Physiology and Endocrinology of Spermatogenesis

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2.1 Introduction

Spermatogenesis is the process by which the primordial germ cells, called the spermatogonial stem cells, transform into highly specialized mature spermatozoa. This is a complex process which takes place in the male gonad (Testis) and involves several steps. Spermatogenesis is controlled by several endocrine and paracrine/autocrine testicular factors (Mahmoud and Eitan 2004). Most of the current knowledge on the exact sequence of mammalian spermatogenesis and its regulation emerge from studies on rodent models. Though the basic sequence of spermatogenesis remains the same among mammals, the number and type of cells, their relationship with each other, and the duration of different stages vary greatly among the species (Kerr and De Kretser 2010). Differences in the life expectancy and offspring number play a major role in the differences seen in spermatogenesis among different species, i.e., primates and rodents (Ehmcke et al. 2006).

2.2 Testis

Testis, the male gonad, subserves two important functions in an adult male: (1) production of male sex hormones, specifically testosterone, and (2) production of the male gamete, the spermatozoa. Gametogenesis or spermatogenesis occurs in the seminiferous tubules which occupies the bulk of the testicular volume. These

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tubules are separated by intertubular space which contains Leydig cells, blood vessels, nerves, and lymphatics.

2.3 Stages of Spermatogenesis

The basic steps of spermatogenesis include (1) generation of daughter cells which can transfer the genetic material to the next generation through the process of reproduction and (2) maintenance of a self-renewable germ cell line for sustaining the abovementioned process (Joachim et al. 2007). In order to fulfill both the aims, the germ cells undergo two types of cell division: mitosis and meiosis. The entire process occurs in a specialized compartment of testis called "seminiferous tubules." The spermatogenic process is divided into four important steps:

1. The proliferation and differentiation of spermatogonia into spermatocytes
2. Meiotic division of spermatocytes to spermatids
3. Spermiogenesis – the process of transformation of round spermatids to elongated spermatozoa
4. Spermiation – release of spermatozoa into the lumen of seminiferous tubules

2.3.1 Seminiferous Tubules

Seminiferous tubules constitute around 60 % of the testicular volume indicating their importance in determining the testicular size. There is an ethnic difference in the testicular weights with Hispanic men having greater testicular weight compared to White and Chinese men. This difference is due to the contribution of seminiferous tubules to the testicular volume. The tubule length varies between 450 and 600 m in different ethnic groups. The tubules are divided into basal and luminal compartments and are supported by layers of myofibroblasts. Seminiferous epithelium contains two types of cells: (1) Sertoli cells (SC) and (2) germ cells (GC) (Kerr and De Kretser 2010).

2.3.2 Sertoli Cells

Sertoli cells are somatic cells present in the seminiferous tubules and they constitute 25 % of the tubular cell population. SC increase in number till approximately 15 years of age beyond which their numbers remain constant. They are the supporting cells for the proliferating germ cell population. Each SC supports a defined number of germ cells (Griseold 1995). SC not only provides structural support to germ cells but also give nutritional and immunological support to them. They are the most important paracrine mechanism of spermatogenic control. Structurally, Sertoli cells are tall columnar cells extending from the basement membrane of seminiferous

epithelium. Along with several proteins, they form a physiological barrier for the germ cells called the "blood-testis barrier" (BTB) (discussed below). SC have receptors for gonadotropins (FSH and LH) and testosterone. The important functions of SC include:

1. Providing nutrition to germ cells
2. Maintenance of integrity of seminiferous epithelium
3. Secretion two important hormones – anti-Mullerian hormone (AMH) and inhibin.
4. Production of various factors like androgen-binding protein, plasminogen activator, lactate, transport and functional proteins like transferrin and ceruloplasmin, growth factors, etc.
5. Facilitating spermiation

2.3.3 Blood-Testis Barrier

As mentioned above, the BTB is formed by SC and also by several component proteins like actin filaments, actin-binding proteins, and cisternae of endoplasmic reticulum. Structurally BTB divides seminiferous tubule into basal compartment and ad-luminal compartment. Functionally, it divides the pre-leptotene/leptotene spermatocyte from postmeiotic germ cells. The main purpose of the BTB include (1) maintenance of immunological barrier for developing germ cells to prevent autoimmune orchitis and (2) providing a suitable intratubular milieu for effective germ cell development as against a blood milieu in the intertubular space. While the tight junctions of BTB prevent the exposure of postmeiotic germ cell antigens to the immune system, the gap junctions facilitate the migration of pre-leptotene/leptotene spermatocytes to the ad-luminal compartment and also provide suitable hormonal milieu for gem cell development. The BTB is a dynamic barrier because of the constant restructuring process it undergoes in order to facilitate germ cell migration (Yan et al. 2008).

2.3.4 Spermatogenic Stages

As mentioned above, the spermatogenic cycle is a complex process by which the spermatogonia differentiates into mature spermatozoa. Spermatogonia are derived from primordial germ cells which migrate into the testis during fetal life. They transform into gonocytes which are capable of multiplication by the process of mitosis. After a period of multiplication, they migrate to the basement membrane of the seminiferous epithelium and are called spermatogonia (de Kretser et al. 1998). Based on the morphology, different types of spermatogonia can be identified in the seminiferous tubules. They are pale type A spermatogonia (Ap) containing pale staining nuclei, dark type A spermatogonia (Ad) containing deeply staining, homogenous nuclei, and type B spermatogonia.

Type A spermatogonia form the pool of self-renewable stem cells whereas type B spermatogonia enter into the process of meiosis and are the precursors of spermatocytes. Spermatogonia undergo repeated mitotic divisions to produce daughter cells but these divisions are incomplete. Hence, the cells are interconnected to one another through cytoplasmic bridges. The connection persists throughout the subsequent stages of spermatogenesis until the mature spermatids are released into the lumen of seminiferous tubules. This helps in synchronization of germ cell maturation (Kierszenbaum 2002).

2.3.4.1 Spermatocytes

Type B spermatogonia attach to the basal compartment of the seminiferous epithelium and undergo the process of meiosis to form the primary spermatocyte. Meiosis starts with DNA synthesis of type B spermatogonia which results in the duplication of the chromosomes. Thus, each chromosome contain two chromatids and hence the DNA content of primary spermatocyte is tetraploid (4C). Meiosis is subdivided into (1) prophase, (2) metaphase, (3) anaphase, and finally (4) telophase. Two successive meiotic divisions occur which result in the synthesis of four spermatids from each primary spermatocyte. Prophase is a lengthy process which takes around 1–3 weeks and is further subdivided into (1) leptotene, (2) zygotene, (3) pachytene, (4) diplotene, and finally (5) diakinesis. Leptotene stage of the prophase starts in the basal compartment and further steps are continued after the spermatocytes cross the blood-testis barrier to reach the ad-luminal compartment. The primary spermatocyte undergoes first meiotic division to form secondary spermatocyte which in turn becomes round spermatid through the second meiotic division.

2.3.4.2 Spermiogenesis

The round spermatids become elongated spermatozoa which attains motility. This is accomplished through the following events:

1. Acrosome formation
2. Nuclear condensation
3. Reduction of cytoplasm
4. Formation of tail
5. Reorganization of cellular organelles.

The detailed discussions of steps of spermatogenesis are seen elsewhere.

2.4 Neuroendocrine Regulation of Spermatogenesis

The complex process of spermatogenesis is regulated by two major mechanisms:

1. Extrinsic or neurohormonal regulation
2. Intrinsic or autocrine/paracrine regulation

2.4.1 Extrinsic Regulation

Neuroendocrine regulation of the testis is often termed as "hypothalamo-pituitary-testicular axis." This axis is mainly controlled by the gonadotrophin-releasing hormone (GnRH).

2.4.1.1 Gonadotrophin-Releasing Hormone

GnRH is a decapeptide which is secreted and released from the hypothalamic neurons. It is the major regulator of secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the gonadotrophs of the anterior pituitary. GnRH is secreted in pulsatile manner and each pulse of GnRH is accompanied by a simultaneous pulsatile secretion of LH. The pulses of GnRH are controlled by "GnRH pulse generator" which is under the influence of several neurotransmitters, the most important among them called as "kisspeptin."

2.4.1.2 Kisspeptin

Kisspeptin is a hypothalamic neuropeptide coded by the KiSS1 gene. Kisspeptin is a neuromodulator that acts upstream of GnRH. It is controlled by sex steroid feedback mechanism and is also responsive to metabolic signals. Kisspeptin is recognized as a crucial regulator of onset of puberty, gonadotrophin secretion, and fertility. It acts on the GPR-54 receptor located on the GnRH neurons and increases the GnRH pulsatility and consequently LH secretion. Activation of kisspeptin neurons triggers the onset of puberty in male and female by stimulating the "GnRH pulse generator." However, there is an anatomical and functional dimorphism in kisspeptin neuronal system between male and female, which explains the greater role of kisspeptin in different stages of menstrual cycle in female. Genetic mutations in KiSS1 gene are associated with hypothalamic form of hypogonadism in men (Skorupskaite and George 2014).

2.4.1.3 Gonadotrophins (LH and FSH)

LH and FSH belong the family of glycoprotein hormones with two subunits, the α subunit which is common for both and the β subunit which is unique and offers specificity. They are secreted by the gonadotrophs which constitute 7–10 % of the cells in the anterior pituitary gland. Gonadotrophins are secreted in pulsatile manner in response to GnRH secretion. Their secretion increases during neonatal period which helps in the gonadal development. After a few months of the neonatal gonadotrophin surge, their secretion reaches a nadir level until the onset of puberty. In adults, LH and FSH secretion remain stable in male but markedly vary in female depending on the reproductive cycle. The plasma half-life of FSH (120–240 min) is longer than that of LH (30–90 min). This difference is attributed to the higher sialic acid content in FSH compared to LH (Matsumoto and Bremner 2011).

2.4.1.4 FSH

The most important function of FSH in male is the stimulation of spermatogenesis. FSH acts through the FSH receptor present in the Sertoli cells of testis. FSH

receptor is a G-protein-coupled receptor with an extracellular hormone-binding domain (which binds to FSH), a seven helical transmembrane domain, and an intracellular domain. Binding of FSH to its receptor causes structural alteration in the seven membrane-spanning domains. This in turn stimulates the G proteins coupled with the receptor to activate adenylate cyclase which increases the intracellular cAMP levels. Apart from this mechanism, binding of FSH to FSH receptor triggers several intracellular pathways like protein kinase A (PKA) pathway, MAP kinase pathway, calcium pathway, and the phosphatidylinositol 3-kinase (PI3-K) pathway. Through these multipronged approach, FSH stimulates several transcription factors which are involved in germ cell proliferation, provides metabolic support to germ cells, helps in the transportation of glucose (which is converted to lactate to provide energy), and facilitates transferrin secretion which is vital for maintaining spermatogenesis (Walker and Cheng 2005). Secretion of FSH is regulated by two paracrine factors, activin and follistatin secreted from the pituitary and also inhibin B secreted from the Sertoli cells of testis (see later). These factors belong to the TGF-β family and their combined action determines the effective secretion of FSH. Men with inactivating mutations of FSH-β gene and FSH receptor are generally infertile with azoospermia or oligospermia, respectively (Matsumoto and Bremner 2011).

2.4.1.5 LH

The main function of LH in males is through its action on the Leydig cells of testis which produce testosterone, the most important male hormone. Leydig cells express receptors for LH on their surface and LH binds to these receptors. The downstream pathway is mediated through cAMP-dependent activation of PKA (similar to FSH action). In Leydig cells, this pathway leads to the production of proteins that regulate steroidogenesis and testosterone biosynthesis. Though Sertoli cells and germ cells do not have receptors for LH, spermatogenesis is indirectly regulated by LH through increasing the intratesticular testosterone concentration. Men with inactivating mutations of LH-β gene or LH receptor lack pubertal development with arrested spermatogenesis or azoospermia and infertility. This explains the role of intratesticular testosterone concentration in normal spermatogenic process (Matsumoto and Bremner 2011).

In short though FSH and LH (by increasing the intratesticular testosterone) help in spermatogenesis, their actions differ according to the stage of spermatogenesis. While FSH has greater effect on the maturation of spermatogonia, early meiosis, and conversion of spermatogonia to pachytene spermatocytes, LH has its predominant effect on the completion of meiosis and on spermiation. Both have similar effect on spermiogenesis (Matsumoto and Bremner 2011).

2.4.1.6 Other Factors of Extrinsic Regulation

Another neuromodulator which plays a major role in gonadotrophin secretion is leptin which is produced from peripheral adipose tissue. Leptin receptors are expressed

in GnRH-secreting neurons through which it positively regulates the gonadal axis. Leptin accelerates GnRH pulsatility and in turn stimulate gonadotrophins secretion. Environmental stress, through the secretion of corticotrophin-releasing hormone (CRH) and glucocorticoid, negatively regulates hypothalamo-pituitary-gonadal axis.

2.4.2 Intrinsic Regulation

The two important cells in human testis which are involved in the autocrine/paracrine regulation of spermatogenesis are (1) Leydig cells and (2) Sertoli cells.

2.4.2.1 Leydig Cells

Leydig cells constitute 10–20 % of the intertubular cell population. They are derived from the mesenchymal fibroblasts present in the intertubular space. They are the major source of testosterone synthesis from cholesterol which acts as the substrate. The steps in the steroidogenic pathway and their control are beyond the scope of this chapter. Testosterone, once synthesized from Leydig cells, diffuses into the systemic circulation. The concentration of intratesticular testosterone is several times greater than its systemic concentration. Especially, higher levels of testosterone are found near the basement membrane of the seminiferous tubules indicating its role in spermatogenesis (Kerr and De Kretser 2010).

2.4.2.2 Testosterone

Testosterone is the predominant male hormone secreted from the Leydig cells of testis. It is synthesized from cholesterol, which acts as its substrate, through the steriodogenic pathway, under the control of LH. After synthesis, it is secreted into the circulation, where it is either bound to albumin (54–68 %) and sex hormone-binding globulin (SHBG) (30–44 %) or present in unbound or (free) form (0.5–3 %). The biological actions of testosterone, including its effect on male sexual differentiation and maturation, development of secondary sexual characters, epiphysis growth, and metabolic actions, are mediated by the free form. Intratesticular testosterone concentration is 100–200-fold higher than its serum concentration. As androgen receptors are present in Sertoli cells, testosterone and consequently LH mediate their effect on spermatogenesis (discussed above) through Sertoli cells. Administration of LH as human chorionic gonadotropin (HCG) alone in hypogonadal men maintains spermatogenesis once it is initiated by the combination of FSH and HCG treatment. However, administration of testosterone in hypogonadal men suppresses sperm production by reducing the endogenous LH. This explains the role of LH (HCG) in increasing the intratesticular testosterone and thereby maintaining spermatogenesis. Exogenous testosterone administration in turn suppresses LH secretion by acting at the level of hypothalamus and pituitary. This completes the feedback inhibition loop of hypothalamo-pituitary-testicular axis. Testosterone is aromatized to estradiol by

the enzyme aromatase. Like testosterone, intratesticular level of estradiol is 100-fold higher than the serum level. Recent studies have thrown light on the importance of higher levels of estradiol in testis and its role in spermatogenesis (Matsumoto and Bremner 2011).

2.4.2.3 Estrogen

Estradiol (E2) is the most abundant and active form of estrogen in both male and female. E2 is derived in male by the peripheral aromatization of testosterone by the enzyme called aromatase. Aromatase enzyme is expressed widely in adipose tissue, testis, and brain. Estrogen exerts its genomic action through two major receptors – estrogen receptor 1 (ER1) and estrogen receptor 2 (ER2) which are located in the nucleus. The non-genomic actions of estrogen are mediated through nonclassical membrane-bound G-protein-coupled receptors called as GPR30/GPER. Recent studies have shown the presence of estrogen receptors (ER1, ER2, and GPR30/GPER) in human testicular Sertoli cells and germ cells (Lambard et al. 2004; Carreau et al. 2010). Also, GPER is found in hypothalamus. Through mouse models, it has been proven than estrogen plays a significant role in spermatogenesis. Estrogen modulates germ cell proliferation, differentiation, survival, and apoptosis. Estrogen also acts at the hypothalamic level to regulate the gonadotrophin secretion. Most of these actions are mediated through ER2 and GPER. The functional and clinical significance of these findings needs further detailed studies.

2.4.2.4 Inhibin B

Inhibins are glycoproteins belonging to TGF-β family of proteins. They are composed of α- subunit which is connected to either a β_A or β_B subunit to form inhibin A or inhibin B, respectively. Inhibin B is the predominant inhibin present in male. It is produced by the Sertoli cells in response to FSH stimulation. Inhibin B in turn controls FSH through negative feedback mechanism. Inhibin B is the marker of Sertoli cell function. It is present in childhood and levels rise during puberty to reach the adult range by mid puberty. In contrast to the prepubertal inhibin level which is exclusively indicative of Sertoli cell function, inhibin B production in adult men is dependent on the presence of certain germ cells in the seminiferous tubules, most likely involving the pachytene spermatocytes and early spermatids. While, prepubertal boys with cryptorchidism will have normal inhibin B levels (indicating intact Sertoli cells) when compared to those with congenital or acquired anorchia, adult men with early spermatogenic failure and Sertoli cell-only syndrome will have low inhibin B levels when compared to normal adult men or men with late spermatogenic failure. The physiology of inhibin B levels in adult men helps us to understand the role of germ cells in the control of Sertoli cell function (Andersson and Skakkebaek 2001) (Fig. 2.1).

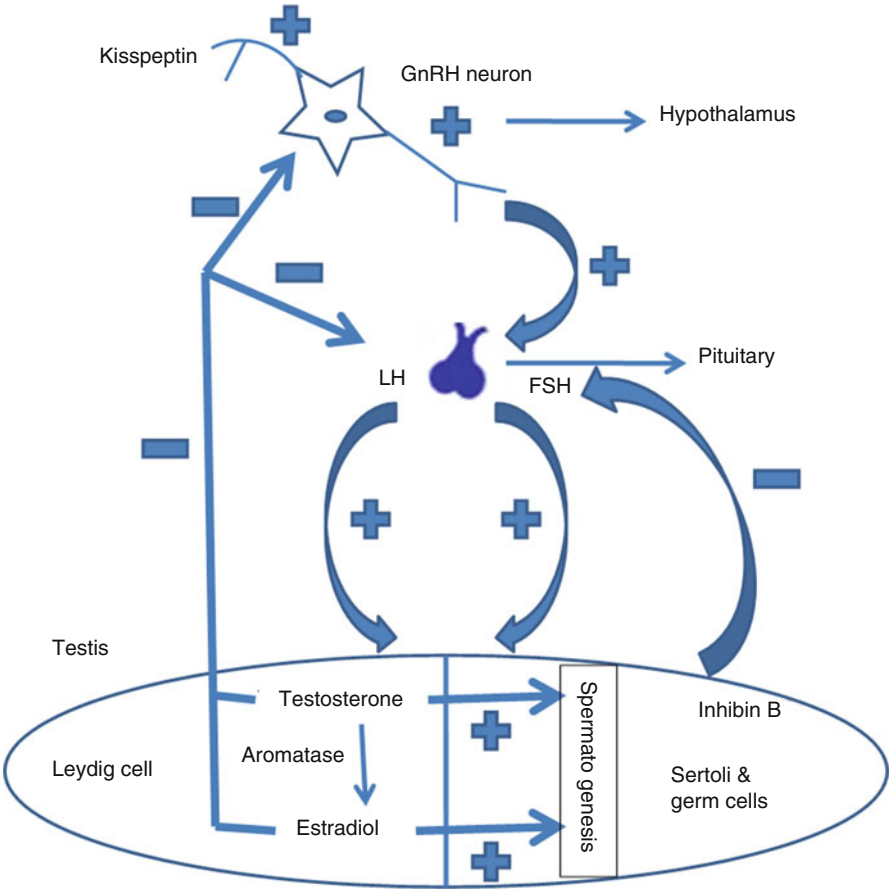

Fig. 2.1 *Hypothalamo-pituitary-testicular axis (graphical representation). GnRH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, – feedback inhibition, + stimulation

Conclusion

The process of spermatogenesis involves a complex interplay of several endocrine, paracrine, and autocrine factors. Endocrine regulation of spermatogenesis is finely controlled by the kisspeptin, gonadotropin-releasing hormone, the gonadotropins, and the testicular steroidogenic pathway. A thorough knowledge of the physiology is the key to successful management of patients suffering from disorders of spermatogenesis.

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