CHAPTER

19

Function of folllicle stimulating hormone and the follicle stimulating hormone receptor

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Follicle stimulating hormone (FSH)

Neuroanatomy

Follicle-stimulating hormone (FSH), is a glycoprotein hormone, synthesized in the anterior pituitary gland, that regulates the development, growth, pubertal maturation and reproductive processes of the human body [1]. Gonadotropin-releasing hormone (GnRH), a tropic peptide hormone produced in the hypothalamus plays an important role in the secretion of FSH, with hypothalamic-pituitary connection leading to the regulation of FSH production. The hypothalamus forms the ventral portion of the diencephalon and is located below the thalamus. GnRH is produced from neuroendocrine cells originating within the pre-optic area of the hypothalamus and is secreted into the hypophyseal portal system where it is transported to the anterior pituitary gland via the hypophyseal portal vessels [2]. GnRH then acts on the GnRH Type 1 receptor on gonadotropic cells of the anterior pituitary, a G-protein coupled receptor. The GnRH type 1 receptor is encoded by a gene on chromosome 14q13.1-q21.1. The anterior pituitary, or adenohypophysis, is derived from embryonic ectoderm and located within the sella turcica, a saddlelike structure of the sphenoid bone. This gland is responsible for secretion of FSH in addition to growth hormone, prolactin, luteinizing hormone (LH), thyroid-stimulating hormone (TSH) and adrenocorticotropic hormone (ACTH) [3]. Gonadotroph cells, which secrete both FSH and LH, are primarily located within the lateral portions of the anterior pituitary and make up 7-10% of cells within this gland [3]. Since the gonadotroph cells secrete both FSH and LH, specific transcription factors are responsible for driving the synthesis of either FSH or LH subunits that dictate FSH or LH secretion [4]. Gonadotropic hormones are stored as secretory granules and alterations in cell membrane permeability result in extrusion of hormones into the blood stream where they travel to reach their receptors in the ovary and testis [4].

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Structure of FSH molecule and FSH receptor

FSH, a pituitary glycoprotein hormone, exerts its action by binding to FSH receptors on Sertoli cells of the testis and granulosa cells of the ovaries. FSH is included in a family of glycoprotein hormones which includes three pituitary hormones (TSH, LH, FSH) and one placental hormone (hCG). The FSH molecule was initially discovered in 1931 [5]. Glycoprotein hormones are disulfide-rich heterodimer proteins involving an alpha and beta subunit. The glycoprotein hormone family is also part of the cystine knot growth factor superfamily. All four of these proteins contain a common α -subunit while their β -subunit remains specific. The gene for the β -subunit of FSH is located on chromosome 11p13 [6,7]. The β -subunit of FSH contains 110 amino acids, which is the smallest β -subunit among the gonadotropin proteins (FSH, LH, hCG) [3]. The total molecular weight of human FSH is approximately 30 kDa. Various types of FSH are secreted according to physiological requirements at a given time and have been identified according to sialic acid content. The amount of sialic acid is mainly influenced by E2 levels and possibly by GnRH. During reproductive years when serum E2 concentration is high, the FSH molecule is less glycosylated with a shorter half-life but greater receptor affinity. Low E2 levels before puberty and after menopause lead to more glycosylated forms of FSH which have a longer half-life [8] (Fig. 19.1).

The FSH receptor is a part of a superfamily of glycoprotein hormone receptors that activates G-proteins intracellularly. The FSH receptor gene is composed of ten exons and nine introns. The interaction of FSH and its receptor is crucial for fertility, therefore defects or variations in the follicle-stimulating hormone receptor (FSHR) could ultimately affect reproductive ability.

The mature FSHR protein is predicted to be 678 amino acids in length [9]. The FSHR is made up of a seven-helical transmembrane domain (each helix is approximately 20-25 amino acids) in addition to a large ectodomain with a molecular mass of 33 kDa [9] (Fig. 19.3). The FSHR is also described to have an "enigmatic" hinge domain that is suggested to be responsible for the binding specificity for FSH [10]. Although the extracellular domain is suggested to be the reason the FSHR binds specifically to FSH, the ability to evaluate this in studies has been challenging [9]. Crystallographic structural analyzes have shown important interaction between the α -subunit of FSH and FSHR, confirming binding is not dependent solely on the β-subunit [11]. After FSH binds to the FSHR, the receptor undergoes a conformational change from inactive to active. The change in the receptor's shape will activate the coupled G-protein and subsequently increase intracellular cyclic-AMP (cAMP). The increase in cAMP in turn activates protein kinase A (PKA) which is able to phosphorylate and activate transcription factors and additional intracellular proteins and enzymes [10]. These factors include p38 MAP kinases, p70-S6 kinase, PI3K and FOXO1 that regulate gene expression in target tissues [12,13].

Mutations in the FSH receptor have been identified which can inactivate FSH activity. The first inactivating FSH receptor mutation was found in individuals from

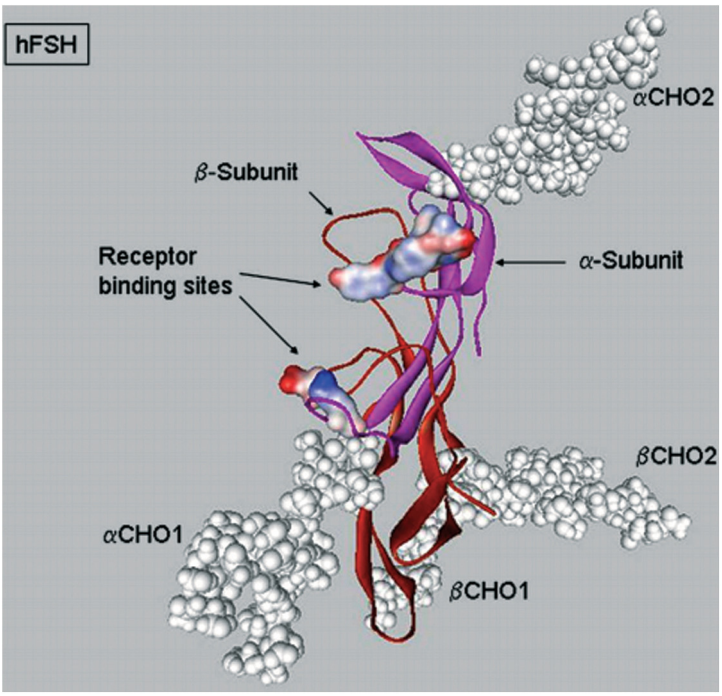

FIGURE 19.1

Molecular structure of human follicle-stimulating hormone

[Credit: Modified from The Practice Committee of the American Society for Reproductive Medicine: Gonadotropin preparations: past, present and future perspectives, Fertility and Sterility 90: S13-20, 2008] [54]

several Finnish relatives who presented with poorly developed secondary sexual characteristics, primary amenorrhea, and recessively inherited hypergonadotropic ovarian failure. A missense Ala189Val mutation was found to be responsible for their presentation [14]. Other inactivating mutations have been described since, involving either the FSHR glycosylation site or the receptor binding affinity [15]. The range of clinical presentation of inactivating FSH receptor mutations further informs the critical biologic role of FSH in human reproductive development and adult function.

The FSH receptor gene includes 731 single nucleotide polymorphisms (SNPs). Several studies have attempted to correlate polymorphisms in this gene with particular reproductive phenotypes. One study examining such polymorphisms concluded that certain genotypes may be a predictor of impaired folliculogenesis and early diminished ovarian reserve [14].

Knockout mice for both the ligand (*Fsh*-β*)* and receptor (*Fshr)* have been used to understand the effects of FSH actions on target cells. Female *Fshr* knockout mice

were infertile, but male knockout mice were normal to sub-fertile. Serum FSH levels were increased 15-fold in female, but only 4-fold in males, and pituitary FSH was elevated in females only. Female *Fshr* knockout mice had severe hypogonadism but without a noted reduction in bone mass. Male *Fshr* knockout mice had reduced serum testosterone levels and small testes but were able to produce offspring when mated to wild type females [16]. In 1997, Tapanainen et al evaluated five males with the same inactivating point mutation of the FSH receptor and found three cases of subfertility and two cases of normal fertility with phenotypes ranging from oligospermia to euspermia. This data suggests only a small percentage of receptor activity is necessary for spermatogenesis in males and compensatory intratesticular factors may be present that maintain spermatogenesis [17].

While it was previously thought that granulosa cells and Sertoli cells were the only cells that expressed FSH receptors [18], recent studies have identified FSH receptors within the cervix, endometrium and myometrium. Within the myometrium, FSH/FSHR pathways are suggested to play a role in regulating uterine contractility. In the non-pregnant state, FSH may help maintain uterine quiescence to improve implantation. In pregnancy, FSH/FSHR pathways may be involved with initiating uterine contractility at term [19]. FSH receptors have also been identified within the placenta. Negative pregnancy outcomes have been demonstrated in FSHR-knock out mice as the FSH/FSHR signaling within the placenta has been associated with fetal vessel angiogenesis [19]. FSH receptors have also been found within malignant tissues, bone and fat. FSH has been found to regulate bone mass and exert action on adipocytes in mouse models [20].

Control/regulation of FSH

Regulation of FSH by GnRH

The regulation of FSH is multifactorial but largely controlled by input from GnRH. Changes in GnRH amplitude and frequency will promote transcription of either FSH or LH within the anterior pituitary. With binding of GnRH to the GnRh receptor, inositol 1,4,5-triphosphate (IP3) and 1,2 diacylglycerol (1,2-DG) stimulate protein kinase and cyclic AMP activity to activate downstream pathways [4]. However, production of FSH is not solely reliant on GnRH stimulation since blockage of the GnRH receptor by GnRH antagonist has been shown to result in only a 40-60% inhibition of FSH. According to early studies by Knobil and colleagues in hypothalamic-lesioned monkeys, intermittent GnRH stimulation lead to production of FSH and LH whereas constant GnRH stimulation resulted in suppression of gonadotropin production [21]. In females, GnRH pulse frequency varies throughout the menstrual cycle whereas GnRH pulse frequency remains constant in males. Studies have demonstrated that slow frequency GnRH stimulation favors FSH production and high frequency GnRH stimulation favors LH production [22]. Several peptides, including oxytocin, CRH and neuropeptide Y can interact with

GnRH at the pituitary and directly affect FSH and LH secretion. Studies have identified intermediate signaling pathways that mediate gonadal steroid feedback at the hypothalamus, since ER- α receptors are not present within the hypothalamus. The KDNy neuron system, coexpresses kisspeptin, neurokinin B and dynorphin, peptide structures that aid in mediating negative estrogen feedback from the gonads [23]. The ER- α receptor is expressed on KNDy neurons and the binding of estrogen or testosterone to this receptor inhibits KNDy neurons, thus preventing GnRH release. The dynorphin peptide acts on K-opioid receptors in KNDy neurons to inhibit NKB and kisspeptin secretion, inhibiting GnRH secretion by acting directly on GnRH receptors. The KNDy neuron system is also involved in positive feedback at the hypothalamus and kisspeptin receptor binding on hypothalamic GnRH neurons causes GnRH secretion mid-cycle prior to ovulation. Neurokinin B (NKB) stimulates pulsatile release of GnRH by activating TACR3 receptors to release kisspeptin which activates GPR54 receptors on GnRH neurons [24]. In mouse models, the *Kiss1* gene is upregulated in gonadotropes by the direct action of estrogen acting through the ER- α receptor [25].

Regulation of FSH by gonadal feedback

Estradiol, via a negative feedback loop to the hypothalamus and pituitary, inhibits FSH release from gonadotropes. Current evidence supports the hypothalamus as being the primary site for estrogen negative feedback on pituitary gonadotropin production but the pituitary is also involved in regulation [23,26]. Studies evaluating rat hypothalamic tissue slices and GnRH neuronal cell lines, show estradiol administration is associated with a decrease in GnRH expression [26]. Studies demonstrate that estradiol reduces GnRH pulse amplitude but not GnRH pulse frequency [27]. Hypothalamic estrogen negative feedback has been shown to be mediated by KNDy neurons of the median eminence upstream of GnRH itself [28,29]. While estrogen predominantly acts to inhibit pituitary gonadotropin secretion, estrogen exerts a stimulatory effect on pituitary gonadotropins mid-cycle resulting in increased production of FSH and LH responsible for the pre-ovulatory LH surge. Sustained levels of estradiol in animal models has been shown to regulate gene expression and second messenger systems within gonadotropes and increase GnRH receptor number [30–32].

Progesterone, primarily produced in the luteal phase of the menstrual cycle, negatively feedbacks at the level of the hypothalamus to slow pulsatile GnRH secretion and suppress LH/ FSH production. However, prior to ovulation, progesterone in the presence of estrogen augments the LH/FSH surge [4]. High levels of progesterone in the mid-luteal phase negatively feedback to progesterone receptors on GnRH neurons. KNDy neurons mediate progesterone negative feedback through dysnorphin signaling [23].

Testosterone acts to negatively feedback gonadotropin secretion. Few GnRH neurons express androgen receptors, therefore the KNDy neuronal network has been

suggested to mediate negative androgen feedback [33]. Androgen feedback may also be mediated by the aromatization of testosterone to estrogen.

Regulation of FSH by other regulating factors

Activins and inhibins are members of the transforming growth factor (TGF) family and act as FSH-regulatory proteins in mediating FSH secretion [3]. Inhibin is a heterodimer peptide molecule with two isoforms, inhibin-A and inhibin-B, that contain similar α -subunits and unique β -subunits. Both isoforms act to regulate FSH release from gonadotropic cells of the anterior pituitary. Inhibin B is produced from the Sertoli cells of the testis in males and granulosa cells in females. Inhibin mRNA has also been identified in pituitary gonadotropes [4]. In females, inhibin-B is produced by granulosa cells of the ovary during the early follicular phase in response to FSH stimulation and acts to suppress FSH during the mid to late follicular phase. Inhibin-A, mainly produced from granulosa cells, rises throughout the follicular phase prior to ovulation and is involved in positive feedback at the level of the pituitary by increasing the number of available GnRH receptors. Inhibin-A is produced from the corpus luteum during the luteal phase, and is found at peak concentrations during the midluteal phase, where it acts to suppress FSH production. Activin, a dimer polypeptide comprised of three isoforms, enhances FSH biosynthesis and secretion. Activin is elevated mid-cycle and during the luteal-follicular phase transition. Activin increases the pituitary response to GnRH by increasing GnRH receptor production. Follistatin is a monomer peptide produced by the anterior pituitary that acts to inhibit FSH by binding, and inactivating, the activin molecule.

Stress and glucocorticoids have been associated with a cortisol-mediated gonadotropin suppression at the level of the hypothalamus and pituitary. This negative feedback may be modulated by sex steroids and the kisspeptin pathway [34,35]. Other hormones, peptides and neurotransmitters that influence secretion of gonadotropins include prolactin, GABA, vasoactive intestinal polypeptide (VIP), vasopressin, catecholamines, nitric oxide, neurotensin, gonadotropin-inhibitory hormone (GnIH)/RFamide related peptide-3 (RFRP-3) and nucleobindin-2/nesfatin-1 [36,37]. Bone morphogenic proteins, BMP-6 and BMP-7 have also been associated with modulation of FSH synthesis in gonadotropes [38,39].

FSH expression from fetal development across the lifespan

During fetal development, GnRH producing neurons migrate from the medial olfactory placode to the hypothalamus at approximately 40 days gestation. GnRH stimulates both LH and FSH production by 9 weeks gestation [40]. While fetal gonadotropin levels peak mid-gestation, high levels of placental estrogens at the end of pregnancy suppress fetal gonadotropin production via the fetal hypothalamic-pituitary-gonadal (HPG) axis. Following birth, a decline in circulating estrogen levels leads to a lack of negative feedback at the hypothalamus and a subsequent rise in FSH and LH levels, primarily during the first 3-6 months of life in both males and females. Gonadotropin

levels then decline and the HPG axis remains dormant until puberty, given the high sensitivity of the HPG axis to negative feedback from even low levels of sex steroids. Prepubertal inhibition of gonadotropin secretion is regulated by brain peptides that include GABA and neuropeptide Y [4]. At puberty, increased pulsatile GnRH frequency, amplitude and regularity leads to increased FSH and LH secretion from the anterior pituitary. The first noted biological change of puberty is pulsatile LH release during sleep.

Recent studies have demonstrated that kisspeptin neurons may release neurokinin B and dynorphin and be involved in initiating the pulsatile secretion of GnRH at puberty in addition to GABA and NMDA receptor signaling [41]. The gonadal steroids, estrogen and testosterone, produced in response to FSH and LH secretion, result in growth and maturation of the gonads and development of secondary sexual characteristics. In both males and females, FSH stimulates the maturation of primordial germ cells. While LH promotes testosterone production from Leydig cells in males at puberty, FSH production promotes Sertoli cells to secrete androgen-binding proteins (ABP), regulated by inhibin's negative feedback mechanism to the anterior pituitary. Activation of Sertoli cells by FSH sustains spermatogenesis in males and stimulates inhibin B secretion feedback. In females, FSH starts and promotes follicular growth, and results in estradiol production from granulosa cells of the ovary. Production of estrogen by the ovaries leads to growth and development of the breasts, ovaries, and uterus in addition to fat redistribution and bone maturation. Once pulsatile GnRH secretion is initiated at puberty, it continues throughout reproductive life. As oocyte quantity declines with age, FSH levels rise. With age, the number of follicles per cycle decreases, leading to lower production of inhibin-B. In response to decreased feedback inhibition from lower circulating levels of inhibin-B (Fig. 19.2), FSH levels rise before a decrease in estradiol is observed. Inhibin A and B levels are undetectable in post-menopausal women. With an increase in circulating FSH levels, there is downregulation of FSH receptors on the remaining follicles [42].

Role of FSH in the menstrual cycle and folliculogenesis

FSH is responsible for driving folliculogenesis, the development and maturation of an ovarian follicle in order for ovulation of a mature oocyte to occur. While the initial steps of folliculogenesis are independent of gonadotropin stimulation, once follicles become antral follicles they are dependent upon FSH for continued growth and development. FSH is then, at this stage, paramount in recruiting a cohort of follicles.

FSH levels are suppressed during the luteal phase of the menstrual cycle, given elevated levels of estradiol, progesterone and inhibin-A. If pregnancy does not occur, corpus luteum function declines, and with it the serum level of progesterone, estradiol and inhibin A. FSH begins to rise during this luteal-follicular transition, which is critical in developing and recruiting a new cohort of developing follicles, before the follicular phase starts again [3]. The early follicular phase is associated with an increase in FSH levels as follicular recruitment is ongoing. FSH exerts its action by

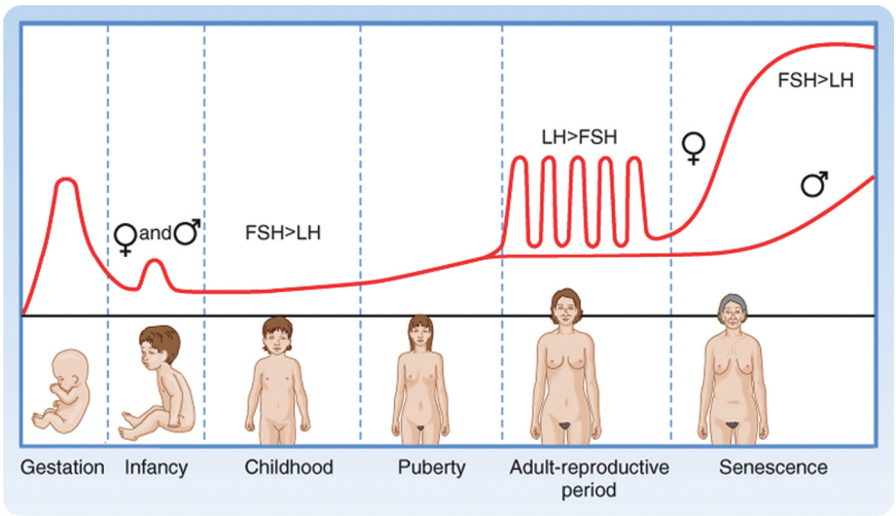

FIGURE 19.2

FSH and LH activity with age

[Credit: Modified from Koeppen B, Stanton B: Hormonal Regulation of Male and Female Reproductive Systems (Chapter 43), Berne and Levy Physiology, 6th Edition: 784-785, 2008] [55]

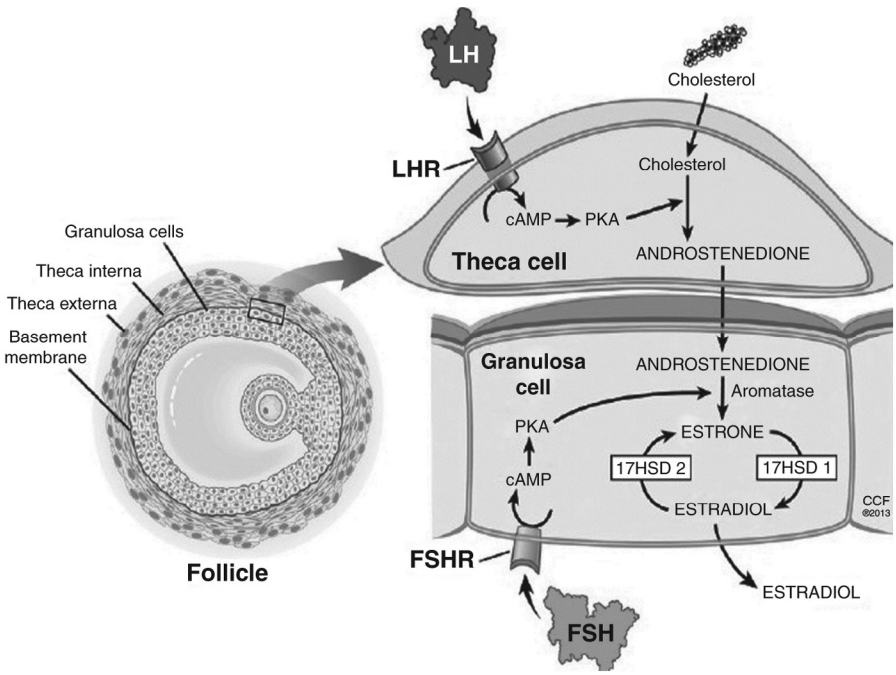

FIGURE 19.3

Synthesis of estradiol via conversion of androgen

binding to receptors on granulosa cells and driving granulosa cell growth, proliferation and development.

In response to FSH, granulosa cells produce estradiol. FSH aids in increasing estrogen production, by increasing receptors on the follicle for LH, leading to a rise in androgen production. LH will stimulate the synthesis of androgens from theca cells (Fig. 19.1), first by initiating the conversion of cholesterol to pregnenolone. Through a series of enzymatic processes, pregnenolone is then converted to progesterone, hydroxy-progesterone, androstenedione and finally, to testosterone. Testerosterone is then aromatized, via the enzyme aromatase in the granulosa cells, to estradiol [43,44] (Fig. 19.3). As follicular development continues, granulosa cells increase their FSH receptor expression. Eventually, the amount of FSH receptors the follicle is able to produce effects the FSH dependent selection of the dominant follicle. With a rise in inhibin B from the developing cohort of follicles, FSH production declines in the late follicular phase, which is a critical step in selecting the follicle that will proceed to ovulation. It is the follicle with the highest sensitivity to FSH and LH that will undergo ovulation, rupture and release of the oocyte. The smaller follicles in the cohort undergo atresia as FSH levels decline. As the dominant follicle produces increasing levels of estradiol, a threshold is reached which results in a surge of gonadotropins; the LH surge being much greater than the surge of FSH [3]. It is this surge that results in resumption of meiosis and causes ovulation to occur with subsequent luteinization.

FSH in spermatogenesis

In males, FSH is necessary to initiate and sustain spermatogenesis. In the neonatal and pre-pubertal stages, FSH stimulates the transcription of genes involved in DNA replication and cell cycle regulation that determine the final number of Sertoli cells that will be present at puberty. FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes [45], and is critical for the initiation of spermatogenesis which occurs within the testicular seminiferous tubules. FSH stimulates spermatogenesis by promoting primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes which then undergo a second meiotic division to become haploid spermatids. FSH provides the structural and metabolic support to spermatogonia, allowing their development into mature spermatids. Androgen-binding protein concentrates testosterone in high levels in the testis which is necessary to sustain spermatogenesis [46]. Completion of spermatogenesis requires preferential LH production, versus FSH production. The Sertoli cells produce inhibins in response to FSH that ultimately feedback and suppress pituitary FSH secretion. Testosterone, produced by the Leydig cells in response to LH, helps to sustain spermatogenesis.

Clinical application of FSH

The exogenous administration of pituitary gonadotropins, FSH and LH, is critical for ovarian stimulation in the management of infertility, specifically assisted reproductive

technologies. The goal of controlled ovarian stimulation is to promote multifollicular growth and development by administering supraphysiologic doses of gonadotropin to extend the physiologic FSH "window" [47,48] and prevent smaller follicles from undergoing atresia. In, ART/IVF, the goal is to retrieve multiple oocytes for fertilization and creation of embryos. Gonadotropin preparations have evolved from pituitary to urinary extracts, and most recently into recombinant forms. As early as 1930, pituitary tissue samples were extracted from various animals including swine, hog and sheep and FSH preparations were used to treat patients with diminished ovarian function. Therapy evolved to treatment with pregnant mare serum gonadotropins and human pituitary gonadotropins prior to the discovery of human urinary menopausal gonadotropins (HMG), containing FSH and LH, in 1949 [49]. In the early 1970s, techniques emerged to separate FSH and LH within urinary HMG in order to adjust specific FSH and LH concentrations. The first recombinant FSH formulation was produced in 1988 with introduction of genes encoding FSH subunits into the genome of the Chinese hamster ovarian cell line (CHO Cells). Recombinant preparations of follicle stimulation hormone (r-FSH) are extremely pure, devoid of urinary proteins and associated with decreased batch to batch variability that can be seen with urinary FSH [50]. The starting dose of rFSH usually ranges between 100 and 450 IU, however multiple studies have suggested no benefit past a maximum dose of 300 IU [51]. Exogenous FSH can also be administered to males suffering from hypogonadotropic hypogonadism to compensate from lack of circulating endogenous gonadotropins. FSH administration in males has been shown to improve sperm quality, improve spontaneous pregnancy rates and pregnancy rates achieved through assisted reproductive technologies [52,53].

Chapter summary

FSH, a glycoprotein hormone produced and secreted by the gonadotrophic cells of the anterior pituitary, is a necessary hormone for sexual development and reproductive function in both males and females. FSH is critical for folliculogenesis in females and spermatogenesis in males and plays an important role in sexual development given its ability to drive steroid hormone production. The regulation of FSH production is multi-factorial and under the control of GnRH, gonadal feedback and regulatory proteins. While FSH receptors are primarily found in the granulosa cells of the ovary and the testis, FSHR are also present in the uterus, within the placenta and in bone and fat. Recombinant FSH technology plays an important role in ovulation induction in anovulatory patients, treatment of male hypogonadotropic hypogonadism and stimulation of multifollicular development in assisted reproductive technologies.

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