Spermatogenesis: An Overview

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Abstract

The purpose of this chapter is to provide a comprehensive overview of spermatogenesis and the various steps involved in the development of the male gamete, including cellular processes and nuclear transformations that occur during spermatogenesis, to provide a clear understanding of one of the most complex cellular metamorphosis that occurs in the human body.

Spermatogenesis is a highly complex temporal event during which a relatively undifferentiated diploid cell called spermatogonium slowly evolves into a highly specialized haploid cell called spermatozoon. The goal of spermatogenesis is to produce a genetically unique male gamete that can fertilize an ovum and produce offspring. It involves a series of intricate, cellular, proliferative, and developmental phases. Spermatogenesis is initiated through the neurological axis by the hypothalamus, which releases gonadotropin-releasing hormone, which in turn signals follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to be transmitted to the reproductive tract. LH interacts with the Leydig cells to produce testosterone, and FSH interacts with the Sertoli cells that provide support and nutrition for sperm proliferation and development.

Spermatogenesis involves a series of cell phases and divisions by which the diploid spermatogonial cells develop into primary spermatocytes via mitosis. Primary spermatocytes in the basal compartment of Sertoli cells undergo meiosis to produce haploid secondary spermatocytes in the adluminal compartment of Sertoli cells in a process called spermatocytogenesis. This process gives the cells a unique genetic identity within the

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population of secondary spermatocytes and subsequent developing cells. After spermatocytogenesis, spermatids elongate to form spermatozoa by spermiogenesis, a morphological development phase in which the nuclear transformations involving chromatin remodeling and compaction occur. Spermatozoa then leave the Sertoli cells through the lumen of the seminiferous tubules, exit through the rete testis, and enter the epididymis for final maturation. This is where spermatozoa acquire motility and acrosomal function. Spermatogenesis in the human male takes about 74 days.

Spermatogenesis is regulated by intrinsic and extrinsic factors. Not all spermatogonia mature into spermatozoa – most are eliminated and phagocytosed in a process called apoptosis. The overall goals of spermatogenesis are (1) to enable the male to transfer genetically recombined DNA by contributing to half of the offspring's genome and (2) to equip the spermatozoa to effectively navigate through the female reproductive tract and deliver the genetic material to the ovum. In the following sections, the complex transformation of the simple single diploid cell into a fully functional haploid cell is described.

Keywords

Spermatogenesis • Male gamete • Neurological pathways in spermatogenesis • Spermiogenesis • Meiosis and mitosis

Neurological Pathways

Spermatogenesis is initiated through hormonal controls in the hypothalamus (Fig. 2.1). The hypothalamus secretes gonadotropin-releasing hormone (GnRH), triggering the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the adenohypophysis or anterior lobe of the pituitary. LH assists with steroidogenesis by stimulating the Leydig cells of the interstitium, and FSH stimulates the Sertoli cells to aid with the proliferative and developmental stages of spermatogenesis. In addition to LH and FSH, the adenohypophysis also secretes adrenocorticotropic hormone, prolactin, growth hormone, and thyroid-stimulating hormone – all of these hormones play important roles throughout spermatogenesis. The primary hormones are responsible for initiating spermatogenesis inside the testes, which is the central organ of the reproductive axis. GnRH stimulations are regulated through three types of rhythmicity: (1) seasonal – peak GnRH production occurs during the spring (2) circardian – daily regulator with the highest output during the early morning and (3) pulsatile – highest output occurring on average every 90–120 min.

Steroid Hormone Interaction and Neurological Axis

Androgens are an integral part of spermatogenesis. Dihydrotestosterone is formed by metabolizing testosterone with 5 alpha-reductase. Both testosterone and dihydrotestosterone regulate various genes and the various developmental stages during gestation [1]. Estrogen is necessary for proper spermatogenesis [2, 3]. During Sertoli cell differentiation, estrogen levels drop to minimum levels. During the prepubescent years, estrogen shuts off androgen production by the Leydig cells. When puberty begins, estrogen levels fall to enable androgen production by Leydig cells and initiate spermatogenesis. Thyroid hormones play a key role in spermatogenesis involving Sertoli cell proliferation and development. All of these hormones interact with one another

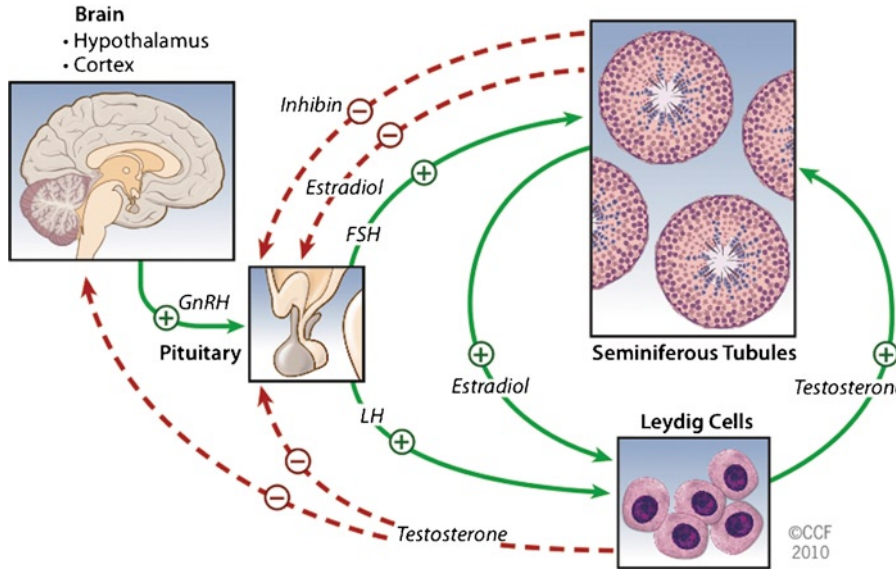

Fig. 2.1 Schematic representation of the hypothalamic pituitary axis and the hormonal feedback system (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

in the testicular axis in both the interstitial region and the Sertoli cells to enable spermatogenesis. In addition to the hormones, growth factors secreted directly by the Sertoli cells also play an important role in spermatogenesis. Transforming growth factor (alpha and beta), insulin-like growth factor, and beta fibroblast growth factor facilitate germ cell migration during embryonic development, proliferation, and regulation of meiosis and cellular differentiation.

Organization of the Testis

The testes are ellipsoid in shape, measuring of 4.5–5.1 cm in length [4, 5], 2.5 × 4 cm in width [6] and have a volume of 15–25 mL [7]. They are engulfed by a strong connective tissues capsule (tunica albuginea) [6] and are the only organs in humans that are located outside the body. Spermatogenesis occurs at temperatures that are optimally 2–4° lower than that the temperature of main body [8]. The testis is loosely connected along its posterior border to the epididymis, which gives rise to the vas deferens at its lower pole [9]. The testis has two main functions: to produce hormones, in particular testosterone, and to produce male gametes – the spermatozoa (Fig. 2.2).

Supporting Cells: Leydig Cells

The Leydig cells are irregularly shaped cells that have granular cytoplasm present individually or more often in groups within the connective tissue. They contribute to about 5–12% of the testicular volume [10–12]. Leydig cells are the prime source of the male sex hormone testosterone [13–15]. LH acts on Leydig cells to stimulate the production of testosterone. This acts as a negative "feedback" on the pituitary to suppress or modulate further LH secretion [15]. The intratesticular concentration of testosterone is significantly higher than the concentration in the blood. Some of the key functions of testosterone are as follows: (1) Activation of the hypophyseal-testicular axis, (2) Masculation of the brain and sexual behaviors, (3) Initiation and maintenance of spermatogenesis, (4) Differentiation of the male genital organs, and (5) Acquisition of secondary sex characteristics.

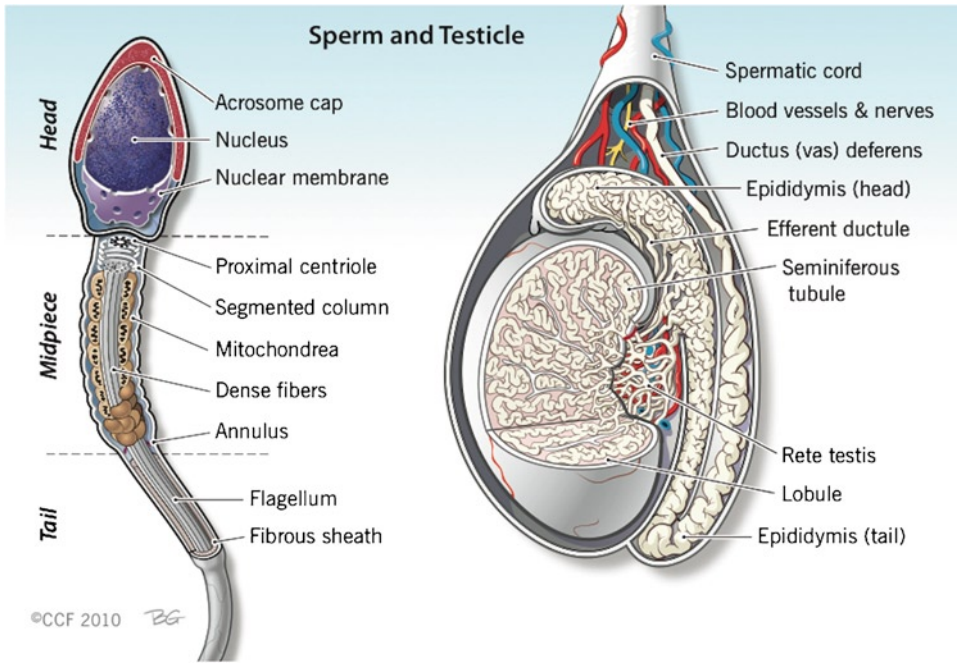

Fig. 2.2 The human testis and the epididymis. The testis shows the tunica vaginalis and tunica albuginea, seminiferous tubule septae, rete testis, and the overlying head, body, and tail of the epididymis. To the *left* is a diagrammatic representation of a fully mature spermatozoon (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

Seminiferous Tubules and Sertoli Cells

Most of the volume of the testis is made up of seminiferous tubules, which are packed in connective tissue within the confines of the fibrous septa. The testis is incompletely divided into a series of about 370 lobules or fibrous septae consisting of the seminiferous tubules and the intertubular tissue. The seminiferous tubules are a series of convoluted tubules within the testes. Spermatogenesis takes place in these tubules, scattered into many different proliferating and developing pockets (Fig. 2.3). The seminiferous tubules are looped or blind-ended and separated by groups of Leydig cells, blood vessels, lymphatics, and nerves. Each seminiferous tubule is about 180 μm in diameter. The height of the germinal epithelium measures 80 μm and the thickness of the peritubular tissue is about 8 μm [16].

Seminiferous tubules consist of three layers of peritubular tissue: (1) the outer adventitial layer of fibrocytes that originates from primitive connective tissue from the interstitium, (2) the middle layer composed of myoid cells that are distributed next to the connective tissue lamellae, and (3) the peritubular layer, a thick, inner lamella that mainly consists of collagen. The seminiferous tubule space is divided into basal (basement membrane) and adluminal (lumen) compartments by strong intercellular junctional complexes called "tight junctions." The seminiferous tubules are lined with highly specialized Sertoli cells that rest on the tubular basement membrane and extend into the lumen with a complex ramification of cytoplasm. They encourage Sertoli cell proliferation and development during the gestational period. Both ends of the seminiferous tubules open into the spaces of the rete testis [17]. The fluid secreted by the seminiferous tubules is collected in the rete testis and delivered into the excurrent ductal system of the epididymis.

Approximately 40% of the seminiferous tubules consist of Sertoli cells, and roughly 40%

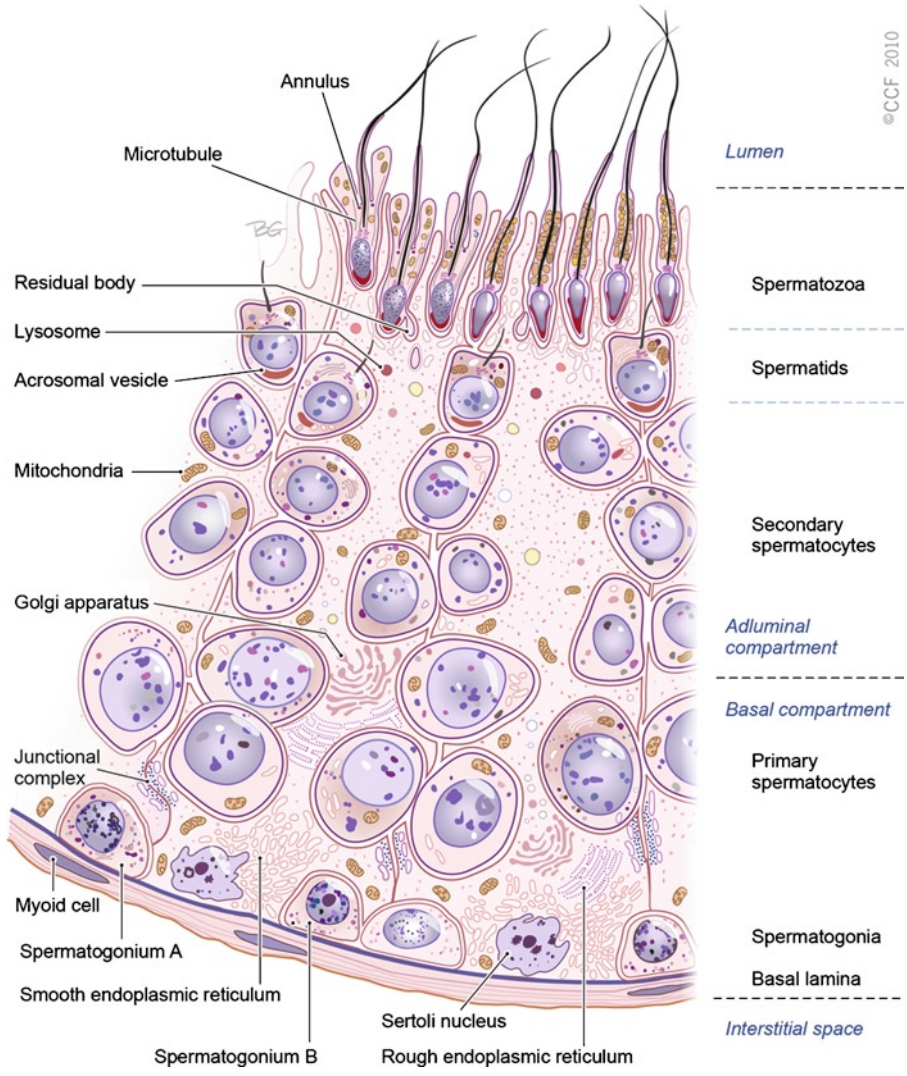

Fig. 2.3 Section of the germinal epithelium in the seminiferous tubule. Sertoli cells divide the germinal epithelium into a basal and adluminal compartment, via the Sertoli cell. Spermatozoa are released into the lumen (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

of the Sertoli cells are occupied with elongated spermatids [18, 19]. Sertoli cells have larger nuclei than most cells, ranging from 250 to 850 cm^3 [18]. Each Sertoli cell makes contact with five other Sertoli cells and about 40–50 germ cells at various stages of development and differentiation. The Sertoli cells provide structural, functional, and metabolic support to germ cells. Functionally and endocrinologically competent Sertoli cells are necessary for optimal spermatogenesis. During spermatogenesis, the earlier germinal cells rest toward the epithelium region of the seminiferous tubules in order to develop and mature while the more developed germinal cells move toward the lumen of the seminiferous tubules in order to exit the seminiferous tubule system and continue with the final phases of spermatogenesis.

Sertoli cells function as "nurse" cells for spermatogenesis, nourishing germ cells as they develop and participating in germ cell phagocytosis. Multiple sites of communication exist

between Sertoli cells and developing germ cells for the maintenance of spermatogenesis within an appropriate hormonal milieu. FSH binds to the high-affinity FSH receptors found on Sertoli cells, signaling the secretion of androgen-binding protein (ABP). ABP allows androgens such as testosterone and dihydrotestosterone to bind and increase their concentrations to initiate and/ or continue the process of spermatogenesis. Sertoli cells also release anti-Müllerian hormone that allows for the embryonic development of the male by reducing the growth of the Müllerian ducts [20, 21]. Sertoli cells also secrete inhibin – a key macromolecule participating in pituitary FSH regulation.

Spermatozoa are produced at puberty but are not recognized by the immune system that develops during the first year of life. The blood–testis barrier provides a microenvironment for spermatogenesis to occur in an immunologically privileged site. The blood–testis barrier is divided into two regions: a basal region located near the seminiferous epithelium and an adluminal region that is positioned toward the lumen region of the seminiferous tubules. The basal region is the spermatogenic site for spermatogonial and primary spermatocyte development, while the adluminal region serves as the site for secondary spermatocyte and spermatid development. The blood–testis barrier has three different levels: (1) tight junctions between Sertoli cells, which helps separate premeiotic spermatogonia from the rest of the germ cells, (2) the endothelial cells in both the capillaries and (3) peritubular myoid cells.

Some of the main functions of the Sertoli cells are as follows:

1. Maintenance of integrity of seminiferous epithelium
2. Compartmentalization of seminiferous epithelium
3. Secretion of fluid to form tubular lumen to transport sperm within the duct
4. Participation in spermiation
5. Phagocytosis and elimination of cytoplasm
6. Delivery of nutrients to germ cells
7. Steroidogenesis and steroid metabolism
8. Movement of cells within the epithelium
9. Secretion of inhibin and ABP
10. Regulation of spermatogenic cycle
11. Provide a target for LH, FSH, and testosterone receptors present on Sertoli cells

Spermatogenesis

The process of differentiation of a simple diploid spermatogonium into a spermatid is known as spermatogenesis [17]. It is a complex, temporal event whereby primitive, totipotent stem cells divide to either renew them or produce daughter cells that are transformed into a specialized testicular spermatozoon (Fig. 2.4). It involves both mitotic and meiotic divisions and extensive cellular remodeling. Spermatogenesis can be divided into three phases: (1) proliferation and differentiation of spermatogonia, (2) meiosis, and (3) spermiogenesis, a complex process that transforms round spermatids after meiosis into a complex structure called the spermatozoon. In humans, the process of spermatogenesis starts at puberty and continues throughout the entire life span of the individual. Once the gonocytes have differentiated into fetal spermatogonia, an active process of mitotic replication begins very early in the embryonic development.

Within the seminiferous tubule, germ cells are arranged in a highly ordered sequence from the basement membrane to the lumen. Spermatogonia lie directly on the basement membrane, followed by primary spermatocytes, secondary spermatocytes, and spermatids as they progress toward the tubule lumen. The tight junction barrier supports spermatogonia and early spermatocytes within the basal compartment and all subsequent germ cells within the adluminal compartment.

Types of Spermatogonia

Fetal spermatogonia become transitional spermatogonia and later spermatogonia type Ad (dark). Spermatogonial stem cells undergo proliferative events and produce a population of cells that have distinct nuclear appearance that can be seen with hematoxylin and eosin staining. Spermatogonia

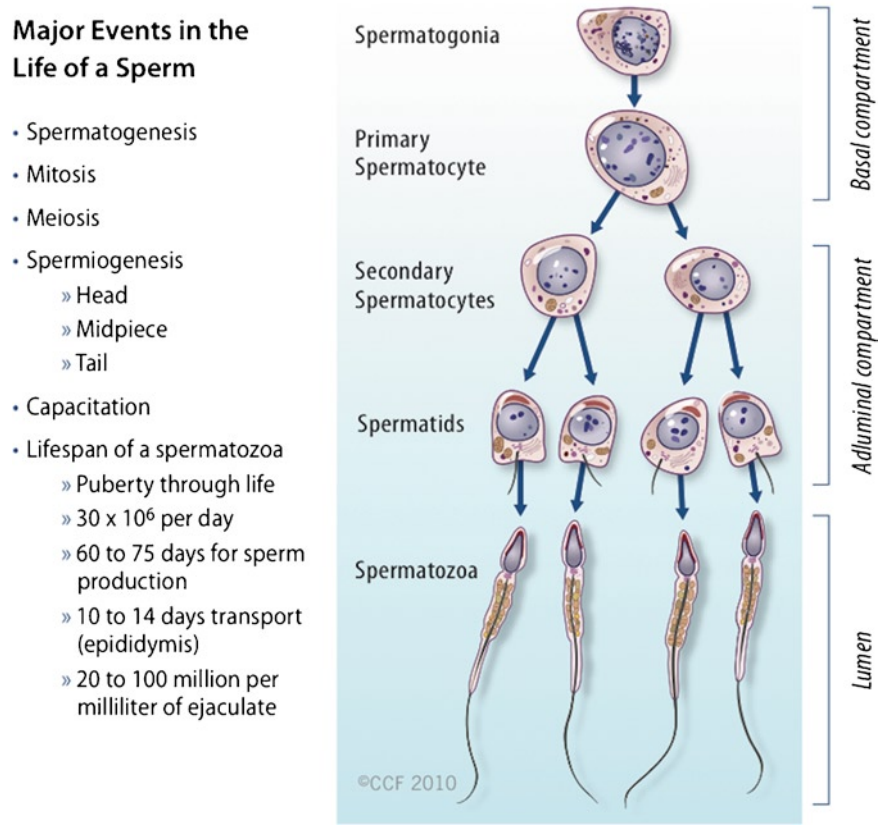

Fig. 2.4 A diagrammatic representation of major events in the life of a sperm involving spermatogenesis, spermiogenesis, and spermiation during which the developing germ cells undergo mitotic and meiotic division to reduce the chromosome content (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

can be categorized into three types: (1) Dark Type A, (2) Pale type A, and (3) Type B spermatogonia (Fig. 2.5).

Dark type A spermatogonia are stem cells of the seminiferous tubules that have an intensely stained dark ovoid nucleus containing fine granular chromatin. These cells divide by mitosis to generate Dark Type A and Pale Type A spermatogonia. Pale Type A spermatogonia have pale staining and fine granular chromatin in the ovoid nucleus. Other proliferative spermatogonia include A_{paired} (A_{pr}), resulting from dividing $A_{isolated}$, and subsequently dividing to form $A_{aligned}$ (A_{al}). Further differentiation of spermatogonia includes Type A1, A2, A3, A4, Intermediate, and Type B, each a result of the cellular division of the previous type. In humans, four spermatogonial cell types have been identified: A_{long}, A_{dark}, A_{pale}, and Type B [22–24]. In the rat, Type $A_{isolated}$ (A_{is}) is believed to be the stem cell [25, 26], whereas in humans, it is unclear which Type A spermatogonia is the stem cell. Type B spermatogonia are characterized by large clumps of condensed chromatin under the nuclear membrane of an ovoid nucleus. Type B spermatogonia divide mitotically to produce primary spermatocytes (preleptotene, leptotene, zygotene, and pachytene), secondary spermatocytes, and spermatids (Sa, Sb, Sc, Sd_1, and Sd_2), [22] (Fig. 2.6). Spermatogonia do not separate completely after meiosis but remain joined by intercellular bridges, which persist throughout all stages of spermatogenesis. This facilitates biochemical interactions and synchronizes germ cell maturation [27].

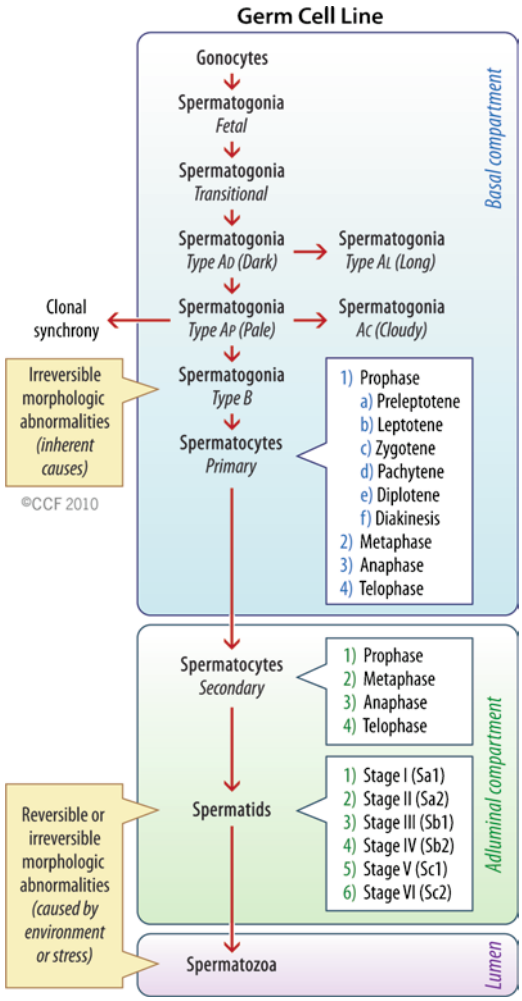

Fig. 2.5 Schematic representation of the development of a diploid undifferentiated germ cell into a fully functional haploid spermatozoon along the basal to the adluminal compartment and final release into the lumen. Different steps in the development of primary, secondary, and spermatid stages are also shown and the irreversible and reversible morphological abnormalities that may occur during various stages of spermatogenesis (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

Spermatocytogenesis

Spermatocytogenesis consists of the meiotic phase in which primary spermatocytes undergo meiosis I and meiosis II to give rise to haploid spermatids. This takes place in the basal compartment. Primary spermatocytes enter the first meiotic division to form secondary spermatocytes. The prophase of the first meiotic division is very long. Primary spermatocytes have the longest life span. Secondary spermatocytes undergo the second meiotic division to produce spermatids. Secondary spermatocytes are short-lived (1.1–1.7 days).

Mitosis

Mitosis involves the proliferation and maintenance of spermatogonia. It is a precise, well-orchestrated sequence of events in which the genetic material (chromosomes) is duplicated, with breakdown of the nuclear envelope and formation of two daughter cells as a result of equal division of the chromosomes and cytoplasm [28] DNA is organized into loop domains on which specific regulatory proteins interact [29–33]. The mitotic phase involves spermatogonia (types A and B) and primary spermatocytes (spermatocytes I). Primary spermatocytes are produced by developing germ cells interconnected by intracellular bridges through a series of mitotic divisions. Once the baseline number of spermatogonia is established after puberty, the mitotic component proceeds to provide precursor cells and initiate the process of differentiation and maturation.

Meiosis

The meiotic phase involves primary spermatocytes until spermatids are formed, and during this process, chromosome pairing, crossover, and genetic exchange take place until a new genome is determined. Meiosis consists of two successive divisions to yield four haploid spermatids from one diploid primary spermatocyte. After the first meiotic division (reduction division), each daughter cell contains one partner of the homologous chromosome pair, and they are called secondary spermatocytes (2n).

Meiosis is characterized by prophase, metaphase, anaphase, and telophase. The process starts when type B spermatogonia lose contact with the basement membrane and form

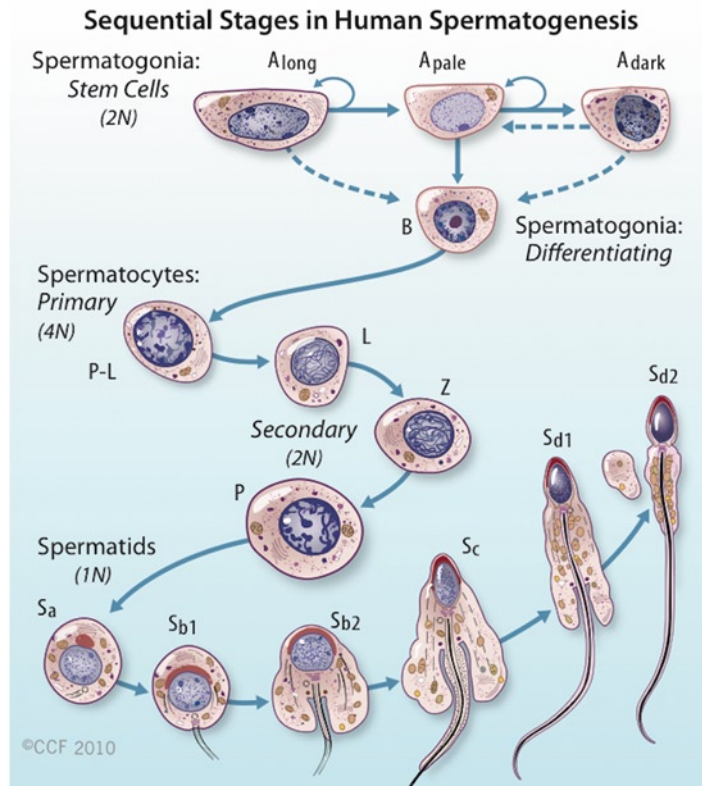

Fig. 2.6 Differentiation of a human diploid germ cell into a fully functional spermatozoon (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

preleptotene primary spermatocytes. During the leptotene stage of prophase, the chromosomes are arranged as long filaments. During the zygotene stage, the homologous chromosomes called tetrads are arranged linearly by a process known as synapsis and form synaptonemal complexes. Crossing over takes place during this phase, and the chromosomes shorten in the pachytene stage. The homologous chromosomes condense and separate from sites of crossing over during diakinesis. This random sorting is important to maintain genetic diversity in sperm. At the end of prophase, the nuclear envelope breaks down, and in metaphase, chromosomes are arranged in the equatorial plate. At anaphase, each chromosome consists of two chromatids migrating to opposite poles. In telophase, cell division occurs with the formation of secondary spermatocytes having half the number of chromosomes. Thus, each primary spermatocyte can theoretically yield four spermatids, although fewer actually result, as the complexity of meiosis is associated with a loss of some germ cells. The primary spermatocytes are the largest germ cells of the germinal epithelium.

The prophase of the second meiotic division is very short, and in this phase, the DNA content is reduced to half as the two chromatids of each chromosome separate and move to the opposite poles. At the end of telophase, the spermatids do not separate completely but remain interconnected by fine bridges for synchronous development. These spermatids are haploid with (22, X) or (22, Y) chromosome and undergo complete differentiation/morphogenesis known as spermiogenesis.

Spermiogenesis

Spermiogenesis is the process of differentiation of the spermatids into spermatozoa with fully compacted chromatin. During this process, morphological changes occur once the process of meiosis is completed. In humans, six different

stages have been described in the process of spermatid maturation; these are termed as S_{a-1} and S_{a-2}, S_{b-1} and S_{b-2}, and S_{c-1} and S_{c-2} (Fig. 2.6). Each stage can be identified by morphological characteristics. During the S_{a-1} stage, both the Golgi complex and mitochondria are well developed and differentiated. In addition, the acrosomal vesicle appears, the chromatoid body develops in one pole of the cell opposite from the acrosomal vesicle, and proximal centriole and axial filament appear. During the S_{b-1} and S_{b-2} stages, acrosome formation is completed, the intermediate piece is formed and the tail develops. This process is completed during the Sc stages. During the postmeiotic phase, progressive condensation of the nucleus occurs with inactivation of the genome. The histones are converted into transitional proteins, and finally, protamines are converted into well-developed disulfide bonds.

Spermiation

A mature spermatid frees itself from the Sertoli cell and enters the lumen of the tubule as a spermatozoon in a process called spermiation. Spermatids that originate from the same spermatogonia remain connected by bridges to facilitate the transport of cytoplasmic products. Sertoli cells actively participate in spermiation, which may also involve the actual movement of the cells as the spermatids advance toward the lumen of the seminiferous tubules [18]. The mature spermatids close their intracellular bridges, disconnect their contact with the germinal epithelium, and become free cells called spermatozoa. Portions of the cytoplasm in the Sertoli cell known as the cytoplasmic droplet are completely eliminated, or at times, they may be retained in the immature spermatozoon during the process of spermiation [34].

The Cycle or Wave of Seminiferous Epithelium

A cycle of spermatogenesis involves the division of primitive spermatogonial stem cells into subsequent germ cell types through the process of meiosis. Type A spermatogonial divisions occur at a shorter time interval than the entire process of spermatogenesis. Therefore, at any given time, several cycles of spermatogenesis coexist within the germinal epithelium. Spermatogenesis is not a random but well orchestrated series of well-defined events in the seminiferous epithelium. Germ cells are localized in spatial units referred as stages. Each stage is recognized by development of the acrosome; meiotic divisions and shape of the nucleus and release of the sperm into lumen of the seminiferous tubule. A stage is designated by Roman numerals. Each cell type of the stage is morphologically integrated with the others in its development process. Each stage has a defined morphological entity of spermatid development called a step, which is designated by an Arabic number. Several steps occur together to form a stage, and several stages are necessary to form a mature sperm from immature stem cells [35, 36]. In rodent spermatogenesis, only one stage can be found in a cross section of seminiferous tubule.

Within any given cross section of the seminiferous tubule, there are four to five layers of germ cells. Cells in each layer comprise a generation or a cohort of cells that develop as a synchronous group. Each group has a similar appearance and function. Stages I–III have four generations comprising Type A spermatogonia, two primary spermatocytes, and an immature spermatid. Stages IV–VIII have five generations: Type A spermatogonia, one generation of primary spermatocyte, one generation of secondary spermatocytes, and one generation of spermatids. Thus, a position in the tubule that is occupied by cells comprising stage I will become stage II, followed by stage III, until the cycle repeats. The cycle of spermatogenesis can be identified for each species, but the duration of the cycle varies for each species [22].

The stages of spermatogenesis are sequentially arranged along the length of the tubule in such a way that it results in a "wave of spermatogenesis." Although it appears that the spatial organization is lacking or is poor in the human seminiferous tubule, these stages are tightly organized in an intricate helicine pattern [37]. In addition to the steps being organized spatially within the seminiferous tubule, the

stages are organized in time. Spermatozoa are released only in certain cross sections along the length of the seminiferous tubule. In rat, all stages are involved in spermatogenesis, but spermatozoa are released only in stage VIII. In humans, this wave appears to be a spiral cellular arrangement as they progress down the tubule. This spatial arrangement probably exists to ensure that sperm production is a continuous and not a pulsatile process. The spermatocyte takes 25.3 days to mature. Spermiogenesis occurs in 21.6 days, and the duration of the cycle is 16 days. The progression from spermatogonia to spermatozoa or spermatogenesis is 74 days or 4½ cycles of the seminiferous cycle.

Chromatin Remodeling/Alterations During Sperm Differentiation

Mammalian sperm chromatin is unique in that it is highly organized, condensed, and compacted. This feature protects the paternal genome during transport through the male and the female reproductive tracts and helps ensure that it is delivered to the ova in good condition. Mammalian sperm DNA is the most tightly compacted eukaryotic DNA [38]. This feature is in sharp contrast to the DNA structure in somatic cell nuclei. Somatic cell nuclear DNA is wrapped around an octamer of histones and packaged into a solenoid structure [39]. This type of packaging adds histones, which increase the chromatin volume. The sperm nucleus does not have this type of packaging, and the volume is highly compacted. Chromatin changes occur in the testis during meiosis in which copies of the genome are partitioned into haploid spermatid cells and during spermiogenesis in which spermatids elongate to form sperm with fully compacted chromatin. These events are largely controlled by posttranslational events for transcription. Translation greatly subsides as DNA becomes compacted and the cytoplasm is jettisoned during spermiogenesis [40, 41]. After meiosis, sperm DNA experiences extreme chromosome compaction during spermiogenesis.

Chromatin modeling is accompanied by changes in the nuclear shape, conversion of negatively supercoiled nucleosomal DNA into a nonsupercoiled state [42], induction of transient DNA breaks [43], and chromatin condensation. It is mediated by drastic changes at the most fundamental level of DNA packaging where a nucleosomal architecture shifts to a toroidal structure [44]. This change is implemented by sperm nuclear basic proteins (SNBs) that include variants of histone subunits, transition proteins, and protamine proteins [45, 46]. Chromatin proteins do not act exclusively to compact sperm DNA. This transition occurs in a stepwise manner, replacing somatic histones with testis-expressed histone variants, transition proteins, and finally protamines [47]. Histone localization and posttranslational modification of histones encode epigenetic information that may regulate transcription important for sperm development [48]. They may also serve to mark the heterochromatin state of specific regions of the genome that may be important after fertilization, when somatic histones are incorporated back into paternal chromatin or during subsequent zygotic development [49]. Male infertility can result from deficits of SNBs [50–52].

Histone and Basic Nuclear Protein Transitions in Spermatogenesis

During spermatogenesis, histone proteins in developing sperm are replaced by testis-specific histone variants that are important for fertility [53]. The cells depend on posttranslational modifications to implement subsequent stages of sperm formation, maturation, and activation as de novo transcription in postmeiotic sperm is largely silenced [54]. During spermiogenesis, sperm chromatin undergoes a series of modifications in which histones are lost and replaced with transition proteins and subsequently with protamines [54–56]. Approximately 15% of the histones are retained in human sperm chromatin, subsequently making chromatin less tightly compacted [57, 58]. Chromatin remodeling is facilitated by the coordinated loosening of the chromatin by histone hyperacetylation and by the DNA topoisomerase II (topo II), which produce temporary nicks in the sperm DNA to relieve torsional stress that results from supercoiling [43, 59–61]. The same enzyme Topo II

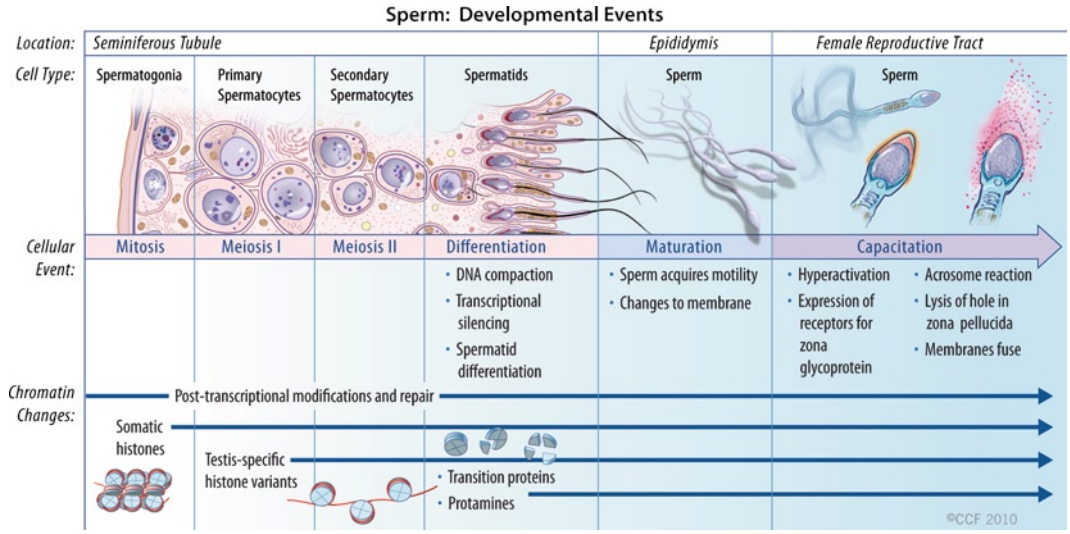

Fig. 2.7 Diagrammatic representation of the series of cellular and chromatin changes during the development of the germ cell into a spermatozoon and its subsequent release and storage into the epididymis and its journey into the female reproductive tract (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

normally repairs these temporary nicks prior to completion of spermiogenesis and ejaculation. However, if these nicks are not repaired, DNA fragmented sperm may be present in the ejaculate [62].

Role of Transition Proteins

The histone-to-protamine transition is important in the formation of spermatozoa [63]. This occurs in two steps in mammals: replacement of histones by transition nuclear proteins (TPs) – TP1 and TP2 – and replacement of TPs by protamines (protamine 1 and protamine 2). TPs are required for normal chromatin condensation, for reducing the number of DNA breaks and for preventing the formation of secondary defects in spermatozoa and the eventual loss of genomic integrity and sterility. TP1 is a 6.2-kDa, highly basic (about 20% each of arginine and lysine) protein with evenly distributed basic residues [64, 65], whereas TP2 is a 13-kDa basic (10% each of arginine and lysine) protein with distinct structural domains. The only similarity between the two is their high basicity, exon–intron genomic patterns, and developmental expression [66].

The transition nuclear proteins are localized exclusively to the nuclei of elongating and condensing spermatids [67] and were first detected in step 10–11 spermatids [68, 69] (Figs. 2.7 and 2.8). The maximum levels of TPs are acquired during steps 12–13, during which they constitute 90% of the chromatin basic protein, with the levels of TP1 being about 2.5 times those of TP2 [51]. They are not detected in the nucleus after the early part of step 15 [68, 69].

Some of the possible roles of TPs are as follows:

1. TP1 can destabilize nucleosomes and prevent binding of the DNA, both of which could contribute to displacement of histones [70, 71]
2. The zinc fingers of TP2 selectively bind to CpG sites and may be responsible for global expression of RNA synthesis [72]
3. Both TPs may play a role as alignment factors for DNA strand breaks, and TP1 is involved in the repair of strand breaks [73, 74]
4. Both TP1 and TP2 can condense DNA, and TP2 is more effective [70, 71, 75]. TP2 is not a critical factor for shaping of the sperm nucleus, histone displacement, initiation of chromatin condensation, binding of protamines to DNA, or fertility, but it is necessary for maintaining the normal processing of P2 and consequently the completion of chromatin condensation [52]

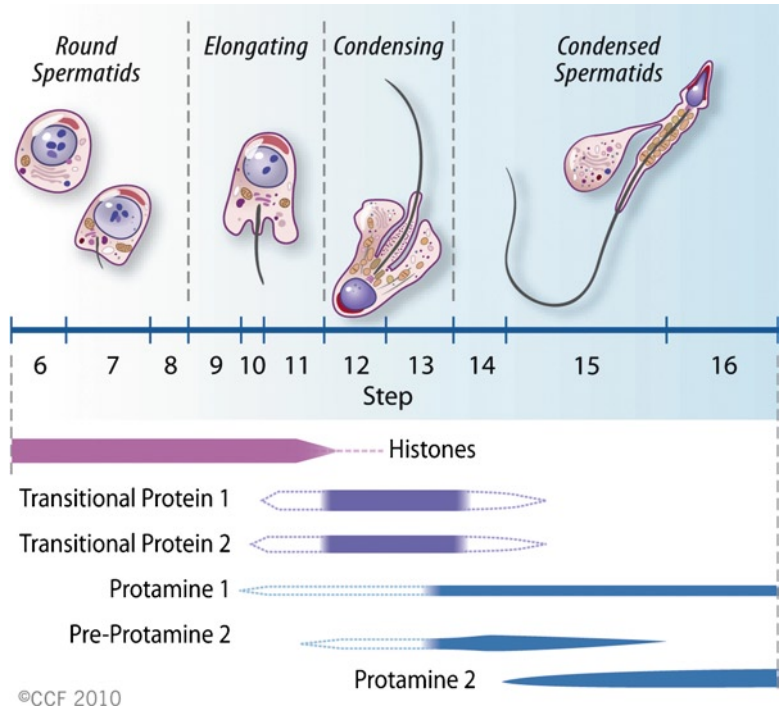

Fig. 2.8 Diagrammatic representation of the steps where the histones are replaced with the transition proteins and protamines in the round spermatid progresses into a condensed spermatid just before it is released into the lumen (reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2010. All Rights Reserved)

Mice lacking either TP1 or TP2 alone had normal numbers of sperm with only minor abnormalities and were fertile, indicating either that the TPs were not essential or that the individual TPs complement each other [51, 52, 76]. Protamine 2 processing defects do not inhibit postfertilization processes because late spermatids containing unprocessed protamine 2 are able to initiate normal development [77]. Defective protamine 2 processing is correlated with infertility in humans [78] and mouse mutants [51, 52] and could be due solely to the secondary cytoplasmic effects on sperm development resulting in a reduced ability to penetrate the egg.

Protamines as Checkpoints of Spermatogenesis

Human sperm chromatin undergoes a complex transition during the elongating spermatid stage of spermiogenesis, in which histones are extensively replaced by protamines. Humans express equal quantities of two protamines: protamine 1 and protamine 2 [79–81]. Protamines are approximately half the size of histones [82]. They are highly basic sperm-specific nuclear proteins that are characterized by an arginine-rich core and cysteine residues [83, 84]. The high level of arginine causes a net positive charge, thereby facilitating strong DNA binding [85]. Cysteine residues facilitate the formation of multiple inter- and intraprotamine disulfide bonds essential for high-order chromatin packaging, which is necessary for normal sperm function [86–90]. P2 protamines contain fewer cysteine groups and thus contain fewer disulfide cross links [81]. This, theoretically, leaves the DNA more susceptible to damage. Altered P2 expression is common in men with infertility [77].

During spermiogenesis, protamines progressively replace somatic histones in a stepwise manner [83]. First, somatic histones are replaced by testis-specific histone variants, which are

replaced by transition proteins (TP1a and TP2) in a process involving extensive DNA rearrangement and remodeling [42]. During the elongating spermatid stage, the transition proteins are replaced in the condensing chromatin by protamines. In humans, ~85% of the histones are replaced by protamines [54, 91–94]. This sequential process facilitates molecular remodeling of the male genome within the differentiating spermatid [40]. In human sperm, the mean P1/P2 ratio is approximately 1.0 [77, 80, 95]. Sperm from infertile men show an altered P1/P2 ratio and/ or no detectable P2 in mature sperm. Protamine abnormalities in sperm from fertile men are extremely rare [78, 95–98].

Two links are proposed between abnormal protamine expression and aberrant spermatogenesis: (1) abnormal protamine expression is indicative of a general abnormality of spermatogenesis, possibly due to abnormal function of the transcription or translational regulator (2) protamines act as checkpoint regulators of spermatogenesis, and abnormal protamine expression leads to induction of an apoptotic process and severely diminished sperm quality [99].

Protamines condense the DNA strands and form the basic packaging unit of sperm chromatin called a toroid. Intramolecular and intermolecular disulfide cross-links between cysteine residues present in protamines result in further compaction of the toroids [100]. Protamines confer a higher order of DNA packaging in sperm than that found in somatic cells. All of these levels of compaction and organization help protect sperm chromatin during transport through the male and female reproductive tract. This also ensures delivery of the paternal genome in a form that allows developing embryo to accurately express genetic information [58, 75, 80, 101]. Protamine replacement may also be necessary for silencing the paternal genome and reprogramming the imprinting pattern of the gamete [102]. Abnormal protamine expression is associated with low sperm count, decreased sperm motility and morphology, diminished fertilization ability, and increased sperm chromatin damage [77, 98, 103]. Infertile men are reported to have a higher histone-to-protamine ratio in their sperm chromatin [95, 104].

DNA Methylation During Spermatogenesis

Nucleohistones are present in human and rat sperm and are absent in mouse sperm. About ~15% of the histones are retained in the mature human spermatozoa [58]. The distribution of these histones within the sperm nucleus may have an important function. Chromatin associated with histones corresponds to specific sequences [58], suggesting that heterogeneity in the sperm nucleus may be the basis for male genetic information [105–107]. There are widespread differences in methylation of specific sequences during oogenesis and spermatogenesis. Maintenance methylases can stably preserve DNA methylation at cytosine residues through rounds of replication [108] and may have a role in gene regulation [109]. Methylation can also provide a mechanism for imprinting the maternal and paternal genomes as seen by the gametic differences in DNA methylation. This results in differential regulation of the paternal genomes during early development [110].The sequences that are highly methylated in pachytene spermatocytes are also highly methylated in spermatids and epididymal sperm, indicating that this state persists throughout spermatogenesis [111].

DNA methylation may be involved in genomic imprinting in mammals and is one of the major epigenetic marks established during spermatogenesis [112]. Mature sperms show a more unique DNA methylation profile than somatic cells [113]. The level of DNA methylation does not correlate with fertilization but with pregnancy rate after IVF [114].

Sperm Nuclear DNA Strand Breaks

Mammalian spermiogenesis involves important changes in the cytoarchitecture and dramatic remodeling of the somatic chromatin; most of the nucleosomal DNA supercoiling is eliminated [115, 116]. This modification in chromatin structure occurs in elongating spermatids and is an important contributor to the nuclear integrity and acquisition of full fertilization potential of the

male gamete [117]. DNA damage involves (1) abortive apoptosis initiated post meiotically when the ability to drive this process to completion is in decline (2) unresolved strand breaks created during spermiogenesis to relieve torsional stress associated with chromatin remodeling and (3) oxidative stress as a result of reactive oxygen species. Three major mechanisms for the creation of DNA damage in the male germ line have been proposed: chromatin remodeling by topoisomerase, oxidative stress, and abortive apoptosis. DNA damage could arise due to a combination of all the three mechanisms. Furthermore, a two-step hypothesis has been proposed [117, 118]. According to this hypothesis, the first step in the DNA damage cascade has its origin in spermiogenesis during which DNA is remodeled prior to condensation. Defects in the chromatin remodeling process result in the production of spermatozoa that are characterized by reduction in the efficiency of protamination, abnormal protamine 1 to protamine 2 ratio, and relatively high nucleohistone content [101, 119, 120]. These defects in chromatin modeling create a state of vulnerability whereby spermatozoa become increasingly susceptible to oxidative damage. In the second step of this DNA cascade, reactive oxygen species attack chromatin.

One of the first hypothesis concerning the origins of DNA damage in the male germ line focused on the physiological strand breaks created by topoisomerase during spermiogenesis as a means of relieving the torsional stresses created as DNA is condensed and packaged into the sperm head [60, 101]. Normally, these strand breaks are marked by a histone phosphorylation event and are fully resolved by topoisomerase before spermatozoa are released from germinal epithelium during spermiogenesis [121].

Sperm chromatin compaction is believed to play an important role in protecting the male genome from insult. This specific chromatin structure of the sperm essential for proper fertility and is in part due to the proteins that are bound to the DNA, including the protamines, histones, and components of the nuclear matrix [122, 123]. The cascade of events leading to DNA damage involves an error in chromatin remodeling during spermiogenesis. This leads to generation of spermatozoa with poorly protaminated nuclear DNA that is increasingly susceptible to oxidative attack [118].

Efficiency of chromatin remodeling during spermiogenesis has been studied employing DNA sensitive fluorochrome chromomycin (CM3). Chromomycin competes with the nucleoproteins for binding sites in the minor groove of GC-rich DNA and serves as a marker for the efficiency of DNA protamination during spermiogenesis. Staining with this probe is positively related to the presence of nuclear histones [124] and poor chromatin compaction [125] and negatively related with presence of protamines [126]. Impaired chromatin remodeling during spermiogenesis is a consistent feature of defective human spermatozoa possessing fragmented DNA [127–131]. DNA damage depends on fundamental errors that occur during spermatogenesis and may explain the correlation of pathology with sperm count [132].

Sperm Apoptosis

Apoptosis in sperm is different from somatic apoptosis in many ways: (1) spermatozoa are transcriptionally and translationally silent, and therefore, cannot undergo programmed cell death or "regulated cell death," (2) sperm chromatin has a reduced nucleosome content due to extensive protamination and, therefore, lacks the characteristic DNA laddering seen in somatic cells, and (3) endonucleases that are activated in the cytoplasm or released from the mitochondria are prevented from physically accessing the DNA due to the inherent physical architecture of the spermatozoa. However, spermatozoa do exhibit some of the hallmarks of apoptosis including caspase activation and phosphatidylserine exposure on the surface of the cells [133].

Sertoli cells can support only a limited number of germ cells in the testis. In the testis, apoptosis normally occurs to prevent the overproduction of germ cells and to selectively remove injured germ cells [134]. Clonal expansion of the germ cells in the testis occurs at very high levels, and thus, apoptosis is necessary to limit the size of the germ cell population to one which the Sertoli cell is able to

support [135]. Fas Ligand (FasL) is secreted by Sertoli cells. Fas is a protein located on the germ cell surface. Evidence of germ cell apoptosis has been demonstrated in FasL-defective mice [136]. Men with poor seminal parameters often display a large percentage of Fas-expressing sperm in their ejaculate [101]. Some of these sperms with DNA damage and Fas expression may have undergone "abortive apoptosis" in which they started but subsequently escaped the apoptotic pathway [137]. However, other studies have failed to find a correlation between DNA damage and Fas expression and other markers of apoptosis [62]. Recent studies examining loss of function have indicated that DNA damage checkpoints occur during spermatogenesis and may also involve excision repair genes, mismatch repair genes, and p53 [138].

Oxidative Stress in the Testis

Sertoli cells provide nutritional support to the differentiating germ cells in the testis. They are protected from oxidative stress as these cells pass through meiosis and emerge as haploid cells known as round spermatids. At this stage of development, these cells are transcriptionally silent. Even in the absence of any regulated gene transcription, they are able to undergo cellular transformation into fully differentiated, highly specialized cells – the spermatozoa. This is accomplished through a highly orchestrated differential translation of preexisting mRNA species through a process called spermiogenesis. Cells are sensitive to oxidative stress during spermiogenesis. Throughout this phase, they are highly dependent on the nurturing Sertoli cells, which possess antioxidants such as superoxide dismutase, glutathione reductase, transferase, and peroxidase [139]. Isolated spermatozoa have a limited capacity for DNA repair [140].

Spermiogenesis and Etiology of DNA Damage

Spermiogenesis, the process by which haploid round spermatids differentiate into spermatozoa, is a key event in the etiology of DNA damage in the male germ line. During spermiogenesis, the chromatin undergoes extensive remodeling, which enables the entire haploid genome to be compacted into a sperm head measuring 5×2.5 μm. This occurs as physiological DNA strand breaks are introduced by topoisomerase to relieve the torsional stresses involved in DNA packaging during sperm differentiation. These strand breaks are corrected by a complex process involving H2Ax expression, formation of poly(ADP-ribose) by nuclear poly (ADP-ribose) polymersases (PARP) and topoisomerase [141]. If the spermiogenesis process is disrupted for any reason, restoration of the cleavage sites is impaired, and defective spermatozoa with unresolved physiological strand breaks are released from the germinal epithelium. The "transition" proteins play a key role in maintaining DNA integrity during spermiogenesis as they move into the sperm nucleus between the removal of histones and the entry of protamines. Functional deletion of these proteins results in the production of spermatozoa with poor fertilizing ability, poor chromatin compaction, and high levels of DNA fragmentation [63]. DNA damage in human spermatozoa is associated with the disruption and poor chromatin remodeling during spermiogenesis [120, 128].

The efficiency of spermatogenesis is reflected by conventional semen characteristics such as sperm count and morphology and the correlation with DNA damage [132, 142]. Poor protamination results in spermatozoa that possess nucleohistone-rich regions of chromatin, which are vulnerable to oxidative attack [117]. Oxidative stress is a major determinant of the quality of spermiogenesis. When this process is disrupted, spermatozoa are produced that are vulnerable to oxidative stress, 8OHdg formation, and ultimately DNA fragmentation as a consequence of apoptosis [120, 143, 144].

Efficiency of Spermatogenesis

The efficiency of spermatogenesis varies between different species; it appears to be relatively constant in man. The time needed for a spermatogonium to differentiate into a mature spermatid is estimated to be 70 ± 4 days [145].

In comparison to animals, the spermatogenetic efficiency in man is poor, and the daily rate of spermatozoa production is about 3–4 million/g of testicular tissue [146]. Although a much higher sperm count should be expected in the ejaculate than the 20 million/mL described by WHO manual [147], this is not the case. This is largely because most developed cells (>75%) are eliminated as a result of apoptosis. In the remaining cells, more than half are abnormal. Therefore, only about 12% of the spermatogenetic potential is available for reproduction [148]. Furthermore, daily sperm production in men also declines with age; this is associated with a loss of Sertoli cells, an increase in germ cell degeneration during prophase of meiosis, or loss of primary spermatocytes along with a reduction in the number of Leydig cells, non-Leydig interstitial cells, and myoid cells.

Postspermiation Events

The process of spermiation and the journey of a sperm through the excurrent duct of the testis to a site where it can be included in the ejaculate take an additional 10–14 days. The nucleus progressively elongates as its chromatin condenses; the head is characterized by a flattened and pointed paddle shape, which is specific to each species, and involves the Golgi phase where the centrioles migrate from the cytoplasm to the base of the nucleus and proximal centriole becomes the implantation apparatus to anchor flagellum to the nucleus and distal centriole becomes the axoneme. In the cap phase, the acrosome forms a distinct cap over the nucleus covering about 30–50% of the nuclear surface [149]. The acrosome contains the hydrolytic enzymes necessary for fertilization. The manchette is formed, and the spermatids are embedded in Sertoli cells. During the maturation phase, mitochondria migrate toward the segment of the growing tail to form the mitochondrial sheath and dense outer fibers. A fibrous sheath is formed to complete the assembly of the tail. Most of the spermatid cytoplasm is discarded as a residual body, and the spermatid moves toward the lumen of the seminiferous tubule. Once elongation of the spermatid is complete, Sertoli cell cytoplasm retracts around the developing sperm, and all unnecessary cytoplasm is stripped. The spermatozoon is finally released it into the tubule lumen. The mature spermatozoon is an elaborate, highly specialized cell produced in large numbers – about 300 per gram of testis per second.

Spermatozoa

Spermatozoa are highly specialized and condensed cells that do not grow or divide. A spermatozoon consists of a head containing the paternal material (DNA) and the tail, which provides motility. The spermatozoon is endowed with a large nucleus but lacks a large cytoplasm, which is characteristic of most body cells. The heterogeneity of the ejaculate is a characteristic feature in men [150–152].

Head

The head is oval in shape, measuring about 4.0–5.5 μm in length and 2.5–3.5 μm in width. The normal length-to-width ratio is about 1.50–1.70 [153]. Under bright-field illumination, the most commonly observed aberrations include head shape/size defects (including large, small, tapering, pyriform, amorphous, and vacuolated (>20% of the head surface occupied by unstained vacuolar areas)) and double heads, or any combination thereof [154].

Acrosome

The acrosome is represented by the Golgi complex and covers about two thirds or about 70% of the anterior head area [151, 152]. When observed under the scanning electron microscope, the sperm head is unequally divided into the acrosomal and postacrosomal regions. Under the electron microscope, the sperm head is a flattened ovoid structure consisting primarily of the nucleus. The acrosome contains several hydrolytic enzymes, including hyaluronidase and proacrosin, which are necessary for fertilization [150]. During fertilization of the egg, the fusion of the outer acrosomal membrane

with the plasma membrane at multiple sites releases the acrosomal enzymes at the time of acrosome reaction. The anterior half of the head is covered only by the inner acrosomal membrane, while the posterior region of the sperm head is covered by a single membrane called the postnuclear cap. The overlap of the acrosome and the postnuclear cap results in an equatorial segment. The equatorial segment does not participate in the acrosome reaction. The nucleus comprises 65% of the head and is composed of DNA conjugated with protein. The chromatin is tightly packaged, and no distinct chromosomes are visible. The genetic information, including the sex determining X or Y chromosome, is "coded" and stored in the DNA [150].

Neck

This forms a junction between the head and tail. It is fragile, and a common abnormality is the presence of a decapitated spermatozoon.

Tail

The sperm tail arises at the spermatid stage. During spermatogenesis, the centriole is differentiated into midpiece, principal piece, and endpiece. The mitochondria reorganize around the midpiece. An axial core composed of two central fibrils surrounded by a concentric ring of nine double fibrils continues to the end of the tail. An additional outer ring is composed of nine coarse fibrils. The main piece is comprised of 9 coarse outer fibrils that diminish in thickness until only the inner 11 fibrils of the axial core surrounded by a fibrous sheath remain. The mitochondrial sheath of the midpiece is relatively short but slightly longer than the combined length of the head and neck [150].

Endpiece

The endpiece is not distinctly visible by light microscopy. Both the tail sheath and coarse filaments are absent. The tail, which contains all the motility apparatus, is 40–50 μm long and arises from the spermatid centriole. It propels the sperm body via waves generated in the neck region. These waves pass distally along like a whiplash.

Under bright-field illumination, common neck and midpiece defects include bent tails, distended or irregular/bent midpieces, abnormally thin midpieces (no mitochondrial sheath), the absence of the neck or midpiece, or any of these combinations [154]. Tail defects include short, multiple hairpin broken tails, irregular widths, coiled tails with terminal droplets, or a combinations of these defects [154]. Cytoplasmic droplets greater than one third the area of a normal sperm head are considered abnormal. They are usually located in the neck/midpiece region of the tail [152].

Under scanning electron microscopy, the tail can be subdivided into three distinct parts, i.e., midpiece, principal piece, and endpiece. In the midpiece, the mitochondrial spirals can be clearly visualized. The midpiece narrows toward the posterior end. The short endpiece has a small diameter due to the absence of the outer fibers [150]. Under transmission electron microscopy, the midpiece possesses a cytoplasmic portion and a lipid-rich mitochondrial sheath that consists of several spiral mitochondria surrounding the axial filament in a helical fashion. The midpiece provides the sperm with the energy necessary for motility. An additional outer ring of 9 coarser fibrils surrounds the central core of 11 fibrils. Individual mitochondria are wrapped around these fibrils in a spiral manner to form the mitochondrial sheath, which contains the enzymes needed in the oxidative metabolism of the sperm. The mitochondrial sheath of the midpiece is relatively short and slightly longer than the combined length of the head and neck [150].

The principal or mainpiece is the longest part of the tail, and it provides most of the propellant machinery. The coarse nine fibrils of the outer ring diminish in thickness and finally disappear, leaving only the inner fibrils in the axial core for most of the length of the principal piece [155]. The tail terminates in the endpiece with a length of 4–10 μm and a diameter of <1 μm due to the

absence of the outer fibrous sheath and distal fading of the microtubules.

Regulation of Spermatogenesis

Both intrinsic and extrinsic regulations influence spermatogenic process.

Intrinsic Regulation

Testosterone, neurotransmitters (neuroendocrine substances), and growth factors are secreted by Leydig cells to neighboring Leydig cells, blood vessels, the lamina propria of the seminiferous tubules and Sertoli cells [12, 148, 156] Leydig cells help maintain the nutrition of the Sertoli cells, and the cells of the peritubular tissue influence the contractility of myofibroblasts and regulate the peristaltic movements of seminiferous tubules and transportation of the spermatozoa. Leydig cells also help regulate blood flow in the intertubular microvasculature [6]. Sertoli cells deliver different growth factors, and various germ cells participate in the development and regulation of germ cells. These factors represent an independent intratesticular regulation of spermatogenesis.

Extrinsic Influences

The hypothalamus and hypophysis control local regulation of spermatogenesis by pulsatile secretion of GnRH and release of LH. Leydig cells produce testosterone, which influences spermatogenesis and provides feedback to the hypophysis, which regulates the secretory activity of Leydig cells. FSH action on the Sertoli cells is necessary for maturation of the germ cells. Both FSH and LH are necessary for complete spermatogenesis. Testicular function is determined by interaction between the endocrine and paracrine mechanisms [157–159]. Sertoli cells secrete inhibin, which functions in the feedback mechanism directed to the hypophysis. Thus, both growth and differentiation of testicular germ cells involve a series of complex interactions between somatic and germinal elements [157–159].

Immune Status of the Testis

The spermatozoa, late pachytene spermatocytes, and spermatids express unique antigens that are not formed until puberty, and therefore, immune tolerance is not developed. The blood–testis barrier develops as these autoantigens develop. The testis is considered to be an immune privileged site, i.e., transplanted foreign tissue can survive for a period of time without immunological rejection. An immune surveillance is present in the testis and the epididymis, which shows an active immunoregulation to prevent autoimmune disease [160, 161].

Disturbances of Spermatogenesis

Disturbances in both proliferation and differentiation of the male germ cells and the intratesticular and extratesticular mechanisms regulating spermatogenesis can occur as a result of environmental influences or as a result of diseases that directly or indirectly affect spermatogenesis [162, 163]. In addition, nutrition, therapeutic drugs, hormones and their metabolites, increased scrotal temperature, toxic substances, and radiation can reduce or completely inhibit spermatogenesis.

Sperm Transport in the Epididymis, Storage, and Capacitation

The epididymis lies along the dorsolateral border of each testis. It comprises the vasa efferentia, which emanates from the rete testis and the epididymal ducts. The primary function of the epididymis is posttesticular maturation and storage of spermatozoa during their passage from the testis to the vas deferens. The epididymal epithelium is androgen-dependent and has both absorptive and secretory functions. The epididymis is divided into three functionally

distinct regions: the head, body, and tail, otherwise known as the caput epididymis, corpus epididymis, and cauda epididymis, respectively. Much of the testicular fluid that transports spermatozoa from the seminiferous tubules is reabsorbed in the caput, thereby increasing the concentration of the spermatozoa by 10- to 100-fold. As the newly developed spermatozoa pass through these regions of the epididymis, many changes occur including alterations in net surface charge, membrane protein composition, immunoreactivity, phospholipid and fatty acid content, and adenylate cyclase activity.

Epididymal Sperm Storage

As many as half of the spermatozoa released from the testis die and disintegrate within the epididymis and are reabsorbed by the epididymal epithelium. The remaining mature spermatozoa are stored in the cauda epididymis, and this provides a capacity for repetitive fertile ejaculations. The capacity for sperm storage decreases distally, and the spermatozoa in the vas deferens may only be motile for a few days. After prolonged sexual activity, caudal spermatozoa first lose their fertilizing ability, followed by their motility and then their vitality. They ultimately disintegrate. Older, senescent spermatozoa must be eliminated from the male tract at regular intervals. Otherwise, their relative contribution to the next ejaculate(s) increases, reducing semen quality, even though such ejaculates do have a high sperm concentration. The vas deferens is not a physiological site of sperm storage and contains only about 2% of the total spermatozoa in the male tract. Sperms transit through the fine tubules of the epididymis in approximately 10–15 days in humans.

Sperms mature outside the testis. The spermatozoa within the testis have very limited motility, or none at all, and are incapable of fertilizing an egg. Both epididymal maturation and capacitation are necessary before fertilization. Capacitation – the final step required for fertilization – may be an evolutionary consequence of the development of a storage system for inactive sperm in the caudal epididymis. Preservation of optimal sperm function during this period of storage requires adequate testosterone levels in the circulation.

Sperm Entry into Cervical Mucus

At the moment of ejaculation, spermatozoa from the cauda epididymis are mixed with secretions of the various accessory glands in a specific sequence and deposited around the external cervical os and in the posterior fornix of the vagina. The spermatozoa in the first fraction of the ejaculate have significantly better motility and survival than the later fractions. Most of the spermatozoa penetrate the cervical mucus within 15–20 min of ejaculation [164, 165]. Spermatozoa enter the uterine cavity from the internal "cervical os" by virtue of their own motility [166]. From here, the spermatozoa traverse to the site of fertilization in the ampulla of the Fallopian tube or the oviduct.

Capacitation and Acrosome Reaction

Capacitation is a series of cellular or physiological changes that spermatozoa must undergo in order to fertilize an egg [167, 168]. It is characterized by the ability to undergo the acrosome reaction, bind to the zona pellucida, and acquire hypermotility. Capacitation per se does not involve any morphological changes, even at the ultrastructural level. It does, however, represent a change in the molecular organization of the intact sperm plasmalemma, which gives spermatozoa the ability to undergo the acrosome reaction in response to the induction of the stimulus. During capacitation, the seminal plasma factors that coat the surface of the sperm are removed, and the surface charge is modified along with the sperm membrane, sterols, lipids, and glycoproteins, and the outer acrosomal membrane lying immediately under it. Levels of intracellular free calcium also increase [169, 170].

The acrosome reaction enables sperm to penetrate the zona pellucida and also spurs the fusogenic state in the plasmalemma overlying the nonreactive equatorial segment, which is needed for interaction with the oolemma. The changes

termed as "acrosome reaction" prepare the sperm to fuse with the egg membrane. The removal of cholesterol from the surface membrane prepares the sperm membrane for the acrosome reaction [171, 172]. In addition, d-mannose binding lectins are also involved in the binding of human sperm to the zona pellucida [173, 174]. Thus, all these series of changes are necessary to transform the stem cells into fully mature, functional spermatozoa equipped to fertilize an egg (Fig. 2.7).

Conclusion

The testis is an immune privileged site. The blood–testis barrier provides a microenvironment for spermatogenesis to occur. The seminiferous tubules are the site of sperm production. The process of differentiation of a spermatogonium into a spermatid is known as spermatogenesis. It involves both mitotic and meiotic proliferation as well as extensive cell remodeling. In humans, the process of spermatogenesis starts at puberty and continues throughout life. Spermatogenesis produces genetic material necessary for the replication of the species. Meiosis assures genetic diversity. Along the length of the seminiferous tubule, there are only certain cross sections where spermatozoa are released. Sperm production is a continuous and not a pulsatile process. Spermatozoa are highly specialized cells that do not grow or divide. The spermatogenic process is maintained by different intrinsic and extrinsic influences. Spermatozoa have to undergo a series of cellular or physiological changes such as capacitation and acrosome reaction before they can fertilize. The epididymis is limited to a storage role. Nutrition, therapeutic drugs, hormones and their metabolites, increased scrotal temperature, toxic substances, or radiation can reduce or entirely inhibit spermatogenesis.

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