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Male reproductive medicine: anatomy and physiology

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Introduction

Reproduction is a highly specialized function of a healthy living organism. This concept may sound ordinary and simplistic, but it needs to be emphasized at a time when reproduction is undervalued by many who consider it a matter pertaining exclusively to sperm and eggs. As a result, it is a great achievement that the World Health Organization (WHO), when releasing its *Glossary of Terminology in Assisted Reproduction*, has defined infertility as **a disease of the reproductive system** [1], raising it to a level that clinicians have the responsibility to treat.

Treating male infertility means treating people not cells. Although assisted reproduction techniques may represent a solution for some infertile couples with male factor infertility, when a doctor is faced with an infertile male patient, he or she has the duty to try to improve and if possible restore their reproductive health.

Understanding the physiology of the male reproductive system results in a better understanding of the physiopathology of male infertility. This is also the basis of learning how to manage an infertile male patient.

Anatomy

It may be surprising, but the male reproductive system does not only comprise the testes, the accessory glands, and the penis, but also the brain and other structures located in the cranium (Figure 1.1). Before involving the genitourinary tract, reproduction depends on the interplay between neural and endocrine

An Introduction to Male Reproductive Medicine, ed. Craig Niederberger. Published by Cambridge University Press.

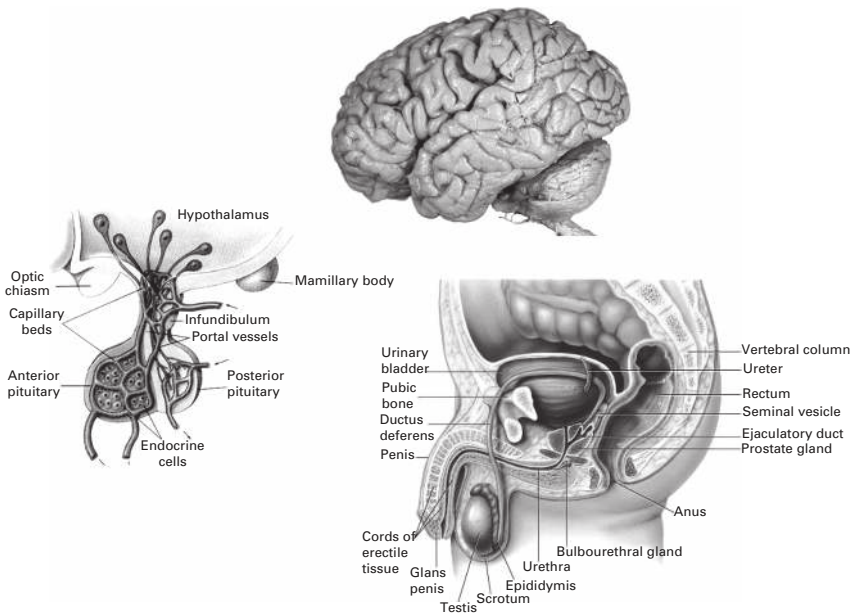

Figure 1.1 Anatomy of the male reproductive system.

events. The **brain** determines the timing of onset of puberty but also controls parenting behavior: a child may be first conceived in his/her parent's brain. A crucial role in controlling sexual behavior and reproduction is played by the **hypothalamus**, the ventral-most part of the diencephalon, which is located on either side of the third ventricle, with the hypothalamic sulcus delineating its dorsal border. It extends from the rostral limit of the optic chiasm to the caudal limit of the mamillary bodies.

The first impulse to spermatogenesis and secretion of sex hormones arises from the arcuate nucleus, located in the tuberal region of the hypothalamus, which contains many of the neurons that control the endocrine functions of the adenohypophysis. Terminals of gonadotropin-releasing hormone (GnRH)-secreting neurons release their secretions in the median eminence and infundibulum, where they enter the **hypophyseal portal system**. This is a capillary network originating from the superior hypophyseal arteries, which recombines in long portal veins draining down the pituitary stalk to the anterior lobe, when it breaks up into another capillary network and re-forms into venous channels. GnRH is then driven to the anterior pituitary, a gland originating from Rathke's pouch that lies in the sella turcica, precisely to the gonadotrophs, basophil-staining cells, which constitute 10%–15% of anterior pituitary cells and are located throughout the entire anterior lobe The gonadotrophs synthesize follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and release them into the systemic circulation; both hormones reach the **testis** by testicular arteries. The arterial supply to the testes follows the lobular division of

seminiferous tubules, so that each lobulus is supplied by one recurrent artery; segmental arteries and capillaries become branched between the Leydig cells and then give rise to the venous system. The pattern of blood supply to the testis is also essential for maintaining a lower testicular temperature compared with body temperature. In the pampiniform plexus, the convoluted testicular artery is surrounded by several veins coiling around the artery many times, so arterial blood is cooled down by surrounding venous blood.

The testis measures 15–25 cm^3 in volume. It is surrounded by a fibrous capsule, the tunica albuginea, from which septations extend toward the testicular mediastinum, dividing the testis into 200–300 lobules. Each of these lobules contains several highly convoluted seminiferous tubules. Each seminiferous tubule consists of a basement membrane lined by Sertoli cells, interspersed with germ cells at various stages of maturation. Seminiferous tubules arise and end at the rete testis, which is an anastomosing network of tubules that empties into the **efferent ductules**. Spermatozoa are then transported through efferent ductules into a single duct, the **epididymis**, and then enter the **vas deferens**, which propels its contents by peristaltic motion into the ejaculatory ducts. These receive fluid from the **seminal vesicles** (two large lobulated glands 5–10 cm in length), which lie inferiorly and dorsally to the bladder wall. They consist of blind-coiled alveoli with several diverticuli; their secretions make up 1.5–2 ml of the average 3 ml ejaculate and provide fructose, prostaglandins, and coagulating proteins. The ejaculatory ducts terminate in the prostatic urethra, where 30–50 tubuloalveolar glands forming the **prostate** open. The prostate is inferior to the bladder and surrounds the proximal portion of the urethra, where it emerges from the bladder. The prostate averages 3.4 cm in length, 4.4 cm in width, and 2.6 cm in depth. It is divided by the urethra and ejaculatory ducts into three major zones: peripheral zone (comprising about 70% of the glandular prostate); central zone (accounting for approximately 20% of the glandular prostate); and transition zone (represents 5% of the prostatic tissue). The prostatic contribution to the ejaculate is approximately 0.5 ml, and it is rich in citrate, zinc, polyamines, cholesterol, prostaglandins, and various proteases important in the liquefaction of semen. Fluid is also added to the seminal plasma by the **bulbourethral glands** and **glands of Littré** during its transit through the penile urethra.

The **penis** is built by paired crura forming the corpora cavernosa and by the bulb containing the urethra and becoming the corpus spongiosum, which is expanded at the tip to the glans. At the base of the penis the corpora cavernosa are covered by the ischiocavernosus muscles, whose contractions, under the control of the pudendal nerve, enhance penile rigidity, and the corpus spongiosum is surrounded by the bulbocavernosus muscle, whose contractions are involved in the ejaculation. Blood supply to the penis is provided by a dorsal superficial and cavernosal arterial system, derived from the internal pudendal artery, which gives off a perineal branch and continues as the penile artery; additional blood supply may be found in the form of accessory pudendal arteries. The deep penile arteries enter the crura cavernosa and stream on both sides centrally within the corpora cavernosa. The coiled helicine arteries then

directly supply the sinusoidal spaces, and the smaller arteries travel between the trabeculae. Subtunical venous plexus collect blood from the sinusoidal spaces, leading into emissary veins, which drain into spongiosal veins, circumflex veins, or directly into the deep dorsal vein and further into cavernosal and crural veins ending in the internal pudendal vein. The retrocoronal plexus drains the glans penis into the deep dorsal vein, which finally enters the periprostatic venous plexus.

Embryology

This section will briefly review the embryology of the male reproductive system, whose knowledge is required to understand the physiopathology of cryptorchidism and of hypospadias. A more detailed description of this argument can be found in [2] (chapters 1 and 6).

Testis development begins during the fifth week of gestation, but remains undifferentiated for the first 2 weeks. It arises from a mix of mesothelium, mesenchyme, and primordial germ cells, and becomes apparent with the formation of the gonadal ridge, which is composed by the epithelial layer (cortex) and the mesenchymal area (medulla). **By the sixth week Sertoli cells develop from the medullar sex cord under the influence of the sex-determining region of the Y chromosome (SRY), and start producing anti-Müllerian hormone (AMH), which will halt the development of the paramesonephric ducts**. Between the eighth and 10th week of development, seminiferous cords are separated by mesenchyme, which is stimulated to become Leydig cells by SRY proteins. Leydig cells start producing testosterone, initially under the stimulation of placental chorionic gonadotropins, but eventually the embryo's own hypothalamic–pituitary axis takes over control with the pituitary secretion of human chorionic gonadotropin (hCG). **Testosterone's effect is the development of the mesonephric duct in the Wolffian duct**. The distal portion of the Wolffian duct becomes the vas deferens. Near the urogenital sinus, a pair of lateral buds develops from the duct and become the seminal vesicles. The most cranial of the duct will disintegrate and remnant tissue will form the appendix epididymis, while the mesonephric tubules that grew near developing testes will become incorporated into the testis–epididymis structure as efferent ductules.

The **descent of the testis is a complex, multistage process requiring interaction of anatomical and hormonal factors**. The testis descends from an intraabdominal location into the bottom of the scrotum in two major phases, the transabdominal and the inguinoscrotal descent [3]. This two-stage process is guided by two mesenteric ligaments: the cranial suspensory ligament and the caudal genitoinguinal ligament or gubernaculums. The first stage occurs between 10 and 23 weeks of gestation. Initially, the undifferentiated gonads are attached to the abdominal wall in a pararenal position. Cranial suspensory ligament attaches the gonad to the posterior abdominal wall, whereas gubernaculums connects the testis via the epididymis to the future intraabdominal inner ring

of the inguinal canal. Under the effects of hormones, cranial suspensory ligament regresses, whereas gubernaculums develops its caudal segment into the gubernacular bulb, a reaction called the "swelling reaction," protruding into the forming scrotal sac. The swelling reaction of the gubernaculum holds the testis very close to the future internal inguinal ring, which causes the transabdominal migration of the testes into the inguinal region. The second, inguinoscrotal, phase occurs between 26 and 28 weeks of gestation, when the testes move from the inguinal region to the scrotum. This phase is due to shortening of the gubernacular cord and outgrowth of the gubernacular bulb. There is no consensus about the factor regulating the two phases of testicular descent; nevertheless, androgens play an important role in this matter.

External genitalia begin their development in an undifferentiated state: the presence of Y chromosome with *Sry* gene and the synthesis of testosterone will drive differentiation to a male phenotype. The first step is the formation of the genital tubercle from the mesenchyme, which will subsequently elongate and become the phallus. Additional proliferating tissue on either side of the cloacal membrane forms the labioscrotal swelling and the urogenital folds. In the presence of testosterone, the phallus lengthens and enlarges to form the penis. The urethral groove, originating from the urogenital membrane of the cloaca, grows as well: more medial endodermal folds will fuse in the ventral midline to form the urethra; the more lateral ectodermal folds will fuse over the developing urethra to form the penile shaft skin and prepuce. As these two layers fuse from posterior to anterior, they leave behind a skin line, the median raphe. By 13 weeks, the urethra is almost complete. A ring of ectoderm forms just proximal to the developing glans penis. This skin advances over the corona glandis and eventually covers the glans entirely as prepuce or foreskin.

A brief introduction to the regulation of hormone secretion and action

Feedback regulation

The endocrine glands constituting the hypothalamus–pituitary–gonadal axis do not continuously synthesize and secrete their hormones in a shower fashion. On the contrary, one distinctive feature of hormone secretion through the hypothalamus–pituitary–gonadal axis is that they regulate their own secretion through negative feedback inhibition. What this means is that a hormone (testosterone) secreted from a peripheral gland (the testis) binds to its receptor on cells in the hypothalamus and hypophysis, and inhibits the secretion of tropic hormones (GnRH and LH). Less GnRH secretion leads to less LH secretion, which leads to less stimulation of testosterone secretion by Leydig cells. Depending on the distance taken by hormones to deliver their feedback message to its destination, there are three kinds of feedback: long loop feedback, short loop feedback, and ultrashort loop feedback (Figure 1.2).

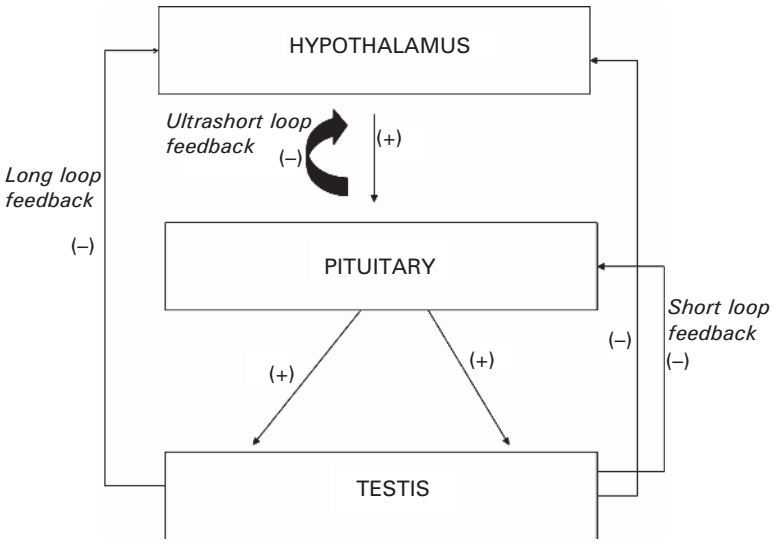

Figure 1.2 Schematic representation of feedback regulation mechanisms.

The usefulness of negative feedback inhibition is that it results in hormonal homeostasis, which is the maintenance of hormone levels within a particular appropriate physiologic range. In the case of a damaged testis, a decreased secretion of testosterone would be expected; however, the homeostatic model would bring testosterone back up towards its normal daily level of secretion, as lower testosterone levels would lead to a consequent decrease in the degree of negative feedback inhibition on the hypothalamus and anterior pituitary. In addition, the release from negative feedback inhibition would lead to an increase in GnRH and LH secretion, and more LH will stimulate the healthy testis to secrete more testosterone.

Downregulation, upregulation, and desensitization

Apart from the feedback pathway of hormone secretion control, other important factors affecting the response to hormone stimuli are downregulation or upregulation of receptor levels and desensitization. **The downregulation mechanism is intended to prevent an excessive response to a higher-than-normal hormone level**. The exposure of testicular cells expressing the endogenous LH receptor (LHR) to a high concentration of hCG or LH downregulates the levels of cell surface receptor. Concomitant with the downregulation of cell surface LHR, a decrease in the abundance of all LHR mRNA transcripts is observed. An important clinical application of this mechanism is that an excessive exogenous hormone administration in order to increase target gland activity will be useless, as it will lead to a downregulation of hormone receptors. **Instead, upregulation occurs when an increase in receptor level is required**: an example of upregulation in

the testis is the permissive role of FSH on LH–Leydig cell interaction: FSH stimulates Sertoli cells to secrete insulin-like growth factor (IGF)-1, which acts to induce LHRs, leading to an enhancement in steroidogenesis.

Desensitization is an important component of the regulation of hormone actions and can occur at multiple levels. For example, very old experiments demonstrated the existence of two steps in the steroidogenic pathway that contribute to the desensitization of steroidogenic responses observed in male rats injected with LH/CG, or in freshly isolated rat or mouse Leydig cells or cultured Leydig tumor cells exposed to LH/CG. These include a reduction in the activity and/or levels of 17α-hydroxylase/17,20-lyase and a reduction in the amount of cholesterol available for steroidogenesis [4], so that an excessive LH/hCG-stimulated testosterone secretion is prevented.

Autocrine, paracrine, and endocrine regulation

The sophisticated control of hormone synthesis and release involves also mechanisms played at autocrine, paracrine, and endocrine levels. Autocrine control is applied when a hormone controls its own secretion through local action on the cell in which it is produced. Paracrine control is a form of bioregulation in which secretion produced by one cell type in a tissue diffuses through the tissue and affects another cell type in the same tissue. Endocrine control is modulated by hormones secreted by other glands, transported through the bloodstream to the target gland, where they play their modulating role.

Hypothalamus–pituitary–gonadal axis

The following sections will focus on the hypothalamus–pituitary–gonadal axis hormone pattern and regulation of secretion. Figure 1.3 and Table 1.1 summarize the concepts exposed below. For a more detailed description of this argument, please refer to [2] (chapter 2).

Gonadotropin-releasing hormone

GnRH is a decapeptide produced in the GnRH neurons and released in the portal blood in discrete pulses. It has been demonstrated that the frequency and amplitude of GnRH stimulation provide signals for the differential regulation of LH and FSH secretion. At higher GnRH pulse frequencies, LH secretion increases disproportionately more than FSH secretion, whereas at lower GnRH pulse frequencies, FSH secretion is favored. In particular, LH-β subunit RNA levels seem to be stimulated by a GnRH pulse frequency every 120 minutes. The pattern of GnRH pulsatile secretion seems to be intrinsic to GnRH neurons. GnRH neurons have been found to display rhythmic activity in multiple time domains, ranging from burst firing on the order of seconds to episodes of increased firing rate that occur on the order of many minutes.

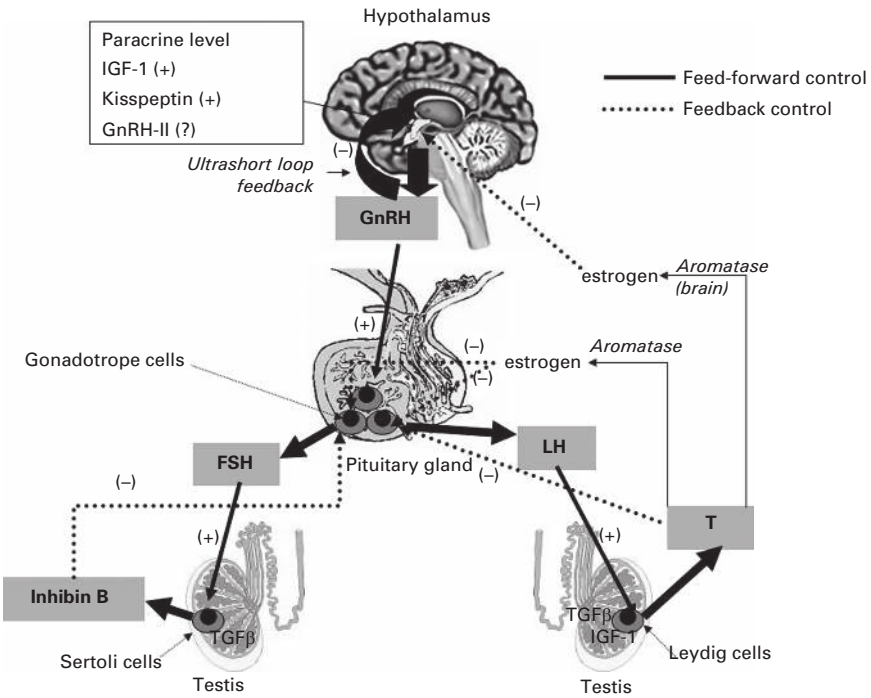

Figure 1.3 Schematic representation of human hypothalamus–pituitary–testis axis. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IGF, insulin growth factor; LH, luteinizing hormone; T, testosterone; TGF, transforming growth factor.

GnRH is able to regulate its own release (*autocrine regulation*) **by means of an ultrashort loop feedback mechanism** [5]. **GnRH neurons are able to activate and suppress their own activity using GnRH itself as an intra-GnRH neural network signal, a strategy that could have important implications for generation of the GnRH surge in addition to the regulation of pulsatile release. As a matter of fact, a substantial subpopulation of adult GnRH neurons expresses GnRH receptors (GnRHR-1), which can be inhibited by a low dose of GnRH. GnRH neurons could also modulate their own secretion through the synthesis of factors other than GnRH, such as endocannabinoids.**

Regulation of GnRH secretion, however, does not only apply to GnRH neurons. A *paracrine control* of GnRH secretion has been postulated, involving the role of a second GnRH subtype, GnRH-II, which differs from GnRH (also called GnRH-I) by three amino acid residues. The actual role of GnRH-II is still to be established: a role in reproductive behavior has been suggested, but it has been also found to stimulate FSH and LH release, probably via the GnRH-I pathway. In addition, IGF-1 may be involved in the paracrine control of GnRH secretion, directly acting on GnRH neurons in the hypothalamus to enhance GnRH gene transcription and play a crucial role in controlling the onset of

Table 1.1 Regulation of hypothalamic–pituitary–gonadal axis hormone release

Hormone	Autocrine regulation	Paracrine regulation	Endocrine regulation
GnRH	GnRH itself (−)	GnRH II (+), IGF-1 (+), kisspeptin (+)	Testosterone (−), estrogens (−), neurotensin (+), norepinephrine (+)
FSH	–	Activin (+), follistatin (−)	GnRH (+), estrogens (−), inhibin B (−)
LH		Activin (+), follistatin (−)	GnRH (+), testosterone (−)
Testosterone	–	IGF-1 (+), GH(+), CRH (−), TGF-β (−), IL-1α (±)	LH (+)

+ Stimulatory effect, – Inhibitory effect. Transforming growth factor-β (TGF-β), corticotropin-releasing hormone (CRH), interleukin 1α (IL-1α), growth hormone (GH), insulin-like growth factor 1 (IGF-1).

puberty. Finally, kisspeptin, the RF-amide peptide ligand, may be involved in the paracrine control of GnRH release.

Testosterone, through its aromatization to estradiol, plays an important role in the *endocrine regulation of GnRH secretion*. Estradiol orchestrates the activity patterns of several neurotransmitter systems within the GnRH network to bring about the GnRH surge; it uses the excitatory amino acids neurotensin and norepinephrine to activate GnRH electrical activity at the level of the GnRH cell body, and neuropeptide Y at the level of GnRH terminals, and reduces the β-endorphin neurons level of activity to disinhibit GnRH neurons. Estradiol is also supposed to influence GnRH pulse generator function: experiments on brain slice preparation demonstrated that it reduces the frequency of GnRH secretion through a pathway involving GABAergic neurons.

Gonadotropins

Gonadotropins FSH and LH are glycoproteins consisting of a common α-subunit and a hormone-specific β-subunit, which are associated through noncovalent interactions. While the β-subunits determine the functional specificity of gonadotropins, **their intrinsic bioactivity is largely determined by their degree of glycosylation. In each blood sample, at least 20–30 different FSH isoforms can be separated by electrophoresis. Weakly glycosylated forms of the hormones have a short circulatory half-time, and although totally deglycosylated gonadotropins are able to interact with their cognate receptor, they are unable to evoke**

generation of a second messenger signal. Highly glycosylated isoforms (acidic) display longer elimination half-life and stronger biologic power.

Gonadotropins are essential for spermatogenesis and secretion of testicular androgens: lack of gonadotropin results in suppression of spermatogenesis, as demonstrated by hypophysectomy, by GnRH immunization, and by GnRH analog treatment. Both FSH and LH play an important role in regulating spermatogenesis. FSH is essential to promote spermatogenesis in men, as it maintains normal testicular size, seminiferous tubular diameter, and sperm number and motility: inactivating FSH mutation or inactivation of FSH receptor gene lead to spermatogenic failure. LH participates in regulating spermatogenesis by stimulating the synthesis of testosterone, which plays an essential role in spermatid maturation: inactivation of LH was found to cause arrest of spermatogenesis and absence of Leydig cells. A permissive role of FSH is postulated, as FSH-stimulated Sertoli cells to secrete IGF-1, which acts in an autocrine and paracrine fashion to induce LHRs and enhance proliferation and steroidogenesis in mice Leydig cells. LH is secreted primarily in pulses: GnRH secretory bursts are followed uniformly by a slightly time-delayed pulse of LH secretion. LH pulses occur in an ultradian fashion, with a mean frequency of approximately one event per hour or one every 90–120 min [6]. On the contrary, FSH secretion is predominantly basal, and it seems to be not directly coupled to GnRH pulses.

Regulation of gonadotropin secretion in the human involves a complex interplay between feed-forward stimulation by GnRH from the hypothalamus, feedback control by sex steroids and inhibin from the testes, and, probably, autocrine/paracrine modulation by other factors within the pituitary. The pattern of GnRH feed-forward regulation of gonadotropin secretion has been illustrated in the previous section. Members of the transforming growth factor (TGF)-β superfamily, activin, inhibin, and follistatin, are produced in the anterior pituitary and seem to be involved in the paracrine/autocrine regulation of FSH secretion. Testosterone feedback control activity at the pituitary level can be direct (mediated by its binding to the androgen receptor [AR]) as well as indirect (mediated by aromatization to estrogens and binding to pituitary estrogen receptors). Testosterone exerts a direct feedback control of LH secretion, while its action on FSH secretion is mostly indirect; estradiol inhibits LH secretion by decreasing LH pulse amplitude and LH responsiveness to GnRH consistent with a pituitary site of action. Sex steroids seem to play a minor role in the feedback control of FSH secretion. On the other hand, inhibin B, the principal form of circulating inhibin in men, is the key factor involved in the testicular regulation of FSH secretion, as demonstrated by the inverse relationship between its circulating level and FSH ones.

Testicular factors

Testosterone is the main secretory product of the testis, the daily production rate being 5–7 mg in men. As the testicular content of testosterone in adult men is approximately 50 mg/testis, it is assumed that testosterone is continuously