MJC 4 (Physiology)

Physiology of Testicular Cycles

In some species, mature males are capable of copulating with a female whenever she is receptive. Secretion of GnRH and hence of GTHs is more or less continuous in these males but with daily and often seasonal fluctuations occurring in circulating levels of some GTHs. Daily secretory patterns for GTHs show considerable variation among different species. Hourly fluctuations of LH have been reported in bulls, and these variations in LH are correlated with following increases in circulating testosterone. In human males, FSH shows no cyclic variation in blood levels, although LH and testosterone exhibit obvious daily patterns with peak levels occurring during early morning hours and minimum values reported for the afternoon. Most wild mammals exhibit distinct seasonal breeding, and active spermatogenesis may be restricted to only a few months of the year or less.

A. Spermatogenesis

Each testis develops primarily from the medullary portion of an embryonic gonadal blastema as described previously. Differentiation of the medullary portion with concomitant regression of the cortical components (progenitor of the ovary) appears to be controlled by local embryonic androgen secretion activated by the sry gene. The medullary region differentiates into seminiferous tubules and interspersed masses of interstitial cells. These interstitial cells are located between the seminiferous tubules and synthesize and release androgens into the general circulation. The seminiferous tubules consist of large Sertoli cells, germ cells, spermatogonia, and cells derived from the spermatogonia. Each tubule is surrounded by a thin layer of connective tissue. Under the influence of androgens, peritubular myoid cells develop in this connective tissue layer during puberty. They surround and provide support for the seminiferous tubules and are believed to be responsible for contractile activity of the tubules that propels sperm to the epididymis. The Sertoli cell has an extensive cytoplasm extending from the outer edge to the lumen of the tubule. The angular nucleus of the Sertoli cell is located at the outer edge of the tubule. Sertoli cells form tight junctions with adjacent Sertoli cells and together with the peritubular myoid cells they secrete the various collagen and laminin proteins that form the basement membrane. This basement membrane along with their tight junctions together form the blood/testis barrier, quite literally a cellular fence that isolates the seminiferous tubules into a ‘backyard” (basal) compartment containing Sertoli cells and spermatogonia and a “frontyard” (adluminal) compartment containing the spermatogenetic cells. As a result of the blood testis barrier, nutrients and leukocytes cannot move between the Sertoli cells into the seminiferous tubules, thereby protecting developing spermatogenetic cells from exposure to cells and antibodies of the immune system. All chemicals must pass through the Sertoli cells in order to reach the spermatogenetic cells.

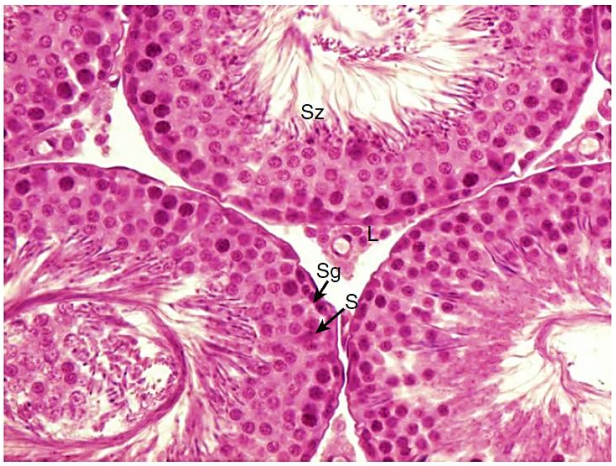

FIGURE 10-8 Spermatogenesis in rat testis. Section of rat testis showing edges of adjacent seminiferous tubules. Note the position of the Leydig cells adjacent to a capillary. Abbreviations: L, Leydig cells; S, nuclei of Sertoli cells; Sg, spermatogonia; Sz, sperm.

Germ cells are present along the outer margins of the seminiferous tubules and differentiate into spermatogonia. Spermatogonial cells proliferate mitotically under the influence of FSH to

produce more spermatogonia. Eventually some of these spermatogonia will undergo differentiation characterized by nuclear enlargement and will become primary spermatocytes that are capable of entering spermatogenesis. Testicular androgens are somehow necessary for initiation of meiosis in primary spermatocytes that undergo the first meiotic division to give rise to two smaller secondary spermatocytes. These latter cells are infrequently observed in histological preparations because, once formed, they quickly enter the second meiotic division to yield four haploid spermatids which are transformed to sperm (spermatozoa) by concentrating the chromatin material into the sperm head and by elimination of the majority of the cytoplasm. The process of transformation of spermatids to sperm is termed spermiogenesis.

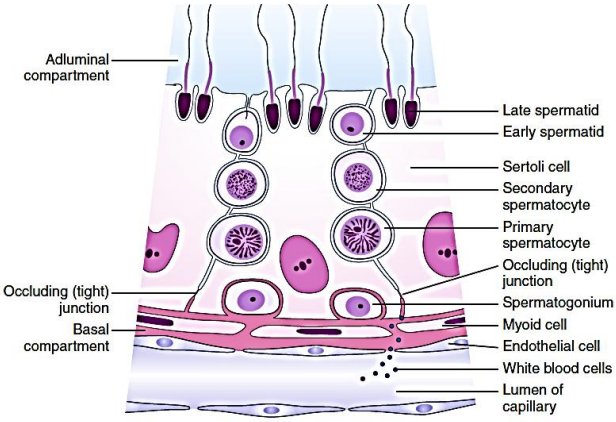

FIGURE 10-10 Occluding (tight) junctions between adjacent Sertoli cells form the blood testis barrier. The presence of the occluding junctions establishes an adluminal compartment isolating developing spermatocytes, spermatids, and spermatozoa from materials or white blood cells, leaving the capillaries in the basal compartment. Substances or leukocytes may diffuse or move by diapedesis through capillaries in the interstitium but cannot move past the occluding junctions linking the Sertoli cells. Spermatogonia lie basal to the occluding junctions in the basal compartment. *(Adapted with permission from Junqueira, L.C. and Carneiro, J., "Basic Histology," 11th ed., McGraw-Hill, New York, 2005.)*

Spermatogenesis is a temperature-sensitive process, and high temperatures such as found within the body cavity of terrestrial eutherians can impair normal spermatogenesis and produce temporary sterility. Consequently, at some time prior to the attainment of sexual maturity or prior to the annual breeding season, the testes descend into the scrotum, where spermatogenesis can proceed at a slightly lower temperature. The failure of the testes to descend, a condition known as cryptorchidism (crypto-, hidden; orchi, testis), may cause irreparable damage to the seminiferous epithelium in most species. Some mammals lack a scrotum (e.g., elephants, whales, seals), and the testes are permanently located within the abdominal cavity. Male elephants, however, are capable of producing viable sperm and copulating with a female at any time of year. In such species, either spermatogenesis does not exhibit the same temperature sensitivity characteristic for scrotal species or these animals possess other mechanisms to reduce testicular temperature.

A given histological section of a seminiferous tubule may show varying numbers of spermatogonia, primary spermatocytes, possibly a few secondary spermatocytes, spermatids, and sperm in sequence from the outer margin (gonia) to the lumen (sperm). The tails of the sperm extend into the lumen, and the heads of the sperm typically are still surrounded by highly folded margins of Sertoli cells.

Millions of maturing sperm may be sloughed off into the lumina of the seminiferous tubules each day. This process is termed spermiation and is stimulated by LH. These sperm pass through the tubules that eventually coalesce into larger ducts of the rete testis that eventually join the epididymis associated with each testis. Vast numbers of maturing sperm are stored in the epididymis. Under the influence of androgens and PRL, the epididymis secretes materials into its lumen where the sperm are being held. Included in this secretion are protein-bound sialic acids (sialomucoproteins), glycerylphosphoryl-choline, and carnitine. These particular substances are involved directly in maturing and maintaining sperm in viable condition until

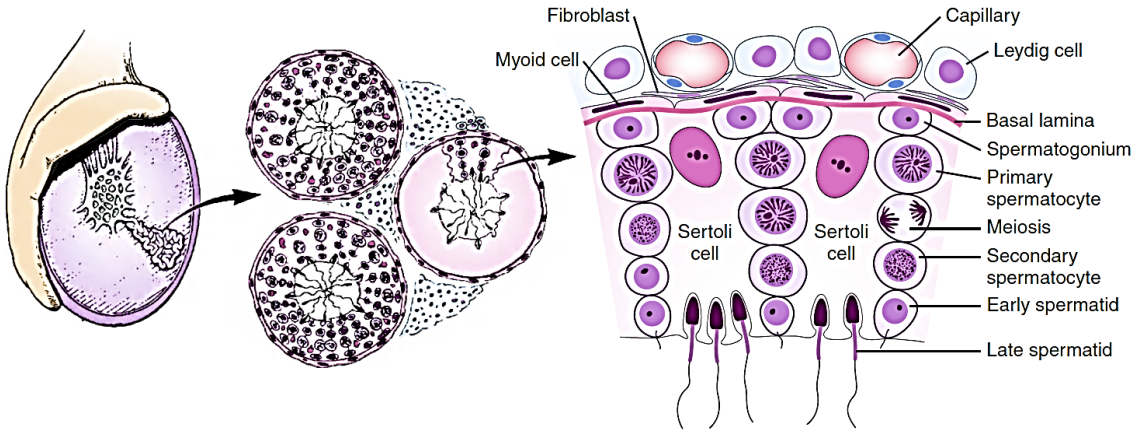

FIGURE 10-9 Organization of mammalian testis. Detail at right shows events of spermatogenesis and spermiogenesis in relation to Sertoli cells. The endocrine roles of interstitial (Leydig) cells, peritubular myoid cells, and Sertoli cells are described in the text. *(Adapted with permission from from Skinner, M.K.,* Endocrine Reviews, ***12****, 45–77, 1991; Junqueira, L.C. and Carneiro, J., "Basic Histology," 11th ed., McGraw-Hill, New York, 2005.)*

ejaculation. Androgens and androgen-binding protein (ABP) produced by Sertoli cells in the seminiferous tubules are released along with sperm and travel to the epididymis.

Androgen molecules freed from ABP in the lumen or androgen-ABP complexes or possibly both are absorbed by the epididymal cells. These androgens stimulate epididymal cells to secrete materials involved in maintenance of the sperm.

Contraction of the smooth muscles of the epididymis and vas deferens causes ejection of sperm (ejaculation). The mature sperm leave the epididymis, enter the vas deferens, travel to the urethra, traverse the length of the penis via the urethra, and are deposited in the female's vagina during coitus. Seminal vesicles and the prostate gland add their fluid secretions to the sperm and epididymal secretions to form a watery mixture of sperm and various organic and inorganic substances known as semen. The bulbourethral gland or Cowper's gland, which is homologous to the Bartholin's gland in females, produces a pre-ejaculate that cleanses and lubricates the urethra prior to the arrival of the semen. The entire ejaculatory event may be induced by the release of OXY from the pars nervosa in response to a neural reflex initiated by mechanical stimulation of the penis.

B. Endocrine Regulation of Testicular Functions

GTHs and a number of paracrine factors including testosterone control production of sperm. Many of the details of the endocrine regulation of these processes are not clear, but a generalized picture is emerging. Relatively separate roles have been defined for LH and FSH in males, although FSH and testosterone work cooperatively in some cases. Spermatogenesis is initiated indirectly by FSH through mitotic proliferation of spermatogonia and formation of primary spermatocytes. Spermatogonia lack FSH receptors, and the mechanism of spermatogonial activation is mediated by paracrine factors from the Sertoli cells that do have FSH receptors. One paracrine factor identified as having an important role in proliferation of spermatogonia is glial cell line-derived neurotrophic factor (GDNF). The intermediary role of the Sertoli cell is supported by observations that FSH stimulates mitosis in Sertoli cells whose number in the adult testis is directly proportional to sperm abundance. Testosterone may initiate meiotic divisions of primary spermatocytes that differentiate from spermatogonia, resulting eventually in formation of spermatids.

The production of ABP, cytoskeleton proteins (actin, vinculin), and $P450_{aro}$ by the Sertoli cell also is stimulated by FSH. Testosterone is the major circulating androgen in mammals, although other androgens such as androstenedione or 5-α-dihydrotestosterone (5α -DHT) may circulate in significant amounts. Some 5α-DHT also is produced and stimulates red blood cell

production in bone marrow and contributes to the higher hematocrit found in males as compared to females. Prior to attainment of puberty in bulls, androstenedione is the principal circulating androgen, but it is gradually replaced by testosterone at puberty. Testosterone within the testis, however, seems to be the most important androgen influencing sperm production.

Testosterone has several important paracrine effects on spermatogenesis and spermiogenesis. Testosterone concentrations within the testis are generally orders of magnitude greater than circulating testosterone levels and, for reasons still not understood, much higher than needed in order to maintain spermatogenesis. Perhaps testosterone is in part a precursor for testicular estrogen synthesis; for example, testicular estrogens reach dramatic levels in the stallion. Androgens are required for spermatogenesis to proceed normally, in part through the activation of a multitude of genes, one of which produces gonadotropin-regulated testicular helicase (GRTH/DDX25) in Leydig cells and germ cells. GRTH is a 56-kDa protein that is transported into the nucleus, where it binds to nuclear RNAs as a component of messenger ribonucleoprotein (mRNP) complexes. It regulates the activity and turnover of certain mRNAs

TABLE 10-6 Possible Local Actions for Gonadal Secretions

Factor	Source	Proposed Action
Activin	Granulosa cells Sertoli and interstitial cells	Unknown Specific receptors shown on germ cells
Epidermal growth factor (EGF)	Thecal/interstitial cells of ovary	Stimulates granulosa cell proliferation
Estradiol	Interstitial cells in male epididymis	Blocks androgen synthesis Stimulates fluid resorption
Fibroblast growth factor (FGF)	Granulosa cell	Causes epithelial proliferation in early follicular development and conversion of thecal cells to ovarian interstitial cells after ovulation
	Testicular germ cells	Binds to receptors on Sertoli cell; function unknown
Gonadotropin-releasing hormone (GnRH)	Ovary	Working through IP_3 second messenger, GnRH alters steroidogenesis by granulosa cells in certain follicular stages; may signal atresia
	Sertoli cell	Alters androgen synthesis by interstitial cells; increases local permeability of capillaries
Growth differentiation factor-9 (GDF-9)	Ovary	Maintains FSH receptors on oocyte
	Oocyte	Follicle growth and maturation (folliculogenesis)
Growth hormone-releasing hormone (GHRH)	Corpora lutea, oocyte	Promotes follicular development and ovulation
	Germ cells of tests	Stimulates sertoli cells to make stem cell factor
Insulin-like growth factor (IGF-I)	Granulosa cells	Increases number of LDL receptors on granulosa cells; stimulates cholesterol and inhibin synthesis
Interleukin 1(IL-1)	Sertoli cells	Decreases steroidogenesis in interstitial cells
PmodS protein	Peritubular myoid cells	Non-mitogenic factor that regulates differentiation and function of Sertoli cells
Interleukin 6 (IL-6)	Ovarian T cells	Suppresses response of granulosa cells to FSH,i.e., decreased progesterone synthesis; induces apoptosis and atresia of granulosa cells
KIT ligand	Oocyte	KIT ligand is a cytocrine that acts on a tyrosine kinase receptor (KIT) to promote folliculogenesis
Nerve growth factor (NGF)	Ovarian cells	Stimulates follicular formation and organization as well as differentiation of ovarian interstitial cells
Testosterone	Interstitial cell	Regulates functions of Seroli cells; stimulates meiosis in primary spermatocytes
Transforming growth factor (TGF-α)	Thecal cells	Facilitates proliferation of thecal and granulosa cells but slows their GTH-induced differentiation
	Sertoli and peritubular myoid cells	Causes EGF-like growth stimulation in interstitial cells; decreases steroidogenesis

involved in meiosis. GRTH-knockout mice are incapable of producing functional sperm. Androgen actions probably are indirect, as testosterone receptors appear to be absent or occur in very low numbers on germ cells.

However, estrogen receptors (ERs) are present on germ cells that also possess aromatase activity. Furthermore, at least in rodents, the local metabolism of DHT to an androgen metabolite 5α-androstane-3β, 17β-diol (3β-diol) has been reported in brain, prostate, and testes. 3b-Diol binds and activates ERs. Furthermore, aromatase knockout male mice exhibit disruption of spermatogenesis, supporting a local role for estrogen receptor-binding regulators. The attachment of Sertoli cells to spermatids involves cytoskeletal actin and vinculin interactions with the spermatids, as well as indirect effects of testosterone. Peritubular myoid cells are stimulated by testosterone to release two proteins, PModSA and PModSB, that cause Sertoli cells to secrete additional paracrine regulators that may alter spermiogenesis.

The Leydig cells of the testis also synthesize and release small quantities of estrogens. Locally, estradiol can block androgen synthesis by interstitial cells and can influence the responsiveness of these cells to GTHs. Estradiol also binds to receptors in the epididymis, where it regulates resorption of excess testicular fluid that was used to conduct sperm to the epididymis. The ratio of testosterone to estradiol in the general circulation may alter the ratios of FSH and LH being released from the pituitary through negative feedback. Finally, conversion of androgens to estrogen occurs in certain brain cells, and circulating estrogens themselves may influence male sexual behavior.

C. Actions and Metabolism of Androgens in Males

Circulating androgens influence development and maintenance of several glands and related structures associated with the male genital tract, such as the prostate gland and seminal vesicles, and induce development of certain secondary sexual characters such as growth of the beard and development of skeletal muscles in men. Androgens also exert a negative feedback effect upon the secretion of GTHs primarily through actions at the level of the hypothalamus.

The action of testosterone in some of its target cells involves its cytosolic conversion to DHT by the enzyme 5α-reductase. DHT has a greater affinity for the androgen receptor than does testosterone and hence is more potent than testosterone. Timely development of prostate and bulbourethral glands, the penis, and scrotum are dependent upon conversion of testosterone to DHT by target cells in these structures. This conversion of testosterone to the stronger androgen DHT is necessary due to the low circulating level of testosterone at this time that is insufficient to activate these tissues. The testes generally do not synthesize DHT until puberty. Neurons in some brain areas express 5α-reductase, and some effects of testosterone on behavior may involve prior conversion to DHT. Many androgenic responses, however, are not mediated by DHT, and this conversion is not necessary for testosterone to produce these effects. For example, development of wolffian duct derivatives (the epididymis, vas deferens, and seminal vesicles) is accomplished by a local level of testosterone that is sufficient to activate receptors without prior conversion to DHT. This effect may occur because local testosterone levels from the developing testes are sufficiently high to effectively activate androgen receptors in these structures. In the tammar wallaby, a marsupial, testosterone is converted to DHT in the wolffian duct, but this has not been shown to occur in a eutherian mammal.

In some target tissues, androgens have been shown to undergo conversion to estrogens through aromatization of the A-ring and removal of the C_{19} carbon atom. Conversion of androgens to estrogens also occurs in both Sertoli and Leydig cells. This process also occurs in the central nervous system, where aromatization may be essential to some androgen actions. Induction of

some male behaviors in castrates requires aromatization and cannot be induced by essentially non-aromatizable androgens such as DHT, whereas others may be induced by either aromatizable androgens or by DHT. The Sertoli cells, under the influence of FSH, secrete two forms of inhibin that selectively block FSH release from the adenohypophysis. Inhibin activity also has been found in rete testis fluid, seminal plasma, testicular extracts, and ejaculate, suggesting local actions. Two forms of inhibin have been isolated: inhibin A and inhibin B. These molecules are glycoprotein heterodimers of 31 to 35 kDa that possess a common α-subunit combined with one of two β-subunits (A or B). Inhibins are believed to be the major factor responsible for negative feedback in the selective regulation of FSH release in both males and females. In addition, a β-subunit heterodimer called activin has been isolated from gonads. Activin is composed of two β-subunits and is a potent releaser of FSH from the pituitary gland in laboratory experiments, although its physiological role is still undetermined.

Activins bind to serineethreonine kinase type I and type II receptors on the plasma membrane, leading to phosphorylation of downstream signaling proteins (so-called R-SMADs for receptor-regulated SMADs, short for an isoform of the humorously named "mothers against decapentaplegic") and changes in gene transcription. Inhibins interfere with activin receptor interation by binding to activin type II receptors and blocking recruitment of type I receptors.

Activin may be a local regulator, as activin levels within the testes are highly modulated during development and activin receptors occur on spermatogenetic cells in the testis.

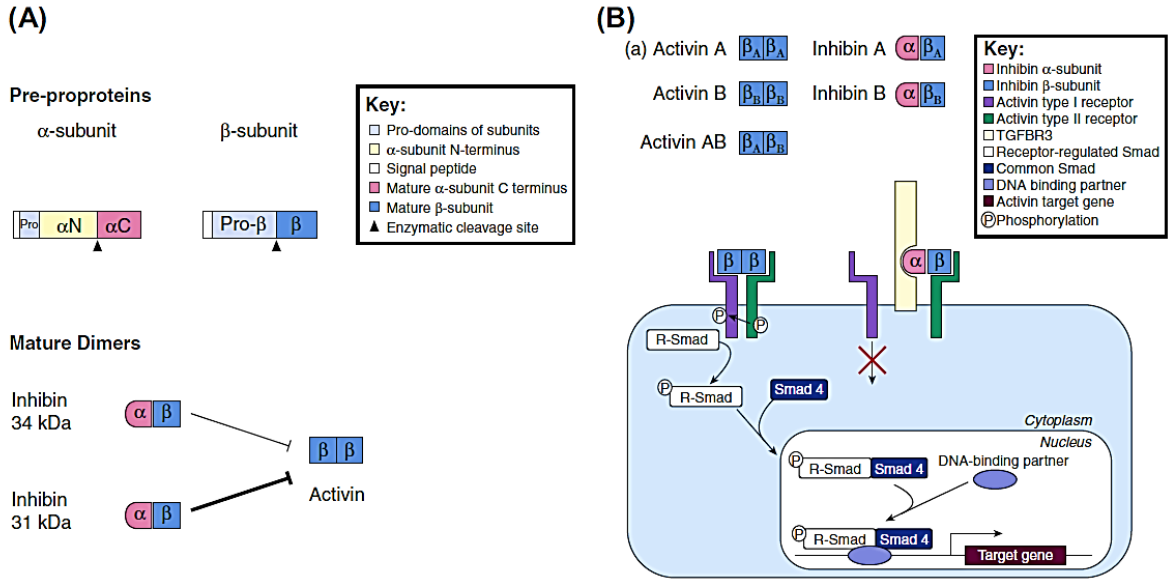

FIGURE 10-12 **Activin and its receptors.** (A) Activin and inhibins are formed by dimerization of two subunits, α and β, that are extensively modified by glycosylation and proteolysis after translation from either the α or β subunit mRNA. Activin is formed from two β subunits. Activins and inhibins act as natural inhibitors of each other's activity. (B) Activins bind to serine—threonine kinase type I and type II receptors on the plasma membrane. Binding to the type II receptor (green) leads to phosphorylation of the type I receptor (purple) and subsequent phosphorylation of downstream signaling proteins (R-SMADs). Inhibins interfere with activin receptor interaction by binding to activin type II receptors as well as TGFBR3 thereby blocking recruitment of type I receptors. Abbreviations: R-SMAD, receptor regulated mothers against decapentaplegic homolog 1; SMAD-4, SMAD family member 4; TGFBR3, transforming growth factor, beta receptor III. *(Adapted with permission from Stenvers, K.L. and Findlay, J.K.,* Trends in Endocrinology & Metabolism, ***21****, 174—180, 2010.)*

References

- Norris DO & James AC (2013). Vertebrate Endocrinology (5th edition). Academic Press, USA. http://dx.doi.org/10.1016/B978-0-12-394815-1.00001-X.