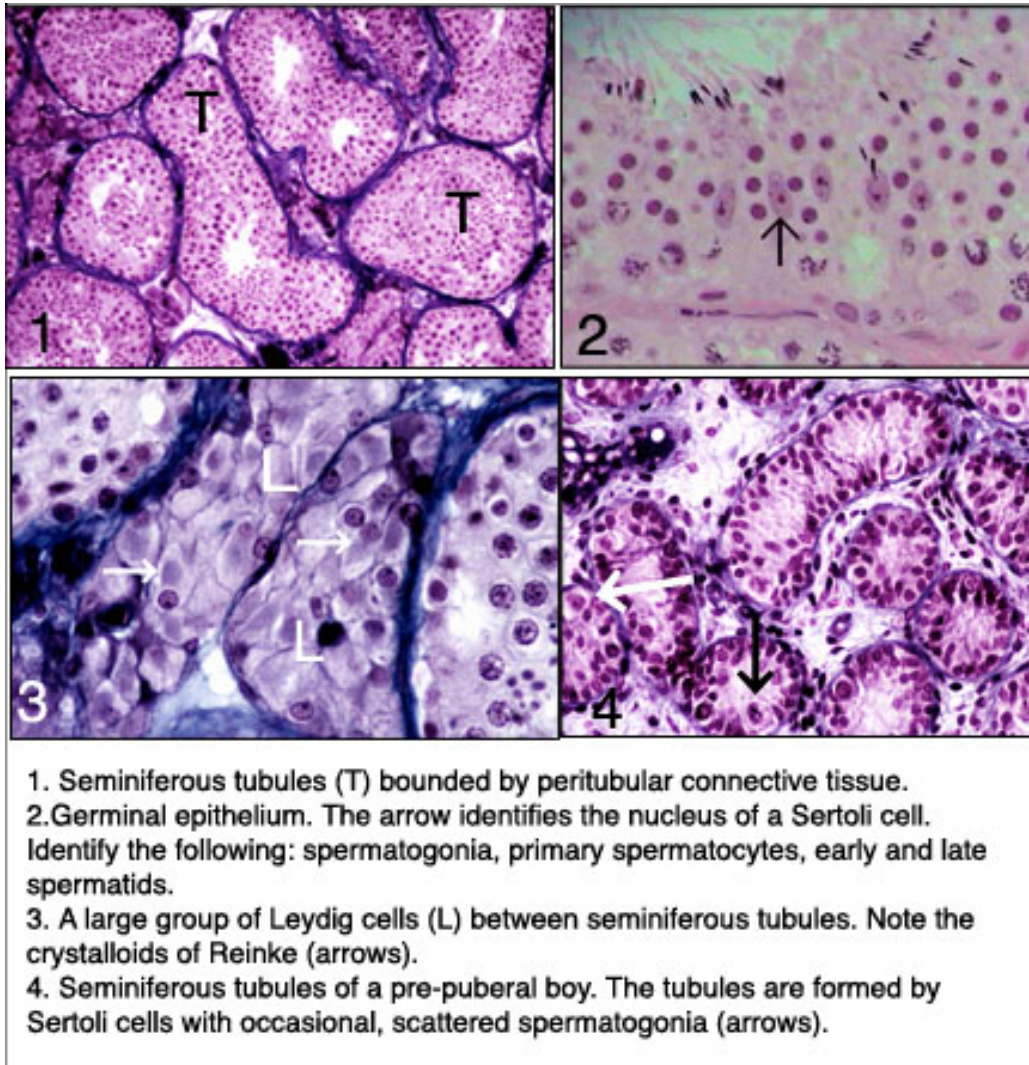


Testes



The testes are compound tubular glands that lie within a scrotal sac, suspended from the body by a spermatic cord. The testes are dual organs that act as exocrine glands producing a holocrine secretion, the sperm, and as endocrine organs that secrete the male sex hormone, testosterone. Each normal adult testis weighs between 12-15 gm and the right testis is commonly slightly larger and heavier than the left testis. Each testis is covered anteriorly and laterally by a simple squamous epithelium (mesothelium) called the visceral layer of tunica vaginalis. On the posterior aspect of the testis, this mesothelium reflects onto the scrotal sac and lines its interior as the parietal layer of the tunica vaginalis. The serous cavity between visceral and parietal layers allows the testes to move freely and reduces the chance of injury from increased pressure on the exterior of the scrotum. A thick, fibrous capsule, the tunica albuginea, lies beneath the visceral layer of tunica vaginalis, separated from it only by a basal lamina. Tunica albuginea consists of a dense fibroelastic connective tissue that contains scattered smooth muscle cells. The muscle cells are concentrated in the posterior region, where the tunica albuginea thickens and projects into the testis to form the mediastinum testis. Connective tissue partitions, the septula testis, extend from the mediastinum into the interior of the testis and subdivide it into approximately 250 pyramid-shaped compartments called lobuli

testis. The apices of the compartments are directed toward the mediastinum, and each lobule contains one to four convoluted seminiferous tubules that represent the exocrine portion of the testis. Their product is whole cells (spermatozoa). The inner region of the tunica albuginea, the tunica vasculosa, consists of loose connective tissue and contains numerous small blood vessels that supply the testis. The connective tissue extends into each lobule and fills the spaces between the seminiferous tubules, forming the interstitial tissue of the testis. The interstitial tissue is rich in extracellular fluid and contains abundant small blood and lymphatic vessels that form a plexus around the seminiferous tubules. In addition to fibroblasts, the interstitial tissue contains macrophages, mast cells, mesenchymal cells, and large polyhedral cells 15 to 20 μm in diameter. These are the interstitial cells (of Leydig), which commonly occur in groups and make up the endocrine portion of the testis. They secrete the male steroid hormone testosterone. Interstitial cells usually contain single, large, spherical nuclei, but binucleate cells are not uncommon. Leydig cells are characterized by numerous LH receptors. In electron micrographs, the cytoplasm shows abundant smooth endoplasmic reticulum, well-developed Golgi complexes, and numerous mitochondria, which contain tubular rather than lamellar cristae. Morphologically, large cytoplasmic crystals called the crystals of Reinke characterize interstitial cells. These proteinaceous bodies are highly variable in shape and size but are readily seen with the light microscope. In electron micrographs they show a highly ordered structure. The crystals appear in the interstitial cells of most postpubertal individuals and vary considerably in number. Their significance is unknown. Most of the enzymes involved in the synthesis of testosterone are located in the smooth endoplasmic reticulum and mitochondria of interstitial cells. Only about 1 to 2% of circulating testosterone is in free form with the remainder being bound to a sex steroid-binding globulin or albumin produced by the liver. Testosterone and its metabolites are essential for the proliferation and the differentiation of excretory ducts and male accessory sex glands and for maintaining these structures in a functional state. Metabolites of testosterone include two potent androgens. Dihydrotestosterone (DHT) produced by the activity of the enzyme 5 α -reductase and 5 α -androstenediol due to the activity of the enzyme 3 α -reductase. Testosterone and its derivatives influence other tissues and are responsible for beard growth, low pitch of the voice, muscular build, and male distribution of body hair. Production of testosterone by the interstitial cells is controlled by a gonadotropic hormone called interstitial cell-stimulating hormone (ICSH) or LH, which is secreted by cells (gonadotrophs) located within the anterior pituitary.

Seminiferous Tubules

Each testicular lobule contains one to four highly convoluted seminiferous tubules that measure 150 to 250 μm in diameter and 30 to 70 mm long. Added together the seminiferous tubules have a tubular length that exceeds 500 m. Each seminiferous tubule is organized into a non-branched closed loop, both ends of which open into the first portion of the excurrent duct system, the rete testis. A seminiferous tubule consists of a complex stratified germinal (seminiferous) epithelium surrounded by a layer of peritubular tissue. The peritubular tissue is separated from the germinal epithelium by a basal lamina and consists of collagenous fibers and flattened cells that, depending on the species, may contain numerous actin filaments. These are the myoid (peritubular) cells. In humans, the myoid cells are said to be only moderately contractile. The production of testicular fluid by the germinal epithelium and its flow through the seminiferous tubules moves the spermatozoa into the excretory duct system. The germinal epithelium of the adult is unique among epithelia in that it consists of a fixed, stable population of supporting (Sertoli) cells and a proliferating population of differentiating

spermatogenic cells. Developing germ cells slowly migrate upward along the lateral surfaces of the supporting cells to be released at the free surface into the lumen of the seminiferous tubule.

Sertoli Cells

The Sertoli cell is a tall columnar cell that spans the germinal epithelium from the basal lamina to the luminal surface. The cell has an elaborate shape with numerous lateral processes with recesses or concavities that surround differentiating spermatogenic cells. The apical portion of the cells also envelops developing germ cells and releases them into the lumen of the seminiferous tubule. The expanded portion of the cell contains an irregularly shaped nucleus distinguished by a large, prominent nucleolus that is readily seen with the light microscope. The basal cytoplasm contains abundant smooth endoplasmic reticulum, and a large, well-developed Golgi complex occupies the supranuclear region. The cytoplasm contains many lipid droplets, lysosomes, thin elongated mitochondria, scattered profiles of granular endoplasmic reticulum, and a sheath of fine filaments that envelops the nucleus and separates it from the organelles. Microtubules are present also, their numbers depending on the state of activity of the cell. Human Sertoli cells feature membrane-bound inclusions called the crystalloids of Charcot-Böttcher; their function is unknown. Sertoli cells are further characterized by FSH receptors. Tight junctions occur between adjacent Sertoli cells near their bases and subdivide the germinal epithelium into basal and adluminal compartments, each of which has a separate, distinct population of spermatogenic cells. The basal compartment extends from the basal lamina of the germinal epithelium to the tight junctions; the adluminal compartment lies between the tight junctions and the lumen of the tubule. The tight junctions between the Sertoli cells appear to form, at least in part, a blood-testis barrier. Germ cells in the basal compartment are contained within an environment that has access to substances in the blood plasma, while the germ cells in the adluminal compartment reside in a specialized milieu that is maintained and controlled by the Sertoli cells. A number of plasma proteins are present in the basal compartment that are not found in the luminal contents of the seminiferous tubule that is, however, rich in other amino acids and ions. Sertoli cells are thought to provide all the nutrients for the avascular germinal epithelium. In addition to secreting testicular fluid, Sertoli cells release androgen-binding protein, whose synthesis is stimulated by a pituitary gonadotropin, follicle-stimulating hormone (FSH). The androgen-binding protein binds testosterone, thus providing the adluminal compartment with the level of hormone needed for the normal differentiation and development of germ cells. The blood-testis barrier helps to confine the high concentration of testosterone to the adluminal compartment and thus allows a different environment to be established in the basal compartment. Many of the developing germ cells in the adluminal compartment are haploid and might be regarded as foreign material by the body if released into surrounding tissues. The tight junctions between Sertoli cells are thought to prevent haploid germ cells from contacting general body tissues and thus prevent an autoimmune response to the individual's own germ cells. Although tight junctions contribute to the blood testis barrier, other factors may be involved. Sertoli cells phagocytize degenerating germ cells and take up the residual cytoplasm that normally is shed during release of mature germ cells into the lumen of the seminiferous tubule. In addition to providing mechanical and nutritional support for developing sperm, Sertoli cells also control the movement of germ cells from the basal lamina through the epithelium to the lumen and are important in the release of germ cells into the lumen. The microtubules and actin filaments in the cytoplasmic processes of the Sertoli cells provide these processes with the mobility they

need to carry out their functions. The numerous gap junctions that occur between adjacent Sertoli cells facilitate communication between cells along specific segments of a seminiferous tubule during migration and release of germ cells. Sertoli cells also secrete a glycoprotein known as inhibin. Inhibin suppresses the secretion of FSH by gonadotrophs in the anterior pituitary and via this feedback loop acts to control the rate of spermatogenesis. Likewise, the concentration of testosterone in the blood plasma is maintained at a specific concentration by circulating levels of this hormone that influence the secretion of pituitary gonadotrophs and gonadotrophin releasing hormone (GnRH) containing neurons located in the arcuate nucleus of the hypothalamus via a negative feedback loop mechanism.

Spermatogenic Cells

The spermatogenic cells of the germinal epithelium consist of spermatogonia, primary and secondary spermatocytes, and spermatids. These are not separate cell types but represent stages in a continuous process of differentiation called spermatogenesis. The term includes the entire sequence of events in the transformation of diploid spermatogonia at the base of the germinal epithelium into haploid spermatozoa that are released into the lumen of the seminiferous tubule. Spermatogonia lie in the basal compartment of the germinal epithelium, immediately adjacent to the basal lamina. The cells are 10 to 20 μm in diameter and round or ellipsoidal in shape and the nucleus of each cell contains the diploid number of chromosomes. Two types of spermatogonia can be differentiated. Type A spermatogonia replicate by mitosis and provide a reservoir of stem cells for the formation of future germ cells. During mitotic division, some type A spermatogonia give rise to intermediate forms that eventually produce type B spermatogonia; these are committed to the production of primary spermatocytes. The two types of spermatogonia can be differentiated by their nuclei. Type A spermatogonia have spherical or elliptical nuclei with a fine chromatin distribution and one or two nucleoli near the nuclear envelope. Many nuclei of this cell type also exhibit a clear nuclear vacuole. The lighter-stained, spherical nuclei of type B spermatogonia contain variably sized clumps of chromatin, most of which are arranged along the nuclear envelope. Only a single, centrally placed nucleolus is found. Primary spermatocytes at first resemble type B spermatogonia, but as they migrate from the basal lamina of the germinal epithelium, they become larger and more spherical, and the nucleus enters the initial stages of division. Primary spermatocytes usually are found in the central zone of the germinal epithelium. How these large cells pass from the basal to the adluminal compartment is unknown. The primary spermatocyte undergoes the first meiotic division to produce secondary spermatocytes. The homologous chromosomes have separated and the number is reduced by half, but the cells contain the diploid amount of DNA. Secondary spermatocytes lie nearer the lumen than the primary forms and are about half their size. Unlike the extended division of primary spermatocytes, secondary spermatocytes divide quickly in the second meiotic division to produce spermatids, which contain the haploid number of chromosomes and the haploid amount of DNA. Because they divide so quickly after being formed, secondary spermatocytes are seen only rarely in the germinal epithelium. Spermatids are about half the size of secondary spermatocytes. Numerous spermatids in different stages of maturation border the lumen of the seminiferous tubule. Early spermatids appear as small spherical cells with round darkly stained nuclei. Late spermatids appear as tailed spermatozoa within the recesses of the Sertoli cell. The cell divisions that take place during formation of male germ cells are unique in that not only is the genetic material reduced by half; the division of the cytoplasm is incomplete. Thus, the cells resulting from a single spermatogonium remain

in cytoplasmic continuity throughout the different stages of differentiation. The continuity is broken only when the sperm are finally released by the Sertoli cell.

Spermiogenesis

The process by which spermatids differentiate into slender, motile sperm is called spermiogenesis. Newly formed spermatids are round cells with central, spherical nuclei, prominent Golgi complexes, numerous mitochondria, and a pair of centrioles. Each of these components undergoes changes during spermiogenesis. At the onset of spermiogenesis, many small granules appear in the Golgi membranes and eventually coalesce to form a single structure called the acrosome. The developing acrosome is bounded by a membrane, the acrosomal vesicle, which also is derived from the Golgi complex and is closely associated with the outer layer of the nuclear envelope. The acrosomal vesicle expands and then collapses over the anterior half of the nucleus to form the head cap. The acrosome, which contains hydrolytic enzymes, remains within the acrosomal membrane. As these events occur, the two centrioles migrate to a position near the nucleus on the side opposite of the forming acrosome. Nine peripheral doublets plus a central pair of microtubules develop from the distal centriole and begin to form the axoneme of the tail. The proximal centriole becomes closely associated with a caudal region of the nucleus called the implantation fossa. As the axoneme continues to develop, nine longitudinal coarse fibers extend around it and blend with nine short, segmented columns that form the connecting piece, which unites the nucleus (head) with the tail of the spermatozoon. The annulus, a ring like structure, forms near the centrioles and migrates down the developing flagellum. Randomly distributed mitochondria migrate to the flagellum and become aligned in a tight helix between the centrioles and the annulus. This spirally arranged mitochondrial sheath characterizes the middle piece of the tail of a mature spermatozoon. Simultaneously, marked changes occur in the nucleus: it becomes condensed, elongated, and slightly flattened. Together with the acrosome, it forms the sperm head. The bulk of the cytoplasm now is associated with the middle piece of the evolving spermatid, and as differentiation nears completion, the excess cytoplasm is shed as the residual body, leaving only a thin layer of cytoplasm to cover the spermatozoon. Sertoli cells phagocytize the residual cytoplasm as the spermatozoa are released into the lumen of the seminiferous tubule. Although morphologically the spermatozoa appear mature, they are nonmotile and largely incapable of fertilization at this time. In most species, spermatids at specific stages of differentiation always are associated with spermatocytes and spermatogonia, which also are at specific stages of development. A series of such associations occurs along the length of the same seminiferous tubule, and the distance between two identical germ cell associations is called a "wave" of seminiferous epithelium. Human germinal epithelium exhibits a mosaic of such areas, and six different cell associations have been described. Such associations represent a fundamental pattern of cycling of the germinal epithelium during sperm production. The time taken for spermatogonia to become spermatozoa is relatively constant and species-specific: in humans it is about 64 days. If germ cells fail to develop at their normal rate, they degenerate and are phagocytosed by adjacent Sertoli cells.

Spermatozoa

Spermatozoa that lie free in the lumina of seminiferous tubules consist of a head containing the nucleus and a tail, which eventually gives motility to the free cell. The chromatin of the nucleus is very condensed and reduced in volume, providing the functionally mature sperm with greater mobility. The condensed form of chromatin also protects the genome while the spermatozoon is in route to fertilize the ovum. The acrosomal cap covers the anterior two-thirds of the nucleus and contains enzymes that are important for penetration of the ovum at fertilization. The size and shape of the nucleus vary tremendously in different species. The sperm tail is about $55\ \mu\text{m}$ long and consists of a neck, middle piece, principal piece, and end piece. The structural details of the different segments are best seen with the electron microscope. The neck is the region where the head and tail unite. It contains the connecting piece that joins the nine outer dense fibers of the tail to the implantation fossa of the nucleus. The region of the connecting piece that joins the implantation fossa is expanded slightly to form the capitulum. The middle piece extends from the neck to the annulus and consists of the axoneme, the nine coarse fibers, and the helical sheath of mitochondria. The principal piece is the longest part of the tail and consists of the axoneme and the nine coarse fibers (a 2 + 9 + 9 arrangement) enclosed by a sheath of circumferential fibers. The circumferential fibers join two longitudinal thickenings (columns) of this sheath located on opposite sides. The end piece is the shortest segment of the tail and consists only of the axoneme surrounded by the plasmalemma. The last step in the physiologic maturation of spermatozoa takes place in the female reproductive tract and is called capacitation. This final activation occurs in the oviduct and is characterized by the removal of a glycoprotein coat and seminal proteins from the plasmalemma overlying the acrosome, by changes in the acrosomal cap, and by changes in the respiratory metabolism of the spermatozoa. Capacitation substantially increases the number of spermatozoa capable of fertilization, but the mechanism of this activation is not fully understood.

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