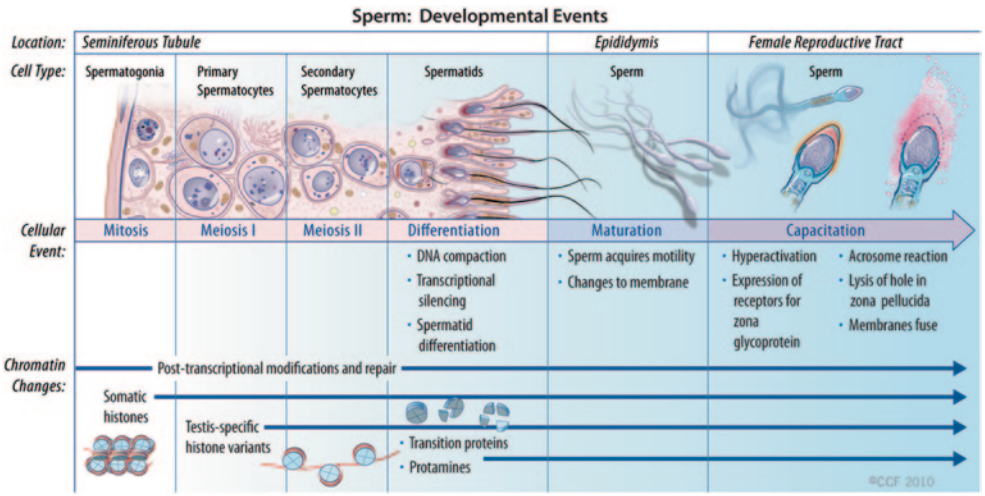

Fig. 5.5 Sperm developmental events. Changes that occur during the development of a germ cell into a spermatozoon leading to its release and subsequent maturation and storage in the epididymis, prior to its journey into the female reproductive tract. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010–2013. All rights reserved.)

microscopy to assess the number, shape, and motility patterns of spermatozoa and to assess other cellular components present in the ejaculate [10].

Immune Status

Despite their biological necessity, spermatozoa are not recognized by the immune system. While immune function is established shortly after birth, surface markers found in late pachytene spermatocytes, spermatids, and spermatozoa develop during puberty. The spermatozoa are protected, however, by the blood–testis barrier, a microenvironment in the seminiferous epithelium that renders them free from immunological attack [43]. Despite the barrier, an immune monitoring system still exists in both the testes and epididymis that defends against autoimmune disease [44].

Disturbances to Spermatogenesis

Several factors can potentially disturb gamete proliferation or differentiation and the intra- or extratesticular mechanisms that regulate spermatogenesis. These include exposure to physical agents such as heat or chemical substances, poor nutrition, obesity, nicotine use, alcohol consumption, ingestion of therapeutic and recreational drugs, bacterial infections, hormonal imbalances, varicocele, cryptorchidism, testicular cancer and radiation [45, 46]. Environmental toxicants such as pesticides, phthlates, polychlorinated biphenyls (PCBs) and endocrine disrupting chemicals (EDCs) can also negatively impact the spermatogenic process [45, 47, 48].

Semen Parameters and Reference Range

A routine semen analysis is the "gold standard" for the initial investigation of male fertility. The following factors are assessed in the seminal ejaculate: physical characteristics (e.g., color, volume, pH, odor, viscosity, and liquefaction time), sperm concentration, motility, progression, viability, and morphology and leukocyte count. Semen parameters such as sperm concentration, motility, and morphology can act as markers of male fertility and may reflect testicular causes of infertility. However, semen analysis must be performed on two or three separate occasions (owing to its large individual biological variability) before any conclusion can be made [49]. The World Health Organization (WHO) normal cutoff values for semen characteristics are shown in Table 5.4.

Conclusion

Spermatogenesis is a highly organized, complex sequence of differentiation events, both mitotic and meiotic, that yields genetically distinct male gametes for fertilization with the female ovum. In a broader scope, it helps to propagate a species and contributes to genetic diversity. In human males, spermatogenesis begins at puberty and persists throughout life. Sperm production is a continuous process that occurs in the seminiferous tubules within the blood–testis barrier of the testis—an immune privileged site. Spermatogenesis involves the transformation of spermatogonial germ cells into spermatids via proliferation and cellular remodeling. The process is regulated by various intrinsic and extrinsic factors. Spermiogenesis converts the spermatids to motile spermatozoa, which are highly specialized haploid cells. Spermatozoa are released along the seminiferous tubules into the epididymis where post-testicular maturation and storage take place.

Table 5.4 Reference values for semen characteristics according to the WHO, 4th (1999) and 5th (2010) edition

Parameter	WHO 1999 (4th edition) [33]	WHO 2010 (5th edition)[33] (Lower reference limits obtained from lower 5th centile values)
Volume (mL)	≥2	1.5
Sperm concentration (10^6 per mL)	≥20	15
Total sperm count (10^6)	≥40	39
Total motility (% motile)	≥50	40
Progressive motility[a](%)	≥25 % (grade a)	32 (grade a + b)
Vitality (% alive)	≥75	58
Morphology (% normal forms)	14	4
Peroxidase-positive leukocytes (10^6 per mL)	< 1.0	< 1.0

[a] Grade a = rapid progressive motility (> 25 μm/s), grade b, slow/sluggish progressive motility (5–25 μm/s); normal, 50 % motility (grade a + b) or 25 % progressive motility (grade a) within 60 min of ejaculation

Before fertilization can occur, spermatozoa must undergo further biochemical changes via capacitation and the acrosome reaction, both of which occur after ejaculation. The entire sperm production process can be inhibited by numerous factors, such as poor nutrition, hormonal imbalances, and therapeutic drug side effects.

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