Hormonal Management of Male Infertility

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**INTRODUCTION**

Various hormones regulate the male reproductive function through well integrated complex set of interactions in various developmental stages. *In utero* sexual differentiation of the male external genitalia and internal reproductive organs is hormonally mediated through fetal testicular secretion of testosterone. Around 7th to 8th week of gestation, fetal Leydig cells begin *de novo* synthesis and secretion of testosterone. Such secretion is initially maintained by placental human chorionic gonadotropin (hCG) and subsequently by fetal pituitary luteinizing hormone (LH) in the latter half of the gestation. However, neither placental hCG nor fetal pituitary gonadotropins have been proved to be essential for *in utero* differentiation of male reproductive organs.

During puberty, testicular steroidogenesis and spermatogenesis are under the control of hypothalamic pituitary gonadal (HPG) axis. Hypothalamic pituitary gonadal axis mainly consists of three endocrine organs: hypothalamus, anterior pituitary gland and testes, secreting peptide, protein and steroid hormones. The hormonal secretions are under positive and negative feedback control at multiple levels of the HPG axis. Furthermore, contribution of higher brain centers, hypothalamic and pituitary hormonal secretion and other endocrine organs, such as thyroid and adrenal glands, also exerts regulatory role. The scope of this chapter is to present a brief physiological overview about hormone structure, transport in the blood, receptor, metabolism and target organ action. Later, the physiological and the pathological conditions of male reproductive endocrinology will be examined with particular emphasis on the role of endocrine therapy in treatment of male infertility.

**PHYSIOLOGICAL OVERVIEW**

Hormones are chemical molecules secreted by specialized group of cells to exert specific action or effect on target cells or body system through binding with specific receptors. The secreting cells, scattered or organized in glands, usually release their contents into the extracellular (EC) space. Based upon the mode of hormone delivery and the target cells, three types of hormonal...
effects are recognized. Paracrine effect is seen when the secreting cells affect other target cells in their vicinity, usually in the same organ or tissue, and the EC fluid is the transporting medium. Autocrine effect is exhibited when the released hormone exerts biological effect on the same cell of origin. Endocrine effect is described when the hormones are transported through the blood stream to bind with their target cell receptors, which are usually in other organs or systems of the body. Lastly, the fourth mechanism, known as intracrine, is demonstrated when the synthesized hormone acts intracellularly in the same cell before release.

Essentially, three classes of hormones are identified based upon the chemical structure: amino acid (usually tyrosine) derivatives, peptides and proteins hormones and steroid hormones. For instance, tyrosine derived hormones encompass thyroxin, epinephrine, norepinephrine (NE) and dopamine (DA). On the other hand, peptide and protein derived hormones constitute the majority of hormones in human body. Specifically, the peptide hormones consists of fewer than 100 amino acids and vary considerably in size, ranging from three amino acids peptide, such as thyrotropin releasing hormone (TRH) to as much as 84 amino acids polypeptide hormone represented by parathyroid hormone. Protein hormone is often composed of more than 100 amino acids and may reach up to 200, such as growth hormone (GH) and prolactin (PRL). Peptide and protein derived hormones are often synthesized in the rough endoplasmic reticulum (RER) as large precursor hormones (preprohormones) that are then cleaved into prohormones and transferred to Golgi apparatus. Inside Golgi apparatus, packaging and storage of prohormones into secretory vesicles occur, where they undergo the final modification and cleavage into active hormones and inactive residues. Certain cellular stimuli, such as increased cytoplasmic calcium or c-AMP trigger fusion of these granules with plasma membranes and release of their contents into interstitial fluid or directly into the blood by the process, known as exocytosis.1 Peptide hormones are generally water soluble and easily transported to their target organs.

Lastly, steroid hormones, a class of lipids derived from cholesterol, include cortisol, aldosterone, testosterone, progesterone and estrogen. Minor biochemical differences among these hormones result in variable physiological role and function. These hormones are synthesized in the smooth endoplasmic reticulum (SER) and secreted from steroidogenic cells in testes, ovaries, adrenal cortex and placenta. Structurally, these steroid hormones consist of three cyclohexyl rings and one cyclopentyl ring combined into a single structure (Figure 1) and are lipid soluble. There is a limited storage capacity of the steroidogenic cells; however, under effect of specific stimuli large amount of cholesterol ester can be mobilized from cytoplasmic ester to SER for immediate synthesis and release of steroid hormones.

Hormones are the second key regulator of the human body systems’ functions after the nervous system. They are instrumental in maintenance of internal homeostasis, response to stressful condition, water and salt balance, metabolic regulation, energy production, growth and behavior, and human reproduction. The later function is fundamentally based on the integrity of complex set of hormonal interaction, known as HPG axis.

**Transport of Hormones in the Blood**

Peptide, protein hormones and some amino acid derived hormones, such as catecholamines, are water soluble and therefore freely distribute in the blood stream, reaching their target receptors without the need for carrier proteins. However, steroid and thyroxine hormones are poorly water soluble and circulate in the blood mainly bound to plasma proteins, such as sex hormone binding globulins (SHBGs), corticosteroid binding globulin, thyroxine binding globulins (TBG) respectively and albumins. The biologically inactive protein-bound hormone constitutes the major hormonal fraction, conferring three important physiological properties. Firstly, it acts as a reservoir replacing the biologically active free hormone when they are bound to their target receptors or lost from the circulation and secondly, prevents rapid clearance of small sized molecules in urine or bile and prolongs their half-lives. Thirdly, it ensures ubiquitous hormonal distribution in various body organs. Interestingly, pathological and physiological increase in the carrier proteins may
ultimately result in false elevation in total hormone measurement assays. Measurement of the physiologically active component, free hormone fraction, is useful in these situations. However, unreliability of some of these assays necessitates simultaneous determination of total hormone level and hormone binding globulin or preferably calculating the free hormones by equilibrium dialysis method.

**Hormone Action at the Target Organ**

Covalent binding of a hormone ligand with its specific receptor in the target organ is the first step in the hormonal action, initiating a cascade of events that ultimately result in expression of the response. For a cell to adequately respond there should be at least 2,000–100,000 high affinity receptors, represented by large proteins undergoing dynamic interaction on binding. The receptor sites and number vary based upon the type of hormone and the receptor itself. Protein, peptide and catecholamines usually attach to cell plasma membrane, receptors. The response is relatively fast and depends on signal transduction and activation of a second messenger. Nonetheless, some protein hormones, such as insulin, PRL and gonadotropins can enter the cells as well. Steroid and thyroid hormones, on the other hand, bind to cytoplasmic receptors (and then translocated to the nucleus) and nuclear receptors respectively, resulting in a slower response in the form of hormone–receptor complex induced activation of DNA transcription and translation. For steroid target cells 3,000–10,000 cytoplasmic receptors should be present to illicit a hormonal response.

Two major classes or families of hormone receptors are recognized: cell surface receptors and intracellular receptors (Figure 2). Cell surface receptors are specifically classified according to molecular mechanism of signal transduction into: group A (the receptor is linked to tyrosine kinase activity inducing intracellular phosphorylation) that is further subdivided into subgroup I receptor that has intrinsic tyrosine kinase activity, such as insulin and growth factors’ receptors, and subgroup II receptors that recruit tyrosine kinase activity, such as cytokines, GH, PR Land leptin’s receptors. On the other hand, group B receptors are coupled with GTP-binding protein (G-protein linked receptor) and there are more than 1,000 known types of G-protein linked receptors. GTP linked receptors have extracellular portion, seven looped transmembrane portion and intracellular cytoplasmic tail. On binding with a ligand, G-protein linked receptors undergo conformational changes replacing GTP for the GDP (already attached to the cytoplasmic tail) and activate either adenylatecyclase with production of cAMP as second messenger or phospholipase C with production of inositol triphosphate (IP3) and diacylglycerol (DAG). Inositol triphosphate increases intracellular calcium as second messenger, while DAG activates protein kinase C with further phosphorylation of intracellular proteins. Examples of G-protein linked receptors that activate adenylatecyclase include LH, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH) and corticotropin-releasing hormone (CRH); whereas, examples of G-protein linked receptors activating phospholipase C encompass LH, FSH, Gonadotropin releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH).

Intracellular receptors are the second major class or family of hormone receptors and include up to 150 members. These receptors are, in fact, hormonally regulated transcription factors which upon binding with specific hormone, bind to specific DNA sequences close to promoter sites controlling the expression of certain genes. This class is further classified into cytosolic receptors, e.g. steroid hormone receptors, and nuclear receptors, e.g. thyroid hormone receptors.

After receptor activation, hormone-receptor complex either dissociates leaving the receptor free or the complex is internalized by endocytosis, degraded inside lysosomes and the receptor is then recycled again. Nevertheless, receptor availability, number and affinity are not static, and instantaneous steady state equilibrium exists between the hormone and its receptors. For instance, increased hormone concentration with consequent increased receptor binding eventually results in decreased availability of the receptors and thus minimizing the target cellular response to the hormone and the process is called ‘downregulation,’ e.g. continuous administration of GnRH results in decrease in the pituitary gland synthesis and release of FSH and LH. Conversely, some hormones or stimulating substances increase manufacturing of receptors and hence the target cell response to stimulating hormone, e.g. thyroxine increases the receptor response to catecholamines.

**Metabolism, Degradation and Clearance of Hormones**

Following activation of target receptor tissues, a small fraction of hormones usually undergoes in situ enzymatic inactivation and degradation; whereas the remaining large portion is inactivated and excreted through the liver and/or the kidney. Less than 1% of any hormone is excreted unchanged in urine. Metabolic inactivation includes oxidation, hydrolysis, hydroxylation, methylation, decarboxylation, sulfation and glucuronidation. However, some hormones can be enzymatically activated to more potent forms at the target tissues, such as conversion of tetraiodothyronine (T4) to
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tri-iodothyronine (T3) and conversion of testosterone to dihydrotestosterone (DHT).

**HYPOTHALAMIC PITUITARY GONADAL AXIS (HPG)**

**Hypothalamus**

Hypothalamus is a vital neuroendocrine organ, representing an important link between nervous and endocrine systems. Hypothalamus is essentially composed of loose network of neurons defined by arbitrary borders, located below the thalamus and above the brainstem forming the ventral portion of diencephalon. It is a small organ of an almond size (1.5 cm x 1.5 cm x 1.3 cm), weighing about 2.5 gm, bound anteriorly by anterior margin of the optic chiasma and lamina terminalis, posteriorly by the posterior margin of the mammillary bodies, and laterally by a diffuse line extending from the optic tracts, internal capsule, pes pedunculi, globus pallidus and ansa lenticularis (Figure 3).

**Figure 2**  A composite diagram showing the different classes of hormone receptors

*Abbreviations: PLC, phospholipase C; TK, tyrosine kinase*

median eminence, whereby vascular networks stemming from superior hypophyseal artery are transporting them through a big vein, hypophyseal portal vein, to another capillary network into the pituitary gland. These releasing and inhibitory factors exert specific effects on a particular group of pituitary cells to elicit a release of a specific hormone.

Specifically, hypothalamic neuropeptides that are mainly concerned with male reproduction are divided into:

- Peptides with direct influence on male reproduction. These include GnRH, CRH, and prolactin inhibitory factor (PIF).
- Peptides with indirect influence on male reproduction. These include TRH, growth hormone releasing hormone (GHRH) and growth hormone inhibiting hormone (GHIH).

**Gonadotropin Releasing Hormone**

Gonadotropin releasing hormone is a hypothalamic decapeptide controlling the secretion of gonadotropins, FSH and LH from the anterior pituitary. GnRH is released in a pulsatile pattern from a diffuse network of 1,500 neurons that are mainly concentrated in medial basal hypothalamus, the infundibulum, and periventricular region. These neurons act as a single pulse generator for the HPG axis. GnRH pulse release lasts for few minutes at frequency of one pulse every 1–3 hours. The amount of GnRH is determined by the quantity of GnRH secreted at each pulse and the frequency of these cycles (Figure 5). Correspondingly, LH is secreted from the anterior pituitary at pulse frequency exactly coinciding with that of GnRH; whereas, FSH shows nonpulsatile mode of secretion and even when it is pulsatile, there is little concordance with GnRH pulses. Exogenous replacement of GnRH should follow similar physiological regulations to stimulate LH and FSH secretion. Chronic continuous administration of GnRH may result in inhibition of LH and FSH.

**Pituitary Gland**

Pituitary gland is an essential endocrine organ in the human body. It is composed of three components: anterior lobe (adenohypophysis, predominant part), intermediate lobe (vestigial component) and posterior lobe (neurohypophysis). Pituitary gland is located in the bony sella turcica at the base of the skull, overlaid by thin diaphragm, through which the pituitary stalk connecting between the pituitary gland and median eminence of hypothalamus is passing through. Its average weight is 400–900 mg and measures about 13 mm in the longest transverse diameter, 6–9 mm vertically, and about 9 mm anteroposteriorly. Adenohypophysis is the major endocrine portion of the
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Figures 4A to C  Relationship between the hypothalamus and the pituitary gland. (A) Connections from the hypothalamus to the posterior lobe; (B) The portal vessels of the pituitary stalk ensure that releasing hormones (factors) are transported from the median eminence in the upper part of the stalk to the epithelial cells of the anterior lobe; (C) Axonal transport of peptide hormones (neuropeptides) from the hypothalamus to the pituitary


pituitary gland, consisting of gonadotrophs (5–10%), somatotrophs, thyrotrophs and corticotrophs. Under the effect of GnRH, gonadotrophs secrete LH and FSH directly into the blood stream to exert their effects on testicular Leydig cells and Sertoli cells, respectively.

Gonadotropins

Luteinizing hormone and FSH belong to a family of glycoprotein hormones that also includes TSH. Each glycoprotein hormone in this family is heterodimeric, i.e. consisting of α-chain and β-chain. The α-chain is a common chain, whereas β-chain confers functional specificity for a particular hormone. These chains are tightly linked by disulfide bonds, yielding specific conformational structure which is necessary for receptor binding and biological activity. Around 60% of gonadotrophs secrete both FSH and LH; whereas, 18% and 22% of cells solely secrete LH and FSH respectively.

Glycosylation of these hormones with various types of oligosaccharides occur before release from the pituitary cells, consisting of various types of sugars, such as sialic acid, mannose, galactose and N-acetylglucosamine. There are two sites of glycosylation in each peptide chain. Single branched; di-,tri- and even tetra-branched oligosaccharides are demonstrated in these glycosylation sites that lead to appearance of different isoforms of the gonadotropin. These sugars have effect on hormone assembly, secretion pattern, mode of action and metabolic clearance. Sialic acid residues are the most critically important sugar in these oligosaccharides.
that leads to appearance of gonadotropin isoforms. Acidic isoforms that have more sialic acid residues in the attached oligosaccharides often have longer half-life and are relatively protected from degradation by the liver, whereas less sialic acid residues render gonadotropins less acidic and more potent. The half-life of LH (20 minutes) is relatively shorter than that of FSH (1–4 hours). GnRH and sex steroids play critical role in regulating the process of glycosylation.

Various studies have shown progressive increase in the serum levels of FSH and LH with aging. FSH secretion increases in both continuous and pulsatile forms; whereas, LH shows modest but inconsistent elevation in the elderly men. Such rise in gonadotropin level is attributed to impaired testicular feedback regulation of the hypothalamus and pituitary gland.

Testes
Testis is the third component of the HPG axis. Functionally, testis is divided into two compartments: interstitial compartment containing Leydig cells, and seminiferous tubules consisting of Sertoli cells and spermatogenic cells in various stages of development.

Leydig Cell
Leydig cells constitute about 10–20% of interstitial compartment that forms 12–15% of testicular volume. Leydig cells secrete group of hormones collectively known as androgens including testosterone, DHT and androstenedione. Testosterone is the most abundant hormone and 75% of blood testosterone is derived from testes. 200 x 10⁶ Leydig cells daily secrete 4–9 gm of testosterone under the effect of LH. Furthermore, insulin-like factor 3 (INSL3) is another protein hormone secreted by Leydig cells, which may be used as an indicator of Leydig cell function. INSL3 has been postulated to play a direct role in regulation of sperm production.

Testosterone: It is the main hormone secreted by the Leydig cells of testes and is the principal androgen in men. It is C19 steroid hormone with OH group at position C17 in the sterol ring. Testosterone is important for regulation and induction of spermatogenesis and development of secondary sexual characteristics. Leydig cells synthesize testosterone, under the effect of LH, from two sources: cholesterol, or synthesized de novo or taken directly from the blood. Androstenedione is synthesized by the adrenal glands. LH stimulates mitochondrial uptake of cholesterol by enhanced activity of steroidogenic acute regulatory protein, the first rate-limiting step in testosterone synthesis. Inside the mitochondria, cholesterol undergoes side chain cleavage to form pregnenolone that diffuses outside the mitochondria towards SER where it is further processed. Two important pathways are embarked toward formation of testosterone inside SER: 3β-pathway (pregnenolone, 17OH pregnenolone and dehydroepiandrosterone (DHEA) and androstenediol) and 4α-pathway (pregnenolone, progesterone, 17OH progesterone and androstenedione). 3β-pathway predominates in human testes. Synthesis of testosterone by testis requires the presence of two specific enzymes: 17β-hydroxysteroid dehydrogenase-3 that catalyzes the conversion of DHEA to androstenediol and conversion of androstenedione to testosterone, and 3β-hydroxysteroid dehydrogenase-2 that directly converts androstenediol to testosterone.

As aforementioned, human testis daily produces 4–9 gm of testosterone. In addition, 500 mg of testosterone produced either directly from the adrenal glands or from peripheral conversion of androstenedione. DHT is another hormone produced by the testis through enzymatic action of Sertoli cell-derived 5α-reductase on testosterone. However, only 20% of serum DHT is secreted by the testes and the rest is derived from peripheral conversion at the target tissues. DHT has more than twice the biological activity of testosterone. Alternatively, testosterone and androstenedione can be converted to estradiol and estrone respectively by the action of aromatase enzyme in the gonadal and extraglandular tissues, as will be described below. The major catabolic pathway of testosterone to less active metabolite is
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mainly through conjugation in the liver and excreted in urine as 17-ketosteroids.

Estrogens: Although small amount (20%) of estrogen in men is directly secreted by the testis, the majority is derived from conversion of testosterone and androstenedione by aromatase enzyme inside gonadal cells, such as Leydig cells and Sertoli cells and extragonadal tissues, such as brain, skin, liver, mammary tissues, and most significantly, the adipose tissue. The plasma level of estradiol is 2–3 ng/dL with production of about 25–40 µg per day. The exact physiological role of estrogen in male reproductive function is under investigation. While estradiol plays an inhibitory role in regulation of HPG axis at the level of hypothalamus and pituitary glands, recent investigations revealed presence of estrogen receptors (ERα) in the efferent ductules, epididymis and Leydig cells. Knockout male mice for ERα suffer alteration of spermatogenesis and infertility. However, exposure to exogenous estrogen can suppress HPG axis and impair spermatogenesis. Further studies on humans may be needed to uncover the complete physiological roles of estrogen. In men, in contrast to women, estradiol level increases with age.

Sertoli Cells

Sertoli cells perform supporting role in orchestrating the process of spermatogenesis and in forming the blood testis barrier. Approximately, 35–40% of the volume of germinal epithelium is represented by Sertoli cells. The intact testis contains 800–1200 × 10⁶ Sertoli cells or approximately 25 × 10⁶ Sertoli cells per gram testis. Sertoli cells secrete variety of proteins, cytokines, growth factors, opioids, steroids, prostaglandins and modulators of cell division. Specifically, Sertoli cells secrete two structurally related proteins, i.e., inhibin and activin. These proteins are dimers belonging to transforming growth factor-beta family. Inhibin consists of an α subunit and a βA or βB subunit, whereas activin is a homodimer (βAβA or βBβB). Inhibin is secreted from Sertoli cells under the effect of FSH and acts to inhibit FSH secretion from the anterior pituitary. Conversely, activin activates secretion of FSH to stimulate spermatogenesis. These hormones are not only secreted by Sertoli cells, but also from other sources, such as Leydig cells, prostate, liver etc.

Regulation of HPG Axis

On each level of HPG axis, three types of regulatory feedback loops are acting to control hormonal output. These regulatory loops are either negative (inhibit hormone secretion) or positive (stimulate hormone secretion). These loops include:

Long feedback loop in which the secretion of testes (the target organ) inhibits or stimulates hypothalamus and anterior pituitary secretion

Short feedback loop in which the anterior pituitary secretions control the hypothalamic hormonal output

Ultrashort feedback loop in which there is intrinsic regulation of hormonal output at each organ or level of HPG axis

In the first loop, testosterone is the major hormone released by testis and regulates GnRH and gonadotropin secretion. Specifically, testosterone or its metabolite DHT reduces the frequency of hypothalamic pulse generator to release GnRH, whereas testosterone or its metabolite estrogen acts on pituitary cells to inhibit GnRH release. Higher doses of testosterone can also exert inhibitory effect on GnRH and LH; however, it directly stimulates pituitary FSH secretion through positive feedback loop interaction. Progesterone, on the other hand, acts on dopaminergic arcuate nucleus neurons that inhibit GnRH neurons. Additionally, other testicular factors, such as activin and inhibin, stimulate or inhibit pituitary release of FSH respectively at the level of pituitary gland (Figure 6).

Evidence for pituitary influence on hypothalamic secretion arises from identification of a direct inferior branch of the hypophyseal artery entering the anterior pituitary gland in some mammals and finding that blood flow within the portal system can occur in the reverse (retrograde) direction—from anterior lobe toward hypothalamus. Therefore, the pituitary gland might regulate the hypothalamus by ‘short-loop’ feedback.

The ultrashort hypothalamic feedback is represented by paracrine and autocrine regulation of GnRH neurons. GnRH neurons can regulate the secretory activity of their own and there is evidence for the presence of receptors for GnRH (GnRHR) in hypothalamic neurons. Neurons from limbic system, brain stem and other hypothalamic areas project to GnRH neurons and influence their secretory activity. Neurons that release glutamate and NE provide stimulatory role to the reproductive axis; whereas, those neurons that release gamma aminobutyric acid(GABA) and endogenous opioid peptides play important inhibitory role.

Within the pituitary gland, nerves and short portal vessels interconnect the pituitary lobes forming potential routes of intrapituitary communication and regulation. Finally, testis is a complex endocrine organ, where various mechanisms of ultrashort regulatory loops exist in the form of paracrine, autocrine and even intracrine systems.
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Figure 6  Schematic figure shows the complex regulation of hypothalamic pituitary gonadal axis. Well-balanced nutrition, moderate exercise and seasonal cues have stimulatory effects on the hypothalamus through higher brain centers, whereas excessive exercise, undernutrition may have inhibitory effect on the hypothalamus. Factors stimulating GnRH neurons to release GnRH include norepinephrine (NE), neuropeptide Y (NPY), leptin, galanine like peptide (GALP) and glutamate; whereas, factors inhibiting central GnRH release include beta endorphins, interleukine1, GABA, corticotropin releasing hormone (CRH) and dopamine (DA). The figure also shows negative feedback regulation of gonadotropin synthesis and release. Testosterone, DHT, estrogen, progesterone and inhibin B are factors released from the testes and inhibit gonadotropin release at the level from the hypothalamus and the pituitary gland. Activin is released from many cells in the male reproductive system and locally produced in the pituitary to stimulate FSH release.

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CLINICAL ENDOCRINE EVALUATION OF INFERTILE MAN

Initial evaluation of infertile couple should aim at determining whether the problem lies within the male or female partner, or both. If the female partner has regular menstrual cycles; patent fallopian tubes; normal FSH, LH, TSH and PRL levels; male factor infertility is the likely cause. Clinical evaluation of endocrine contribution to male factor infertility should commence with detailed history taking and proper conduct of physical examination. Four important goals of endocrinological evaluation are to assess:

1. Whether male reproductive dysfunction is attributed to male hypogonadism or not.
2. Prepubertal or postpubertal onset of hypogonadism.
3. Whether the problem is localized to the HPG axis or outside this axis.
4. Finally, if the defect has been traced to HPG axis, specific testicular, pituitary or hypothalamic etiology should be sought and then treated.

Certain childhood illnesses, such as unilateral or bilateral empty scrotum, testicular torsion, scrotal trauma, pediatric inguinal hernia repair and mumps orchitis could risk not only the spermatogenesis function, but also Leydig cell function as well. Defective testosterone-synthesis or action in the third trimester could result in male live births born with ambiguous genitalia, cryptorchidism and micropenis. Other risk factors for male hypogonadism include testicular tumors; exposure to certain medications, such as anabolic steroids, cimetidine, ketoconazole, digoxin and spironolactone; cancer chemotherapy or radiotherapy; HIV/AIDS; hemochromatosis; pituitary tumor and surgery and certain systemic inflammatory and infectious conditions, such as sarcoidosis, chronic obstructive airway diseases, chronic liver and kidney diseases, histiocytosis, tuberculosis and fungal infections. Furthermore, substance abuse and recreational drugs, such as marijuana, heavy smoking, cocaine and alcohol have been also implicated in the etiology of male hypogonadism.

Absent or delayed pubertal development is ominous sign of male hypogonadism. In approximately 99.5% of white boys, early signs of secondary sex characteristics may at least become evident by the age of 14 years. Increased testicular volume from 1–2 cc to 3–8 cc accompanied by discernible enlargement of penile size are the first signs of puberty and typically such signs occur at the age of 11.5 years in most US boys. Although the most common cause of pubertal delay is constitutional [idiopathic delay (60%)] and delayed but spontaneous pubertal developmental [functional hypogonadotropic hypogonadism (HH) 20%], permanent endocrine disturbances in the HPG axis (24%) should always be sought.

In adults who have already completed pubertal spurt, features of hypogonadism include loss of energy and decrease in libido. Tables 1 and 2 show signs of male hypogonadism before and after puberty.

Physical examination (general and genital) adds a wealth of information. Tanner staging is essential component of physical assessment. Arms span and its discordance with the body height, excessive skin pallor, sparse pubic and facial hair distribution, female fat distribution, diminished testicular size, and presence of gynecostasia may all pinpoint to potential endocrine deregulation as a cause for infertility. Physical examination could even suggest the possible mechanisms and the exact site of endocrine disturbance. Visual field defects, galactorrhea and impotence may indicate prolactinoma associated hypogonadism. Lack of smell sensation is a diagnostic feature of Kallmann’s syndrome. Gynecomastia, bilateral small testes and behavioral problems may indicate Klinefelter’s syndrome. Varicocele, bilateral testicular atrophy, testicular mass, signs of chronic liver or kidney diseases, obesity, signs of Cushing’s syndrome, acromegaly, thyroid dysfunction all can probably shed a light on the etiology behind hypogonadism.

Semen quality should be determined by analyzing semen samples obtained by masturbation after 2–7 days of abstinence. Semen volume, pH, sperm count, and motility should be evaluated. Azoospermia or oligospermia should be sought.

Table 1  Signs of early onset hypogonadism

<table>
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<td>Eunuchoid appearance: arm span &gt; 5 cm than height</td>
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<tr>
<td>Poor musculinization</td>
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<tr>
<td>Lack of recession of hair on temporal lobe</td>
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<tr>
<td>Sparse axillary, pubic, facial and body hair</td>
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<tr>
<td>High-pitched voice</td>
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<tr>
<td>Small testes and possibly maldescended</td>
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<tr>
<td>Infantile external genitalia</td>
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<tr>
<td>Impaired libido and potency</td>
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<tr>
<td>Oligospermia to azoospermia</td>
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Table 2  Signs of postpubertal onset of androgen deficiency

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<th>Sign</th>
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<tr>
<td>Normal body proportions with possibility of osteoporosis</td>
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<tr>
<td>Atrophy and decreased muscle strength</td>
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<tr>
<td>Male-pattern baldness</td>
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<tr>
<td>Sparse facial, pubic, axillary, chest and hair</td>
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<tr>
<td>Normal pitch of voice</td>
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<tr>
<td>Small, often soft testes</td>
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<tr>
<td>No change in the size of external genitalia</td>
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<tr>
<td>Decreased libido and erectile dysfunction</td>
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<td>Oligospermia to azoospermia</td>
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density, motility, morphology and viability are evaluated in accordance with WHO criteria. It is important to obtain multiple semen samples to overcome tremendous variability in sperm parameters.

**Indications of Endocrine Testing**

In two large retrospective analyses of 1,035 infertile men in two infertility centers, endocrine abnormalities, on repetitive testing, had been detected in only 99 patients (9.6%). FSH elevation was the most frequent abnormality. Furthermore, only 1.75% of these men had clinically relevant endocrinopathy in terms of disease management. Interestingly, with presence of relatively normal spermatogenesis, detecting low levels of FSH or LH has no clinical significance. The current recommendations of endocrine testing include:

- Sperm concentration less than 10 million/ml
- Erectile dysfunction and decreased libido
- Hypospermatia (sperm volume < 1 ml)
- Signs and symptoms of endocrinopathies, such as thyroid dysfunction, acromegaly, delayed puberty or hypogonadism
- Presence of gynecomastia
- History suggestive of hyperprolactinemia, such as headache, galactorrhea, impotence, blurred vision
- Testicular mass
- History of exogenous estrogen or androgen exposure
- Significant obesity
- Clinical features of androgen resistance
- Clinical picture consistent with 5-α reductase deficiency

Clinical fertility centers differ in the extent of hormonal evaluation for infertile men. Current American Urological Association (AUA) guidelines recommend the measurements of at least serum FSH levels and serum testosterone levels in patients suspected of having abnormality in HPG axis. Such measurements are reliable in detecting 99% of endocrine abnormalities. Testosterone is considered the indicator of endocrine abnormality in HPG axis. On the other hand, low FSH in the presence of low testosterone may reflect central disorder in the HPG axis, such as HH. In contrast, normal FSH measurement may not be indicative of normal spermatogenesis and does not exclude severe derangement of spermatogenesis. Specifically, men with spermatogenic arrest at late stages, focal Sertoli cell only syndrome and hypospermatogenesis may all have normal FSH level. Even more specifically, normal FSH is not absolute predictive parameter of sperm retrieval by testicular sperm extraction in men with NOA. From the above discussion, it is apparent that the value of serum FSH measurement is particularly limited and determination of other endocrine factors, such as inhibin B, may be required to represent the quality of spermatogenesis.

Inhibin B is a direct product of Sertoli cells and its serum levels have been found to be better correlated to sperm parameters than FSH and thus may serve as a better marker of spermatogenesis. Inhibin B levels can also be useful for monitoring the effects of gonadotropin therapy. However, inhibin B or FSH alone as well as the combination of both hormones cannot predict the finding of spermatozoa by testicular biopsy in patients with azoospermia who are candidates for intracytoplasmic sperm injection (ICSI) treatment.

Testosterone level is, more or less, a good indicator of the integrity of HPG axis and Leydig cell function. Total testosterone level consists of protein (SHBG and albumin) bound fraction (98%) and free fraction (2%), and its measurement correlates well with available testosterone. However, measurement of free testosterone should be considered when alterations in SHBG are expected (Table 3). In order to determine free testosterone reliably, equilibrium dialysis or ultrafiltration techniques are required. These methods are complicated and not routinely recommended at present. However, a simple and reliable method for clinical practice is the estimation of free testosterone from the levels of total testosterone, and SHBG by using a standard equation and special nomograms (Fig. 7). Calculated free testosterone correlates well with free testosterone estimated by equilibrium dialysis. The serum total testosterone concentration is not diagnostic of hypogonadism in obese patients or those with nephrotic syndrome, hyper- or hypothyroidism, chronic liver disease, or on therapy with anticonvulsants or steroids.

The generally approved reference range for total testosterone in adult men is wide, from 260 ng/dl to 1,000 ng/dl (9–34.7 nmol/l). Furthermore, testosterone levels have circadian fluctuations, particularly marked in younger men, where there is a striking difference in testosterone levels measured in early morning versus those
taken later in the day. The mean maximum level of 720 ng/dl (25 nmol/l) is reached at approximately 8 AM, and declines to a mean minimum of 432 ng/dl (15 nmol/l) at approximately 10 PM. However, such degree of change between morning and evening testosterone levels is less striking in older men. Nevertheless, it is best in all cases to measure the concentration in the morning so that peak results can be compared with the usual standards. In general, because of this circadian variations in secretion, serum samples for total testosterone determination should be obtained between 7:00 AM and 9:00 AM. Moreover, because of diurnal variation in testosterone and pulsatile pattern of secretion of LH and FSH, an alternative is to perform hormone assays on three pooled blood samples taken at 10 minutes intervals.

In men who are found to have azoospermia but normal testosterone, LH, and FSH levels and normal testes volume, obstructive disorders should be ruled out by measuring seminal levels of fructose and neutral α-glucosidase. The latter originates in the epididymis. In case of azoospermia or severe oligospermia, normal testosterone and LH levels, but elevated FSH, primary spermatogenic failure should be considered. These patients should get testicular volume assessment, karyotyping and Yq micro deletion screening.

Primary testicular failure (spermatogenesis and steroidogenesis) often presents with low testosterone, and elevated FSH and LH serum levels; whereas, patients

<table>
<thead>
<tr>
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Table 3 Factors that affect circulating sex hormone-binding globulin levels

![Figure 7](image.png)

**Figure 7** Nomogram for calculating free testosterone (CFT) from total testosterone (TT) and (SHBG)

*Source: Carruthers M. Androgen deficiency in the adult male: causes, diagnosis and treatment. London: Taylor & Francis; 2004*
with selective spermatogenic failure have normal testosterone and LH, and only elevated FSH. In cases with low testosterone and low or inappropriately normal LH and FSH, it is important to determine PRL, cortisol, serum ferritin, TSH and free thyroxine levels. These patients may need magnetic brain resonance imaging to determine the cause of HH. Normal MRI, PRL level and other measurements in these men point out to the possibility of hypothalamic disorders, such as anosmic HH (Kallmann’s syndrome), nonanosmic isolated hypogonadotropic hypogonadism (IHH) and adult onset HH. Direct measurement of GnRH is not feasible in humans due to pulsatile mode of secretion, short half-life, very low concentration and dilution of this hypothalamic hormone by the pituitary portal circulation and then by systemic circulation. In unresolved cases when there is low or normal LH and FSH, GnRH stimulation test can be conducted. Subcutaneous injection of intravenous 100 µg of GnRH should normally cause a three fold rise in LH and 1.5 times in FSH after 30–45 minutes of injection. The rise in LH/FSH helps confirm the diagnosis of anosmic and normosmic HH. In contrast, negative response is usually attributed to failure of pituitary gland to respond to the injected GnRH. Such unresponsiveness may occur due to lack of previous exposure to GnRH (Priming effect) or due to mutated gene coding for GnRH receptor (GNRHR). To discriminate between the two conditions, subcutaneous portable GnRH pump releasing 5 µg of GnRH every (90–120) is undertaken for 36 hours a day for 7 days. Then GnRH stimulation test is repeated. Positive rise confirm the HH diagnosis and exclude the possibility of pituitary unresponsiveness.

Normal PRL level in men is usually less than 18 ng/dl (550 mIU/l). However, due to high assay variability, testing should be repeated, if levels are elevated. If hyperprolactinemia is discovered and secondary causes are ruled out or PRL levels are above 150 ng/dl, a gadolinium enhanced MRI with special attention to the region of hypothalamus and pituitary is indicated for revealing a prolactinoma or another space occupying process.

Both in complete and partial forms of androgen insensitivity, serum testosterone and LH levels are usually elevated, but FSH may be normal or elevated. Estradiol is higher than in normal males. Failure of SHBG to decrease after testosterone administration confirms the androgen insensitivity. An hCG stimulating test demonstrating normal testosterone and DHT production can be used to distinguish partial androgen insensitivity syndrome from defects in testosterone biosynthesis and 5-α reductase activity. Karyotyping reveals 46 XY and is indicated especially in cases with ambiguous genitalia and bilateral inguinal hernias. Androgen receptor studies are helpful in cases with incomplete insensitivity.

Serum estradiol determination may be considered in select group of patients, such as ones with Klinefelter’s syndrome and in cases with gynecomastia, a testicular mass, a history consistent with exogenous estrogen exposure, or evidence of androgen resistance.

### DISEASES OF THE HPG AXIS

#### Hypothalamic and Pituitary Disorders

Hypogonadotropic hypogonadism results from failure of the hypothalamus or pituitary to stimulate and maintain normal gonadal function. Pituitary functions may be affected in events of pituitary tumors, infarction, inflammatory and granulomatous diseases, surgery and radiation. However, gonadotropin deficiency may also occur in the presence of otherwise normal pituitary function when the secretion or action of GnRH is altered: IHH

#### Isolated Hypogonadotropic Hypogonadism

Isolated hypogonadotropic hypogonadism is clinically defined as absent or incomplete puberty by the age of 18 years because of low gonadotropin secretion. The GnRH deficiency can be due to impaired migration of the GnRH neurons to the hypothalamus during embryonic development, abnormal maturation or decreased survival of GnRH neurons, or resistance to the action of GnRH at the level of pituitary. IHH can be either sporadic or familial. It may be inherited in an X-linked recessive, autosomal dominant or autosomal recessive mode. However, the genetics are not strictly Mendelian. IHH may be due to mutations in more than one gene, as well as interaction between genes, or between genes and environmental factors. In males, the prevalence is around 1 in 10,000. There are two forms of IHH depending on the presence or absence of the normal sense of smell: normosmic HH and Kallmann’s syndrome.

#### Kallmann’s Syndrome

It is a form of IHH associated with olfactory disturbances (hypo- or anosmia) due to the absence or hypoplasia of the olfactory bulbs and tract. The incidence is estimated to be about 3.7% of all IHH male patients. The male preponderance of cases remains still unexplained. The olfactory and reproductive deficits are combined with various defects, including cryptorchidism, bimanual synkinesis (mirror movements), unilateral renal agenesis, craniofacial or dental abnormalities, syndactyly, sensorineural deafness.

#### Hyperprolactinemia

It is another endocrine cause of secondary hypogonadism commonly seen in clinical practice. PRL is an anterior...
pituitary hormone whose excessive concentrations suppress the secretion of FSH and LH and/or impede their action on the gonads. Hyperprolactinemia can be caused by prolactinomas, pituitary tumors secreting both PRL and GH, processes causing pituitary stalk compression or section, empty sella syndrome, medications, primary hypothyroidism, chronic renal failure among other causes or may be idiopathic. Symptoms include depressed libido, erectile dysfunction, and infertility. Galactorrhea is rare in men.

**Isolated FSH and LH Deficiency**
Rare disorders include isolated FSH deficiency, which may present with oligo- or azoospermia, though such patients have normal virilization and normal testosterone and LH levels. Isolated LH deficiency (Pasqualini syndrome, fertile eunuch syndrome) on the other hand leads to eunuchoid habitus, low testosterone levels, but normal maturation of germinal epithelium with Leydig cell atrophy on testicular biopsy. Serum levels of LH are low, but of FSH are normal.

**Other Complex Congenital Syndrome**
This syndrome associated with HH includes Prader-Willi syndrome where lack of GnRH secretion leads to LH and FSH deficiency. The hypogonadism in the very rare genetic disorders, Laurence-Moon syndrome and Bardet-Biedl syndrome, is not obligate.

**Testicular Diseases**

**Hypergonadotropic Hypogonadism**
This group includes different congenital and acquired disorders primarily affecting the gonads (Table 3). The result in testicular failure and infertility, but some of them can cause only fertility disturbances without obvious signs of hypogonadism. Defects in androgen production, as well as conversion of testosterone to DHT due to the deficiency of enzyme 5-α reductase, affect the phenotype and reproduction. A number of genetic disorders, such as Klinefelter’s syndrome and Y chromosome microdeletions, have been implicated in spermatogenic failure. Table 4 demonstrates causes of HH.

**Disorders of Androgen Actions**
Androgen insensitivity causes undermasculinization of various degrees in 46 XY individuals. The androgen receptor gene is located on the X chromosome between Xq11 and Xq13. Androgen insensitivity syndromes result from defects in androgen receptor number or function. Androgen insensitivity may be complete or partial (incomplete). Complete androgen insensitivity syndrome (testicular feminization syndrome) is characterized by complete feminization of genetic males. Partial androgen insensitivity presents with great variations from normal male phenotype with infertility to individuals with genital ambiguity and gynecomastia.

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### TABLE 4

<table>
<thead>
<tr>
<th>Disorders causing primary or hypergonadotropic hypogonadism</th>
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<tr>
<td>• Klinefelter’s syndrome (47,XXY)</td>
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<tr>
<td>• XX male syndrome</td>
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<tr>
<td>• 47,XXY men</td>
</tr>
<tr>
<td>• Gonadal dysgenesis</td>
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<tr>
<td>• Noonan’s syndrome</td>
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<tr>
<td>• Defects in androgen biosynthesis</td>
</tr>
<tr>
<td>• Bilateral anorchia (vanishing testes syndrome)</td>
</tr>
<tr>
<td>• Acquired anarchy</td>
</tr>
<tr>
<td>• Orchitis</td>
</tr>
<tr>
<td>• Varicocele</td>
</tr>
<tr>
<td>• Adult seminiferous tubule failure</td>
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</tbody>
</table>

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**DISEASES OF THE ADRENAL GLANDS**
Glucocorticoid excess (hypercortisolism) in Cushing’s syndrome of either endogenous or exogenous etiology may also suppress LH secretion and testosterone biosynthesis resulting in testosterone deficiency and hypospermatogenesis. Androgen excess can induce a hypogonadal state by inhibiting gonadotropin production through negative feedback. The source of androgen excess could be either endogenous production from adrenals or testes, or exogenous anabolic steroids. Deficiency of enzyme 21-hydroxylase is the commonest cause of congenital adrenal hyperplasia. The excess of adrenal androgens in this condition may lead to precocious pseudo-puberty and infertility. It can be diagnosed by high basal and ACTH stimulated plasma 17-α hydroxyprogesterone levels. Men with partial enzyme deficiency may remain undiagnosed until late in adulthood, though they are usually fertile. ‘Adrenal or testicular Leydig cell tumors’ can also produce excess serum androgens and require radiological imaging for diagnosis.

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**THYROID DISORDERS**
Both hyper- and hypothyroidism may have an adverse impact on male fertility. Hyperthyroidism is known to cause elevation of SHBG and decline in semen quality especially in semen motility. In literature, hyperthyroid men has shown relative primary gonadal insufficiency that might be due to exaggerated SHBG levels and increased gonadotropin levels with co-pulsatility
between LH and FSH, which was more pronounced than in healthy men. Evidence is weak though about the possible deleterious effects of hypothyroidism on male reproductive system.

**EXCESS ESTROGEN**

Estrogen excess can also produce secondary testicular failure by inhibiting pituitary gonadotropins. It can be derived from either estrogen secreting adrenal or testicular tumors or excess peripheral conversion of androgens to estrogens by aromatase enzyme in patients suffering from chronic liver diseases or obesity. Men with high estrogen levels may present with gynecomastia, erectile dysfunction and testicular atrophy.

**DIABETES MELLITUS AND METABOLIC SYNDROME**

Diabetes mellitus affects the reproductive function mainly through microangiopathy and neuropathy, which in turn leads to erectile dysfunction and ejaculate disturbances. Obesity as well as diabetes mellitus type 2 (DM 2) may cause hypogonadism and infertility. Both low and high body mass index (BMI) are associated with disturbances in spermatogenesis. In obesity, increased peripheral conversion of androgens to estrogens in excess peripheral adipose tissue suppresses the gonadotropin secretion. Another unfavorable effect of obesity may be the oxidative stress leading to impaired spermatogenesis. Dyslipidemia also increases oxidative stress. Metabolic syndrome is not a separate disease by itself but a cluster of abnormalities, including visceral type obesity, dyslipidemia, hypertension and impaired glucose metabolism or DM 2 with insulin resistance as the hypothesized underlying pathogenic mechanism. An association of metabolic syndrome with low testosterone and low SHBG serum levels is widely accepted, but the cause and effect relationship is still unclear. Men with low testosterone and low SHBG levels are more likely to develop insulin resistance and DM 2. On the other hand, insulin is known to inhibit SHBG synthesis, therefore in insulin-resistant individuals SHBG, and consequently total testosterone is decreased. A negative correlation of total testosterone with insulin levels, insulin resistance and BMI in young males with metabolic syndrome was reported.

**HORMONAL INTERVENTION IN MALE INFERTILITY**

Hormonal therapy was utilized in the field of male infertility many years ago; however, the fertility outcomes confront a great deal of disappointment and frustration. Alternatively, the successful conception rates achieved through assisted reproductive technology has led to infrequent and unenthusiastic demand on hormonal intervention. Nevertheless, appropriately prescribed hormonal treatment is cost-effective and attractive mode of therapy. Currently, therapeutic and fertility preservation roles are the two main sorts of hormonal manipulation.

Therapeutic intervention aiming at restoring the fertility potential is used for specific replacement of a particularly deficient hormone, or used in nonspecific manner in idiopathic male infertility with no identifiable endocrine abnormalities.

Adequate replacement therapy either with GnRH or LH and FSH can induce spermatogenesis in patients with HH. Maturation of the human sperm takes approximately 72 days, so the treatment should last at least 3 months for the sperm to appear in the ejaculate. Usually a much longer period (up to 2 years or even more) is required, especially in congenital HH.

**Gonadotropin Releasing Hormone**

*Rationale:* GnRH stimulates anterior pituitary to secrete LH and FSH which in turn regulates T cells production and spermatogenesis. It can thus be used in pulsatile fashion in men with HH caused by hypothalamic dysfunction, but not in those having loss of pituitary function. It can also be used for induction of puberty.

*Method of administration:* GnRH is administered using portable pump in doses of 4–20 µg per pulse administered subcutaneously every 2 hours as pulsatile therapy. Doses are adjusted until serum testosterone reaches mid-normal levels. GnRH as nasal spray is used for treatment of cryptorchidism.

*Indications:* GnRH has been demonstrated to be quite effective in inducing androgenization and spermatogenesis in men with IHH. It did not differ in efficacy in terms of spermatogenesis and pregnancy rates as compared to the gonadotropin therapy. In preliminary investigations involving infertile men who had cryptorchidism, GnRH analogues have been shown to improve spermatogenesis when used as an adjunct to
orchidopexy. On the basis of successful treatment of one single case, Iwamoto et al. concluded that nasal therapy with GnRH analogue buserelin in low doses avoids pituitary down-regulation exerting stimulatory effect on it and therefore may be an effective and well-tolerated therapeutic option for patients with HH of hypothalamic origin.

Side effects and disadvantages: Wearing of the portable pump is cumbersome and hence discouraging for patients. Formation of anti-GnRH antibodies in certain cases has also raised some concern. Furthermore, at present consensus exists that GnRH has no role as empiric therapy in idiopathic infertility.

Gonadotropins
Various urinary, purified, and recombinant forms of gonadotropins have been used including hCG with LH activity, human menopausal gonadotropin [(hMG) FSH analogue], recombinant FSH and LH.

Mixed Gonadotropin Therapy
Rationale: In all forms of hypogonadism testosterone one is sufficient for maturation and maintenance of secondary sex characteristics, libido and erectile function. In HH; however, the anterior pituitary hormones LH and FSH are required together to initiate and maintain spermatogenesis. A combined gonadotropin therapy can thus be used to treat hypogonadotropic infertility arising at the level of pituitary or hypothalamus including IHH, when treatment with GnRH is not desired or indicated. hCG is used as the source of LH activity to stimulate testosterone secretion by Leydig cells, whereas hMG acts as FSH. In recent years, recombinant gonadotropins have been introduced in clinical practice.

Method of administration: The therapy is started with hCG 1,000–2,500 IU two times/week subcutaneously or intramuscularly; adjusting the dose to target mid-normal testosterone levels. Testosterone levels are measured 48 hours after the hCG injection. Alternatively, recombinant LH can be used. After a period of 8–12 weeks of hCG or recombinant human LH therapy, a highly purified hMG or recombinant human FSH is added at the doses of 150–225 IU three times/week subcutaneously. The treatment continues until sperm appear in the ejaculate or pregnancy occurs respectively, but in some cases therapy may be required for 1–2 or more years.

As soon as in men with HH spermatogenesis is induced with combined gonadotropin treatment or with GnRH, it can be maintained qualitatively by hCG alone for long time, but the decreasing sperm counts indicate that FSH is necessary for maintenance of quantitatively normal spermatogenesis.

**Indications:** Several studies Although not placebo controlled have shown induction of spermatogenesis and ability to induce pregnancy with use of mixed gonadotropin therapy and it is presently the most widely used therapy for hypogonadotropic infertility. Testicular volumes of 8 ml or more and post-pubertal onset of gonadotropin deficiency are more likely to respond than those with testicular volumes of less than 4 ml and pre-pubertal onset. Nevertheless, this treatment is also indicated in cases with cryptorchidism or with small testicular volume. According to a recent study men with BMI less than 30 kg/m² have a greater chance of achieving spermatogenesis than men with a BMI equal to or greater than 30 kg/m². Low BMI and advanced sexual maturity, especially large baseline mean testicular volume are predictors of a good response to combined therapy with recombinant human FSH and hCG. Recent observations has demonstrated useful effect of hCG therapy in improvement of sperm retrieval rates in men with Klinefelter’s syndrome as will be described later.

FSH Monotherapy
Rationale: FSH has an established role in promoting spermatogenesis. It enhances the production of androgen-binding protein by Sertoli cells which are required to maintain high local concentration of testosterone in the seminiferous tubules thus supporting spermatogenesis. However, the role of FSH in the maintenance of spermatogenesis remains controversial.

**Method of administration:** Purified or recombinant human FSH is given at doses ranging from 50 IU to 300 IU administered subcutaneously threetimes weekly for over 3 months.

**Indications:** Several randomized controlled trials have evaluated the efficacy of FSH in men with idiopathic infertility with mixed results. In these studies, the gonadotrophic status of the patients was not well...
characterized. Although pregnancy outcomes were not reported in most of these studies, improvement in sperm parameters was noted in some when FSH was used at higher doses.48,63 When used 50 days before ICSI, FSH has been shown to improve fertilization, implantation and pregnancy rates in men with severe oligozoospermia.64

**Side effects and disadvantages:** Evidence is weak and the consensus is that FSH therapy alone has at the best little efficacy in treating idiopathic male infertility.

**Androgen Therapy**

**Testosterone Therapy**

**Rationale:** Although testosterone has contraceptive properties in men due to its negative feedback on hypothalamic-pituitary axis and thus inhibition of LH and FSH, and spermatogenesis respectively. It has been tried to treat subfertile men with testosterone based on two rationales. Raising serum testosterone would improve epididymal maturation of spermatozoa; gonadotropins and sperm concentration respectively increase transiently upon sudden stopping of testosterone, the so-called ‘rebound effect’.

**Method of administration:** Male infertility is treated using testosterone undecanoate or mesterolone in doses of 120–240 mg/day and 75–150 mg/day respectively.

**Indication:** Various meta-analyses have demonstrated no improvement in pregnancy outcomes with androgen therapy in idiopathic male infertility.65,66

**Side effects and disadvantages:** Published literature strongly discourages any role of testosterone monotherapy for men with idiopathic infertility.

**Anti-estrogen Therapy**

**Anti-estrogen Monotherapy**

**Rationale:** Anti-estrogens indirectly stimulate the secretion of GnRH, FSH and LH by binding to ERα in the hypothalamus and pituitary, thereby blocking estrogen feedback inhibition. The resultant increase of gonadotropin concentration is believed to improve the gametogenic function of the testes.

**Method of administration:** The two most commonly used nonsteroidal anti-estrogens are clomiphene citrate and tamoxifen. Clomiphene citrate is usually prescribed in doses of 12.5-50 mg/day either continuously or on a 25-day cycle with a 5-day rest period, each month for 3-6 months. Tamoxifen is administered at a dosage of 10-20 mg daily over a period of 3-6 months.

**Indication:** Cochrane meta-analysis of 10 randomized controlled trials with idiopathic infertility found no improvement in pregnancy rates with anti-estrogen therapy.67 Similarly, another meta-analysis reported no significant change in pregnancy outcomes with clomiphene citrate or tamoxifen therapy of idiopathic infertile men (OR, 1.54; 95% CI: 0.99-2.40).65 However, some studies demonstrated improvement in sperm count and sperm motility.68 Hence, empiric therapy for at least 3 months may have a beneficial effect on fertility status in subfertile men by improving semen parameters which may allow a down-staging of the required ART procedure, i.e. utilizing intrauterine insemination instead of ICSI. Recent reports have shown specific indication and beneficial effect of clomiphene citrate and its isomer enclomiphene in treatment of men with HH and restoration of physiological level of testosterone. Clomiphene citrate can also restore testosterone/estrogen ratio in hypogonadal men.69,70-72 Lastly, men with Klinefelter’s syndrome who have been prescribed clomiphene citrate show an improvement in sperm retrieval rates by testicular extraction as demonstrated below.

**Side effects and disadvantages:** Literature support remains inconclusive awaiting large randomized prospective trials of empiric therapy in idiopathic male infertility. Unfortunately, deterioration in semen parameters of some men with idiopathic male infertility has been reported after using clomiphene citrate. For replacement of testosterone in men with HH, two regimens have been described either low dose daily regimen of 25 mg69 or 50 mg given three times a week.71

**Tamoxifen and Testosterone Combination Therapy**

**Rationale:** Tamoxifen has been shown to primarily increase sperm density without much improvement in other parameters, such as sperm motility and morphology. One of the main reasons could be inferior androgenic environment in the reproductive tract of oligozoospermic men. This in turn may compromise epididymal maturation of the spermatozoa which can be theoretically overcome by supplementing tamoxifen treatment with testosterone.

**Method of administration:** Tamoxifen and testosterone undecanoate are administered as 20 mg and 120 mg respectively in daily divided doses for 6 months.

**Indication:** Treatment with tamoxifen and testosterone undecanoate improved sperm variables and led to a higher incidence of pregnancy in couples with subfertility related to idiopathic oligozoospermia.73
Section 2 Male Factor Infertility

Side effects and disadvantages: Literature is scarce and primarily restricted to single group of investigators.

Tamoxifen and Kallikrein Combination Therapy

Rationale: While tamoxifen improves sperm count, kallikrein has been shown to improve sperm motility; hence a combination can hypothetically be useful in men with idiopathic oligoasthenozoospermia.

Method of administration: Tamoxifen is administered as 20 mg/day along with 600 IU of kallikrein daily for 3 months.

Indication: Improvement in both sperm count and motility with such a therapy has been demonstrated in few trials when used in idiopathic normogonadotropic men with oligoasthenozoospermia.

Side effects and disadvantages: Pregnancy outcomes have not yet been studied and further studies are warranted to draw any inferences.

Therapy with Aromatase Inhibitors

Rationale: Aromatase is a P450 cytochrome enzyme that converts androgens to estrogens. Aromatase inhibitors block its activity thereby reducing serum estradiol concentration and its negative feedback on the hypothalamus and pituitary, resulting in elevated serum FSH levels, which in turn, might improve spermatogenesis. On the other hand, aromatase inhibitors lead to increase in testosterone which also might contribute to achievement of fertility.

Method of administration: Two types of aromatase inhibitors are available, steroidal (e.g. testolactone) and nonsteroidal (letrozole and anastrozole). The latter is more effective in increasing testosterone to estrogen ratio and is less likely to cause interruption of the adrenal axis beyond aromatase inhibition. Testolactone is given in doses of 100–200 mg/day, whereas anastrozole is used as 1 mg/day dosage and letrozole 2.5 mg daily orally for 4–6 months.

Indication: Hyperestrogenemia associated with male infertility constitutes the main indication for the use of aromatase inhibitors therapy. Elevated estrogen level or elevated estrogen to testosterone ratio is obviously observed in men with significant obesity associated with idiopathic HH and Klinefelter’s syndrome.

In idiopathic oligozoospermic men studied in double blind randomized controlled fashion, testolactone therapy failed to show any improvement in semen parameters. Normal spermatogenesis proven by testis biopsy was achieved with letrozole in one case with azoospermia and normal FSH serum levels. Controlled studies evaluating the efficacy of aromatase inhibitors on pregnancy outcomes are still lacking. Furthermore, Ramasamy et al. (2009) indicated that medications leading to physiological endogenous testosterone secretion, such as aromatase inhibitors, clomiphene or hCG result in a better chance of sperm retrieval in men with Klinefelter’s syndrome with either normal or low baseline testosterone than those Klinefelter’s men who used exogenous testosterone to induce secondary sexual characteristics ([77% vs. 55%]) Level C evidence]. Such observation may be attributed to exogenous testosterone induced suppression of release of pituitary FSH and LH and henceforth suppression of spermatogenesis.

Side effects and disadvantages: Further investigation is needed before drawing any conclusions on the use of aromatase inhibitors in male infertility. Elevation of hepatic enzymes has been reported with both these drugs and hence caution is advised in those who have underlying liver disease.

Growth Hormone Therapy

Rationale: GH acts on gonads directly or through hepatic secreted insulin-like growth factor-1 and plays a significant role in sexual growth and differentiation, gonadal steroidogenesis and gametogenesis.

Method of administration: Recombinant GH is given for 12 weeks.

Indication: GH therapy has shown mixed results in terms of improvement in sperm parameters and pregnancy rate when used in oligo- and asthenozoospermic men. When used as an adjunct therapy in a small study of seven men with HH who failed gonadotropin therapy, GH has been demonstrated to help induce spermatogenesis.

Side effects and disadvantages: Available literature is limited and further studies trying combination therapy of GH and gonadotropins for male infertility are awaited.

Oxytocin Therapy

Rationale: Oxytocin has been shown to promote sperm progression through the reproductive tract by improving epididymal contractility. It can thus be used to increase sperm retrieval in men with oligozoospermia.
Method of administration: Oxytocin is given as intravenous injections or intranasal just before ejaculation.

Indication: Oxytocin therapy has failed to improve sperm output in severely oligozoospermic men.82

Side effects and disadvantages: This form of therapy lacks evidence in supporting any role in male infertility.

Endocrine Therapy for Hyperprolactinemia

Dopamine agonists are the primary therapy for both micro- and macroadenomas associated hyperprolactinemia, as well as for non-tumor related hyperprolactinemia. Historically, bromocriptine (2.5–10.0 mg, maximal 30 mg a day) was the first effective medical therapy. Cabergoline, a non-ergot DA agonist, is administered at a dosage of 0.25–1.0 mg twice per week. Both normalize PRL levels, decrease tumor size and restore reproductive function. Cabergoline is better tolerated than bromocriptine. However, there are some recent concerns of heart valvular defects with higher doses of cabergoline.83

Summary: Therapy for subfertile men generally falls under two categories: specific and empiric, depending upon the etiology. Endocrine evaluation of men presenting with infertility should aim at identifying candidates for specific therapy. For men who have HH, both gonadotropin therapy and pulsatile GnRH are equally effective. However, idiopathic infertility which is a much more frequently encountered clinical problem responds poorly to empiric endocrine treatment. Recently, several hormonal intervention studies have shown promise in management of idiopathic male infertility. Further trials with adequate sample size and study design are warranted before it can be put into routine clinical practice.

CYTOPROTECTIVE HORMONAL EFFECTS ON SPERM IN CANCER PATIENTS

Hormonal manipulation inducing a state of quiescence may protect the testicular germ cells from being heavily damaged by exposure to gonadotoxins, such as cancer chemotherapy, or radiotherapy. This theory stems from the observation of relative resistance of prepubertal testes to cancer chemotherapy and animal studies in which GnRH agonists or antagonists were given to suppress spermatogenesis.

In United States 17,000 men in reproductive ages between 15 years and 45 years are diagnosed with cancer, such as Hodgkin’s lymphoma, leukemia, testicular cancer and soft tissue sarcoma each year.84,85 Of these, more than 3,000 patients receive treatment, such as alkylating agents, platinum based chemotherapy or radiation which may render men azoospermic or severe oligozoospermic.84,85 Cytotoxic chemotherapy may also be used in the treatment of autoimmune diseases. The mechanisms of deranged spermatogenesis include direct stem cell aplasia or failure of differentiation of surviving stem cells into mature sperm. Restoration of male fertility is very slow and may take up to 6 years. Endocrine alterations, described in these men, particularly with germ cell aplasia, may include a decreased level of serum inhibin and consequently elevated serum FSH level. Chemotherapy or radiotherapy induced testicular ischemia contributes to decline in the systemic distribution of testosterone, elevated LH level and increased intratesticulal testosterone.86,87 Ultimately, successful resumption of spermatogenesis depends on survival of stem cells and their ability to differentiate into sperm.

Various hormonal regimens have been used for protection of testicular tissues during chemo- or radio-therapy. In animals, GnRH, FSH and steroids were administered, whereas in humans GnRH agonists alone or in combination with testosterone or antiandrogen, testosterone and medroxyprogesterone have been used with mixed results. Animal studies have shown protective effect of GnRH given for several weeks (upto 6 weeks) before cytotoxic chemotherapy and continued thereafter or through administration of GnRH after irradiation or chemotherapy to stimulate spermatogenesis.86,88 Only single small study out of seven trials in humans has shown such promising improvement by the use of testosterone, concomitantly with cyclophosphamide for the treatment of autoimmune nephritis.99 Nevertheless, further studies are actually required and prolonged pretreatment with hormonal therapy for extended period of time for several weeks should be utilized to show a protective effect.

Other options for fertility preservation encompass sperm or testicular tissue cryopreservation. The transplantation of cryopreserved spermatogonia is being studied and preliminary animal studies have shown beneficial effects of GnRH analogues in maintenance of the survival of transplanted spermatogonia.90,91

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