Regulation of Male and Female Reproductive Functions

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Abstract

Reproduction is an important biological process of species evolution, it is leading to new offspring from parents. The pituitary gland (the master gland) in the endocrine system in coordination with the hypothalamus plays a vital role in the reproductive system, differentiation, and different physiological functions in the entire stages of life and its circadian rhythm in both males and females. The chapter deals with the prominent role played by the brain, endocrine system, and gonads axis through complex communicating signals. The male gonads are the location of testicles, interstitial tissue (Leydig cells), and peritubular myoid cells. Sertoli cells act as "nurse and stem" cells, spermatogenesis, spermiogenesis were explained. Gonad's steroid hormones (Androgens) characteristics are specified in male reproductive activity, biological actions along with the regulator hormones (follicle-stimulating hormone, luteinizing hormone, and hypothalamic–pituitary–Leydig cell axis. Ovaries are female reproductive glands. The chapter describes the tissue zones of ovaries, puberty, and two main functions (exocrine and endocrine) controlled and coordinated by the hypothalamus and the pituitary. Female sex hormones in pre-puberty (Estrogen) and post-puberty (estradiol, estrone, progesterone, and inhibin), and sources (ovarian follicle and corpus luteum) were discussed in detail. Structure of steroid hormones discussed with the role of the endometrium, regulation of ovarian functions, and puberty by endocrine and immune system. The different phases of the ovarian cycle are explained to regulate gonadotropins, follicular growth, steroid synthesis, non-functional corpus albicans (infertile ovum), and regulation of the ovarian

cycle (pre-ovulatory phase, ovulation phase, and post-ovulatory phase). The involvement of estradiol metabolites, enzyme aromatase, the hormone oxytocin, matrix metalloprotease, cytokines, and vasoconstriction in corpus luteum formation is related along with maintenance and regression. Synthesis of ovarian hormones (β-estradiol, estrone, and estriol) after puberty discussed with important functions of progesterone, regulatory roles of inhibin, activin, and follistatin in the physiology of testis and ovary. Dehydroepiandrosterone (DHEA) involvement was discussed for menopause in elderly women and andropause in men. The chapter declares that the classic theory of cessation of oocytes production after birth was canceled. Both human neonatal and adult ovarian germline stem-cell precursors (ovarian surface cells) have the capability for oogenic/differentiating and producing functional oocytes, so it renews the oocyte pool (neo-oogenesis) and ensures renewal during the prime reproductive period, with follicular cooperation under the regulation of the endocrine, immune systems, and cellular support. After the prime reproductive period, aging starts, and menopause occurs because of the immunoregulatory changes that cause cessation and terminate neo-oogenesis and follicular renewal in vivo despite the existence of germline stem cell precursors. The rest of the oocytes in the primordial follicles retain ovarian function but advancing age (aging oocytes) correlates positively with the occurrence of fetal chromosomal abnormality. This chapter discusses the topics related to regulation of male and female reproductive functions.

Keywords

Follicle-stimulating hormone · Luteinizing hormone · Ovary · Testis · Reproductive hormone · Stem cell

Abbreviations

16-keto-E2	16-Keto-estradiol
2N	Diploid
2-ME2	2-Methoxyestradiol
4-OHE1	4-Hydroxyestrone
ACTH	Adrenocorticotropic hormone
AMH	Anti-Müllerian hormone
BMP	Bone morphogenetic protein
BTB	Blood–testis barrier
Ca^{2+}	Ionic calcium
Ca^{2+}/CaMK	Ca^{2+}/calmodulin-dependent protein kinase
CaM	Calcium-modulated protein
CRH	Corticotropin-releasing hormone
DFF	Dominant follicular fluid
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
FSH	Follicle-stimulating hormone

FSHRH	Follicle-stimulating hormone-releasing hormone
FSTL1	Follistatin-related protein 1
GDF	Growth differentiation factor
GDF-9	Growth differentiation factor-9
GMP	Guanosine monophosphate
GnIH	Gonadotropin-inhibitory hormone
GnRH	Gonadotropin-releasing hormone
H&E	Hematoxylin and eosin stain
hCG	Human chorionic gonadotropin
ICSH	Interstitial cell–stimulating hormone
IGF-1	Insulin-like growth factor 1
IGF-2	Insulin-like growth factor 2
IGFBP-2	Insulin-like growth factor binding protein 2
IGFBP-3	Insulin-like growth factor binding protein 3
LH	Luteinizing hormone
LHRH	Luteinizing hormone-releasing hormone
mRNA	Messenger ribonucleic acid
N	Haploid
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
TGF	Transforming growth factor
TGF-beta	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha

9.1 Introduction

Reproduction refers to multiple biological processes that maintain the survival of humankind and all living creatures, by producing new individual "offspring" from their parents. Reproduction is a prominent biological feature. The endocrine system, especially the pituitary gland in coordination with the neuroendocrine gland in the central nervous system, the hypothalamus, controls and organizes the reproductive system growth, differentiation, physiological functions and its circadian rhythm in males and females, throughout life, even in the menopausal or andropause phase.

Fully integrative hormonal and neurotransmitter signals act through several systems to produce a synchronized physiological result. The human reproductive function is regulated by a network of complex communication systems including many organs and tissues such as the brain, endocrine system and gonads by circulation and local signals. The hypothalamic-pituitary-gonadal (testicular/ovarian) axis plays a key role in regulating this biological feature with other endocrine, paracrine, and autocrine signals in a very exquisite harmony. The functional paracrine and autocrine signals and mechanisms in the gonads have been recommended as

therapeutic targets for the infertility cases in both females and males (Rudolph et al. 2016a, b).

9.2 Gonad Hormones and Male Reproduction

9.2.1 Testes Structure

The testes are a pair of loose, oval-shaped glands measuring; length 3.6–5 cm, width 2.5–3 cm, and weight 10–45 g. The size varies according the mammal's species (Fig. 9.1).

9.2.2 External Location of Tests

Each testicular gland is surrounded by two fibrous membranes. In order to provide ideal conditions for spermatogenesis, testicular/scrotal thermoregulation is a very important complex process controlled by various local mechanisms within the testes. Spermatogenesis in humans and mammals is a serial biological process that occurs within the seminiferous tubules of the testes. This process involves a controlled

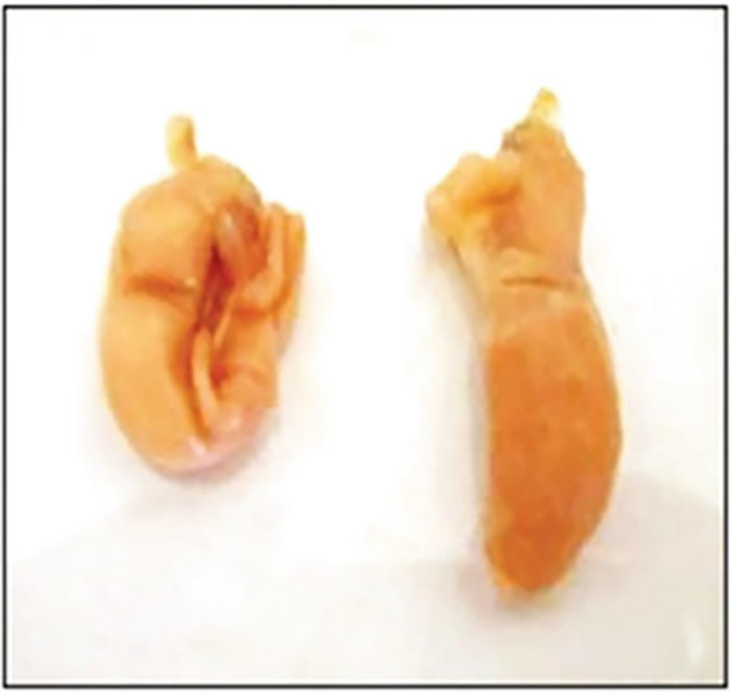

Fig. 9.1 Morphology of the testes in rabbits (Fixed in 10% formalin)

balance of cell proliferation, differentiation, transformation, and apoptosis. For these precise processes, the testes are located outside the body cavity in the scrotum, at a temperature 2–7 °C lower than temperature of the core body, and with a circulation that is not affected by body changes (Reyes et al. 2012). Many studies have reported the damaging effects of heat on the testes' weight, endocrinology, physiology, histology, and biochemistry, also on sperm count, motility, fertilizing ability, and on the growth of embryos produced if sperms from heated testes fertilize normal ova and the possibility of abnormalities in male reproduction in humans and animals. It was found that elevated testis temperature in humans, either due to occupational risks or clothes style may contribute significantly to infertility disorders (Mieusset and Bujan 1995; Parazzini et al. 1995; Thonneau et al. 1997). Heating method was considered as a potential contraceptive tool (Kandeel and Swerdloff 1988). Also, global heating may a potential reason of the decline of sperm counts in human (Setchell 1998).

9.2.3 Puberty

At the time of puberty, certain physical changes occur in human body along with functional changes including various sexual maturations ranging from gonadal differentiation during the first trimester of fetus growth to other changes evident at later stages.

In the second trimester of fetus growth, it is observed that FSH and LH levels are equivalent to those in adults while the levels are found to be lower during the last trimester. This could be because of the maternal and placental sex steroids that regulate negative feedback.

The concentration of serum FSH in infant girls may be similar to adults while the level of LH in such individuals elevates gradually to reach the lower levels in adults. In case of males, FSH levels as well as LH level show a gradual rise in childhood and early puberty.

The levels of FSH and LH are retained at a lower level by the endocrine system during the period from 2 years' age to puberty and the juvenile pause. This may be attributed to the suppression of melatonin secretion by the pineal gland. The pulse amplitude is specifically found to be higher at the time of puberty. Consequently, gonads are stimulated, and pituitary gland becomes more sensitive to GnRH stimulation (Copeland and Chernausek 2016).

In animals, the onset of male puberty is controlled by the timing of early pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Byrne et al. 2017). While in human, the onset of puberty is between the ages of 12 and 16 years, although this can vary from one geographical area in the world to another as a function of diet, growth, genetics, health, and environmental factors. These factors influence several metabolic and reproductive hormones which in turn influence endocrine functions of the hypothalamic–pituitary–testicular axis. Puberty is also influenced by the hormone melatonin as mentioned in Chap. 7. The signs of puberty may not appear until the age of 16 years (Copeland and Chernausek 2016).

In addition, puberty follows a sigmoidal growth curve, with a major variation testicular growth and adult testicular volume. The increase in Sertoli cells caused testicular growth early in puberty. Largest and fastest growth of testicular due to the increase in the diameter of the seminiferous tubules, first due to spermatogonial proliferation phase and then due to the expansion of meiotic and haploid germ cells. Moreover, FSH stimulates Sertoli cells and spermatogonial proliferation, whereas LH or testosterone is mandatory to complete spermatogenesis (Koskenniemi et al. 2017).

9.2.4 Physiological Functions of the Testicles

- Endocrine function: Synthesis of the sex hormones (steroidogenesis), as well as of inhibin, activin, and other hormones.
- Exocrine function: Spermatogenesis and spermiogenesis.

The parenchymatous tissue of the testis consists of two functionally interconnected components (Fig. 9.2). The two tissues are separated by partition tissue which includes the basal membrane (Al-Motabagani 2008; Burkitt et al. 1996; Young and Heath 2000).

9.2.4.1 Seminiferous Tubules

Each testicle is divided deeply by the tunica albuginea into small lobules. Each lobule contains multiple seminiferous tubules. The conical seminiferous tubules number around 250–300. These comprise 90% of the testicular volume and make up a total length of 400 m per testis. They are responsible for the production of around 30 million sperm every day, starting from puberty and continuing throughout life.

Sex Cells (Spermatogonia and Spermatogenesis)

Spermatogenesis occurs according to the following steps.

Two essential phases are required:

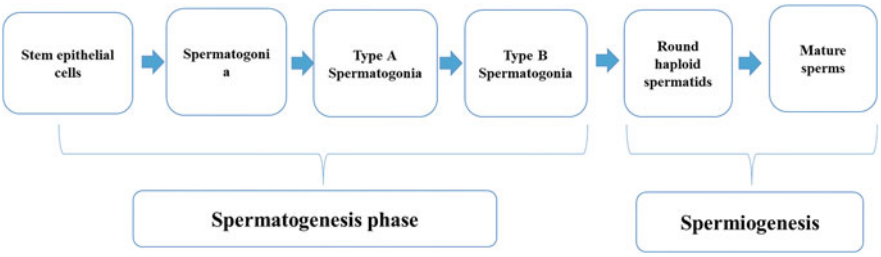

Fig. 9.2 Stages of spermatogenesis and spermiogenesis

Spermiogenesis and Spermiogenesis

- The process starts with the proliferative phase (spermatogonia): Type A spermatogonia, present and in contact with the basement membrane of the seminiferous tubules start to differentiate. Once spermatogonia enter into the process of spermatogenesis, they change from stem cells into germ cells ("progenitor cells") (Fig. 9.5).
- Mitotic phase: After enlargement, spermatogonia develop into type B spermatogonia which undergo one last division by mitosis to produce two primary spermatocytes (2n) (spermatocyte I) and then secondary spermatocytes (1n) (spermatocyte II).

Spermiogenesis

- The day-long process of conversion of a round spermatid into a sperm cell is called Spermiogenesis. The four processes involved in spermiogenesis are as follows:
- Nuclear condensation: In this process, the nucleus shrinks resulting in closely packed contents within nucleus depicting pear shape.
- Acrosome formation: In this process, a cap-like structure called acrosome is created by the Golgi apparatus on the anterior part of sperm cell. These created structures perform the same functions as performed by lysosome and these structures also contain lysosomal enzymes like hyaluronidase and acrosin which are responsible for the disruption of outer ovum membrane.
- Development of flagellum: In this process, the flagellum is produced by a centriole. The four main body parts of a flagellum are neck, middle piece, the main piece, and the tail. It consists of nine outer microtubules doublets as well as two single ones in the center. The flagella move with the help of energy obtained from the mitochondria within the middle piece.
- Cytoplasmic reduction: In this process, the Sertoli cells eliminate the extra and unneeded cytoplasmic components through Phagocytosis. At this point, the sperm cell is not able to swim. It becomes able to swim when it passes through the epididymis (O'Donnell 2015).
- In this phase round-shaped spermatids (**1n**) turn into mature sperms, the process which is free from cellular division. At the end of meiosis, the **haploid spermatids** change biochemically and morphologically without any cellular division, which leads to the formation of **mature sperm (spermatozoa)** (Sèdes et al. 2018; Guyton 1986; Guyton and Hall 2006, 2016). This is summarized in Fig. 9.2.

Somatic Cells of the Testes (Functions of Sertoli Cells and Peritubular)

Spermatogenesis, from the beginning of the formation of sperms to their secretion into the tubule cavity, takes approximately 64 days, but this can vary a great deal. Mature sperms are then transported through the epididymis to the ejaculatory duct over a period of 12–21 days.

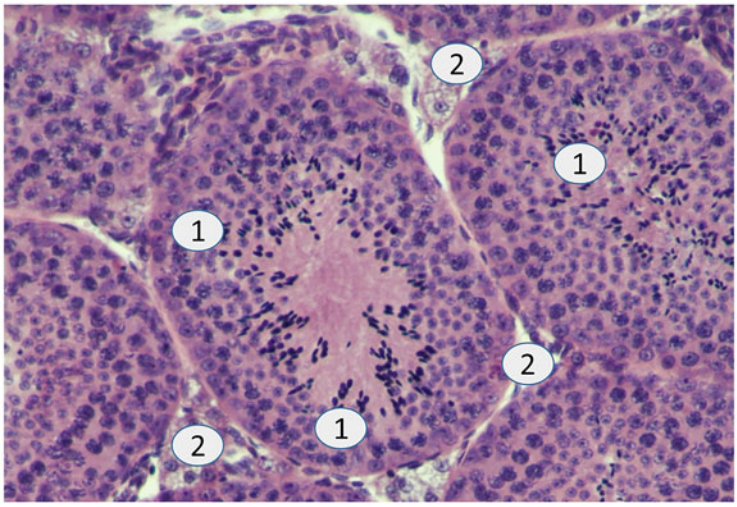

Fig. 9.3 Cross-section of the testis showing the normal structure of the seminiferous tubules in which cells in various stages of spermatogenesis can be seen (1) and the interstitial tissue (2) (H&E magnification 400×)

Stages of Spermatogenesis

Stages of spermatogenesis are explained as showed in Figs. 9.3, 9.4a, b, and 9.5.

The Seminiferous Tubules Contain Three Types of Cells

1. Sex cells (spermatogonia): These are the stem cells that differentiate into different cell lines during spermatogenesis.
2. Different cell lines necessary for the synthesis of spermatocytes and sperms.
3. Somatic cells of the testis, including
   (a) Large, elongated "nurse" cells called Sertoli cells (sustentacular cells) which are found in the seminiferous tubule between sections of spermatogonia. These are the only cells located in the basal membrane.
   (b) Peritubular myoid cells: These cells provide structural integrity to the seminiferous tubules. Thus, these somatic cells surround the seminiferous tubules within the testis. The cells are connected by junctional complexes similar to epithelial cells. Due to their structural function, the peritubular myoid cells contain cytoskeletal proteins, mainly abundant actin filaments, myosin, desmin, or vimentin and alpha-actinin.

Functions of Peritubular Myoid Cells

- They are contractile cells.
- They facilitate the transport of sperms and testicular fluid within the tubules.
- Peritubular myoid cell produce substances such as extracellular matrix components (fibronectin, types I and IV collagen, proteoglycans, and growth factors such as transforming growth factor beta (TGF-beta), insulin-like growth

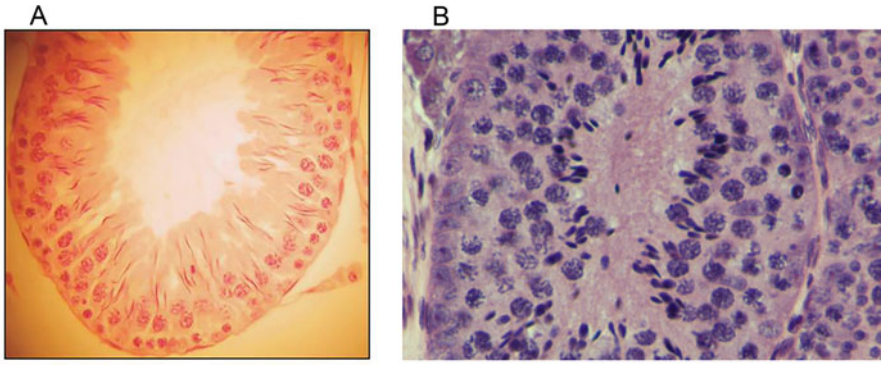

Fig. 9.4 (**a**) Cross-section of the testis: Microscopic structure of seminiferous tubule where spermatids can be seen (one of the stages of spermatogenesis) (H&E magnification 100×). (**b**) Cross-section of the testis: Microscopic structure of seminiferous tubule where spermatids can be seen (one of the stages of spermatogenesis) (H&E magnification 400×)

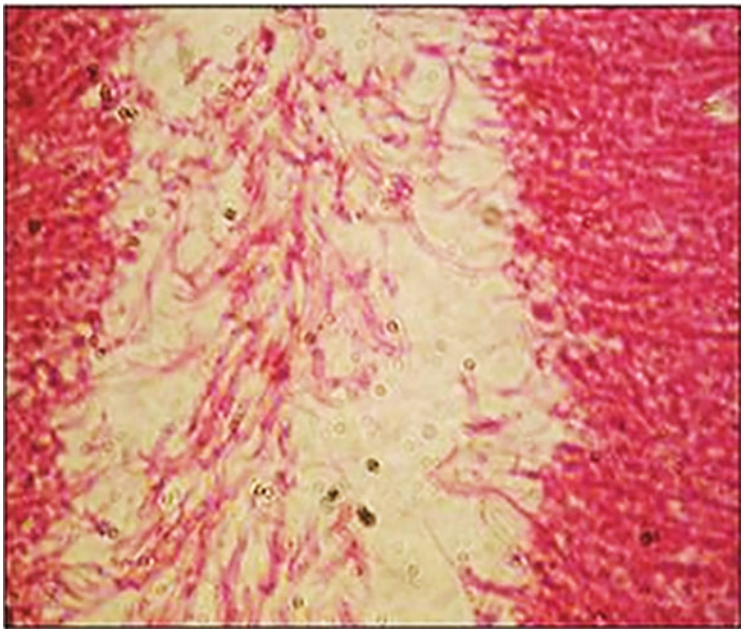

Fig. 9.5 Cross-section of the testis showing the presence of sperms inside the cavity of the seminiferous tubule (H&E magnification 400×)

factor, and activin A). As will be seen by the end of this chapter, these substances affect the functions of Sertoli and Leydig cells, and other hormones.

- Peritubular myoid cells express androgen receptors required in vitamin A/retinol processing.

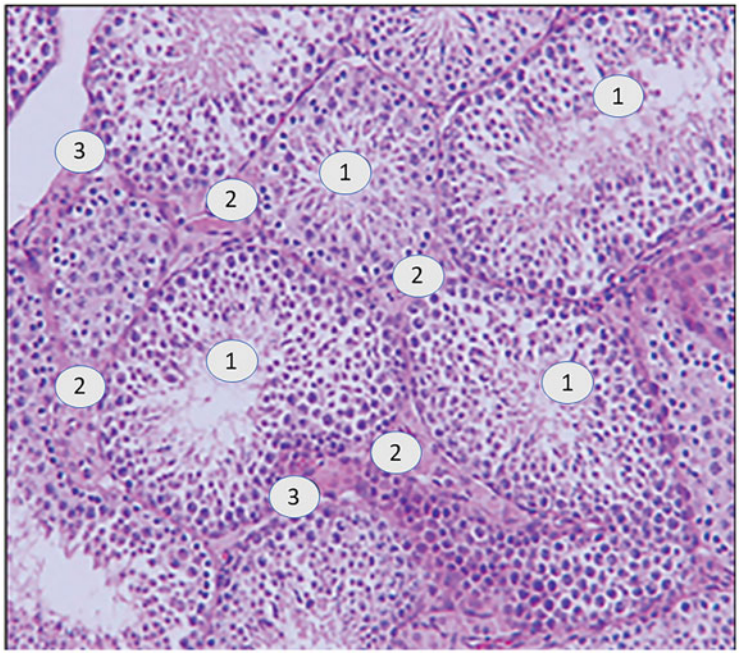

Fig. 9.6 Cross-section of the testes showing the normal structure of several seminiferous tubules (1) and the interstitial tissue between them (2) Peritubular myoid cells (3) (H&E magnification 10×)

- It is clear that peritubular myoid cells don't only provide integrity for the structure of the tubules, but also play a partly regulatory role in spermatogenesis and testicular function (Maekawa et al. 1996).

Functions of Sertoli Cells: The Nurse Cells

1. Sertoli cells act as sustentacular or "nurse" cells, and so are characterized by special properties proportional to their functions. Sertoli cells form Blood–Testis Barrier (BTB).
   - Sertoli cells are located on the basal membrane of the seminiferous tubules and fill the narrow spaces between nests of spermatogonia. Their functionally suitable structure extends between the basal membrane of the tubule and its antrum (Fig. 9.6). The Blood–Testis Barrier (BTB) consists of a unique type of tight junctional complex between adjacent Sertoli cells in the basal seminiferous tubule's epithelium. These connections take the form of secure extensions, with the complexes lying in the direction of the basal membrane to divide the tubule into two portions, a basal portion and the antrum which contains the spermatids. This is a barrier that regulates the passage of nutrients and materials through and within the testis. BTB physically divides the meiotic spermatocytes from post-meiotic spermatids away from testicular blood

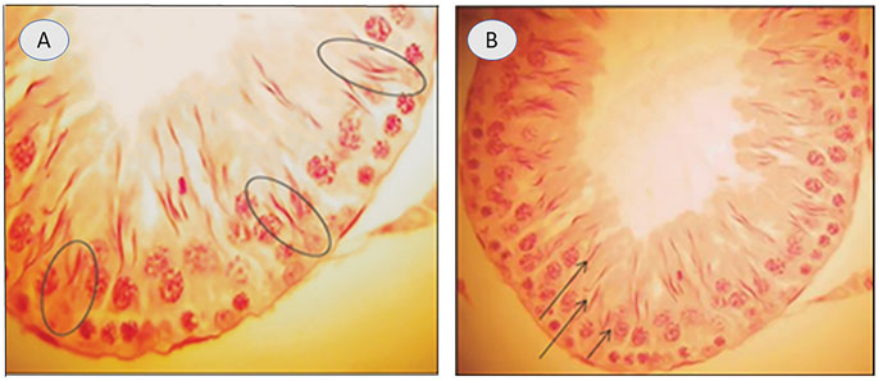

Fig. 9.7 Locations of stem cells within seminiferous tubule—Sertoli cells act as stem cell (**a**). Stem cells distributed equally across all seminiferous tubule stages (**b**). (H&E magnification 100×)

vessels, thereby preventing the autoimmune activity of the immune system against the immunogenic germ cells.

- Under the control of androgens, vitamin A compounds and thyroid hormones, Sertoli cells form the BTB at puberty, which regulates the passage of substances, drugs and toxic materials and prevents autoimmune disorders.
- Paracrine signals, such as the TGF-beta superfamily of cytokines (TGF-beta 3, activin A) and vitamin A (retinoid) signaling, act as potential factors for junction assembly and disassembly during the process of spermatocyte translocation.
- Endocrine and locally-produced testicular factors play a participatory role in the regulation of the BTB (De França et al. 1995; Willems et al. 2010; Tarulli et al. 2012; Stanton 2016).

2. Sertoli cells nuclei are located at the base while the cytoplasm is extensive, irregular, and constantly changing to allow sperms to move upwards to the surface of the tubule cavity (Fig. 9.7).
3. Sertoli cells act as "nurse" cells, and protective cells for developing spermatocytes by providing mechanical support, in addition to metabolic support by providing nutrition. Sertoli secretes a diversity of cytokines and immunosuppressive factors, to provide adequate nutritional supply and immune defense for sperm's development.
   - They secrete a fluid rich in certain ions that contribute to directing mature sperms in the right direction to the epididymis.
   - They are involved in the phagocytosis of excess cytoplasm released from the spermatids after they are enveloped.
   - Sertoli cells release many transporter proteins that facilitate transportation of transferrin, androgen-binding protein, and retinoid-binding protein across BTB (Porter et al. 1985; Sylvester and Griswold 1994; Mruk and Cheng 2004). The androgen-binding protein is synthesized by the cells under the control of FSH.

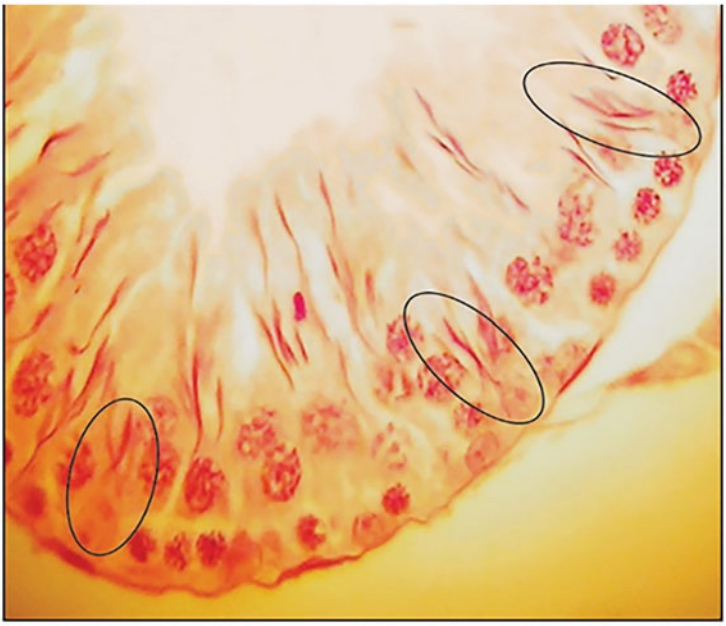

Fig. 9.8 Cross-section of the testis showing the structure of a seminiferous tubule, indicating the direction of the spermatids toward the Sertoli "nurse" cells present on the basal membrane of the seminiferous tubule (H&E magnification 100×)

- They secrete inhibin, which inhibits FSH secretion by the pituitary.
- They produce several substances such as estradiol, insulin-like growth factor, transferrin, and other compounds.
- In male fetuses, they are involved in the organogenesis of the testis.
- During male sex differentiation, fetal Sertoli cells produce a hormone called Mullerian-inhibiting factor or Anti-Mullerian Hormone (AMH). AMH mainly causes the regression of the female organs (Mullerian ducts, the anlage for uterus and Fallopian tubes) (Josso et al. 2005).
- Sertoli cell is a type of mesenchymal stem cells (Fig. 9.8).

9.2.4.2 Endocrine Cells of the Testes (Interstitial Tissue)

- **Interstitial tissue**: This includes Leydig cells and stem cells.
- **Leydig cells:** which are somatic cells and represent the endocrine tissue in the testis. These cells occur in the interstitial tissue either individually or in nests and make up 10% of the space between seminiferous tubules (Figs. 9.4 and 9.6).
- **Stem Leydig cells:** Adult Leydig cells develop from undifferentiated mesenchyme-like stem cells (stem Leydig cells) and exist in the same interstitial tissue during the early postnatal period (Fig. 9.9). Stem Leydig cells have been

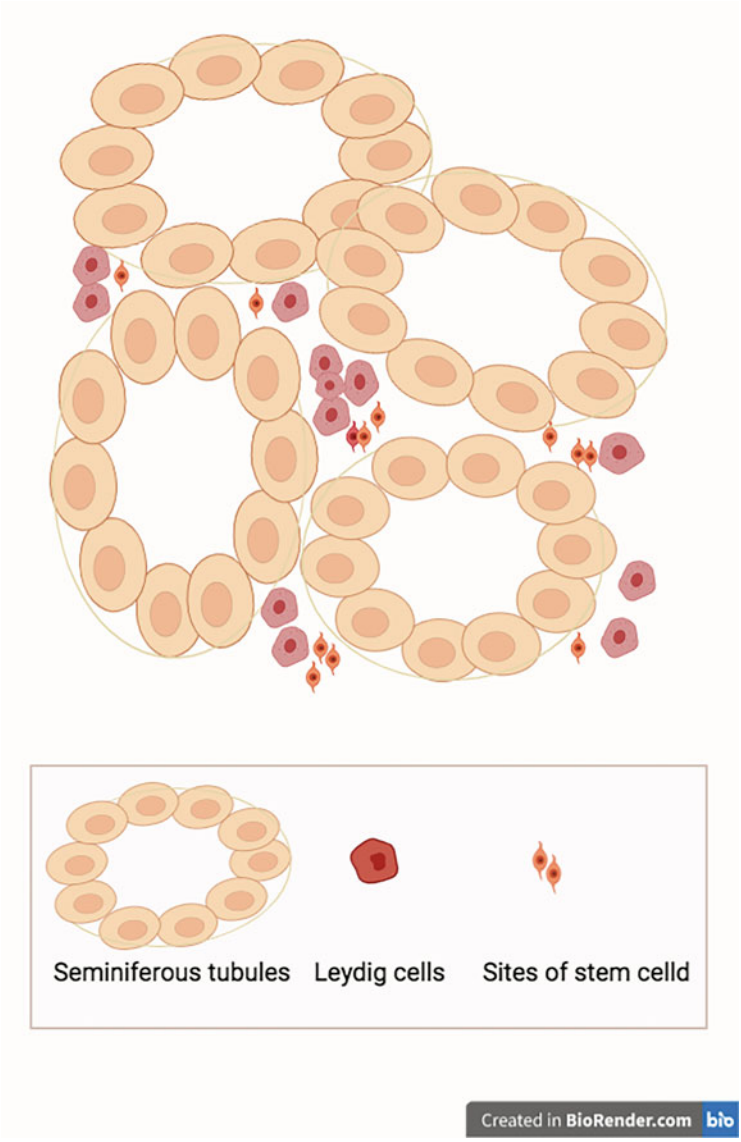

Fig. 9.9 Locations of Testis stem cells in interstitial tissue. (Reproduced from Al-Suhaimi and Aljafary (2019))

identified in peritubular and perivascular sites in adult testis to regenerate new Leydig cells instead of the loss of the adult cells (Chen et al. 2017a, b).

Physiological states for endocrine Leydig cell activity in the interstitial tissue of the testis (Figs. 9.9 and 9.10).

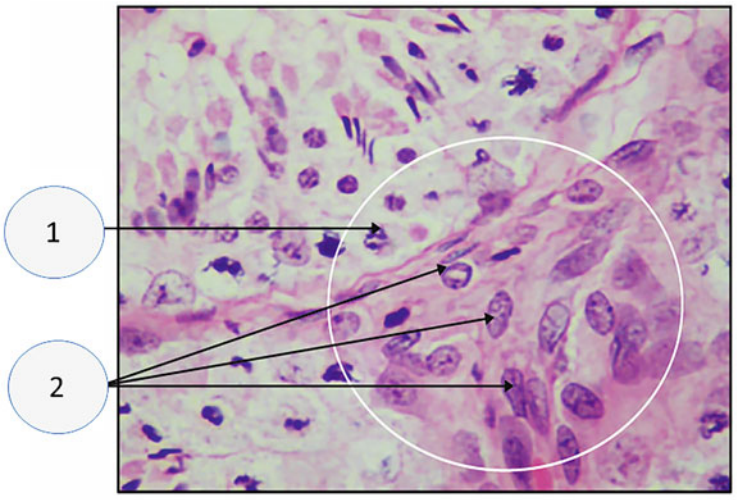

Fig. 9.10 Cross-section of the testis showing the interstitial tissue (1) as well as testosterone-producing endocrine Leydig cells (2) (H&E magnification 200×)

Endocrine Leydig Cells

- The Leydig cells produce testicular hormones called androgens.
- Testosterone and dihydrotestosterone are the main (more than 90%) source of circulating androgens synthesized and released by the testes into the spermatic venous blood; the remaining is synthesized and released by other tissues such as the zona reticularis of the adrenal glands in humans.
- The testes also produce estrogen, estradiol, and progesterone; these hormones contribute to the biosynthesis of androgens from cholesterol as a steroid precursor (Fig. 9.11).

9.2.4.3 Characteristics of Androgens

- Androgens are steroid hormones.
- Synthesis: refer to Fig. 2.4, Chap. 2.
- Testosterone is formed and secreted mainly from the Leydig cells.
- They are secreted in smaller quantities by the adrenal cortex.
- They circulate mainly in the blood, 60% of which is bound to a sex-hormone binding protein, globulin with high affinity and limited capacity, and 38% is bound to albumin which has lesser strongly and great total capacity; 1–3% of testosterone also occurs in the free form, allowing it to enter target cells.
- The bound hormone can enter the cell after it is released from the protein whenever biologically active testosterone is needed more than the less active free form.
- The androgens bind to the cell's cytoplasmic receptors and the hormone-receptor complex allows it to penetrate the target cell nucleus to induce a biological response.

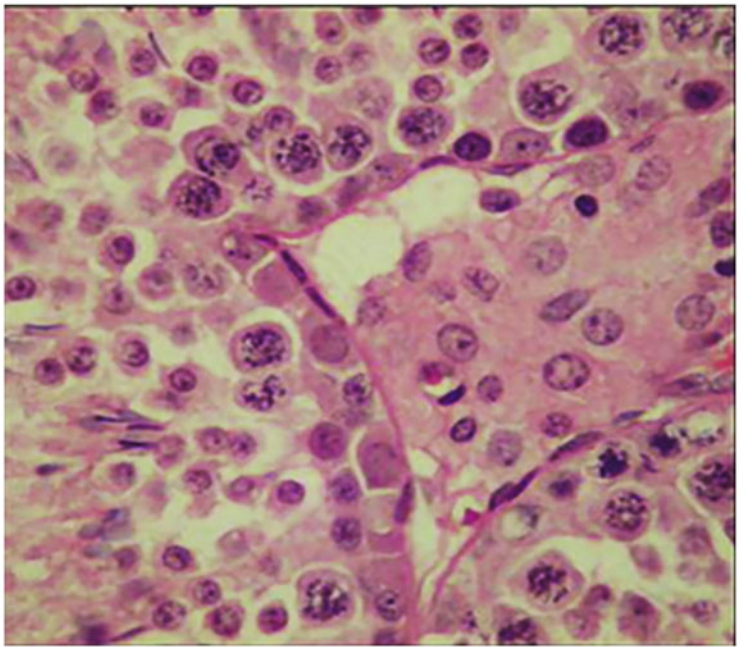

Fig. 9.11 Cross-section of the testis showing a reduction in the secretory activity of the interstitial endocrine cells, indicated by the dark-colored cytoplasm due to the presence of unreleased secretory vesicles (H&E magnification 200×)

- The androgens have a long half-life as they are bound to proteins. They have rapid clearance in the first hour with a half-life of 10 min, thereafter; they have a very slower decline in plasma level (100 min) (Sèdes et al. 2018; Guyton 1986; Guyton and Hall 2006, 2016; Bentley 1980; Bullock et al. 1991, 2001; Yeh et al. 2002).

9.2.4.4 Sexual and Reproductive Functions of the Androgens

The three steroids of main important in male reproductive functions are: Testosterone, dihydrotestosterone, and estradiol.

Sexual and Reproductive Functions

(a) **In the fetal phase**: This is essential for proper differentiation of the male reproductive system. Fetal Leydig cells are responsible for releasing the first peak of testosterone, the highest level required to masculinize the organs of the urogenital tract, like differentiation of vas deferens, seminal vesicles, and the Wolffian duct into the epididymis.

(b) **At puberty**: The hormone is involved in the growth and secretory activity of the reproductive tract such as the scrotum, epididymis, prostate, vas deferens, seminal vesicles, etc. Androgens are necessary for functional complementarity between these organs. At puberty, Leydig cells are also responsible for another secretory peak of testosterone to allow the development of secondary sexual characteristics and the initiation of spermatogenesis by promoting the physiological maturity of Sertoli cells.

(c) **Post-puberty**:

- The androgens are essential for the regulation of spermatogenesis. The androgens and their signals are essential for maintaining the survival and differentiation of the germ cell lineage. Thus, changes in androgenic signaling arrest the process of spermatogenesis.
- Although androgens are mainly expressed in somatic testicular cell types (Leydig, Sertoli, and peritubular myoid cells) in humans and rodents, its expression in germ cells is still debatable (Yeh et al. 2002). Androgens are also necessary for spermiogenesis phase, during which spermatids are transformed into mature sperms.
- After puberty they are necessary for preserving the differentiation and functions of the additional organs of the reproductive system needed for the production, storage, and secretion of sperms, such as the prostate, epididymis, and seminal vesicles, as well as to preserve male secondary sexual characteristics.
- The androgens respond to sexual appetite, and bring about hair growth of the pubis, underarm, face, chest, and areas of the body other than the scalp. They are also important for laryngeal development and increased vocal cord thickness in men. They are also involved in leadership characteristics and influence the social behavior of males.
- Androgens are key compounds for estrogen synthesis as well as for the bioavailability of free estrogen in peripheral tissues.
- Androgens directly affect the central nervous system to modulate other endocrine signals associated with hot flushes (Notelovitz 2004).

9.2.4.5 Anabolic Functions of Androgens

- Testosterone has an anabolic function and supports the development of muscle and bone. The androgens promote cell division, tissue growth and the extraction of nitrogen and certain salts needed for growth.
- The androgens are therefore anabolic hormones as they stimulate the synthesis of proteins in the skeletal muscles.
- In the same way as other sex hormones, the androgens promote the process of calcium deposition in the bone and epiphyseal fusion in the long bones. They do this efficiently but more slowly than estrogens (female hormone) which give males the opportunity for more bone growth, especially in the chest and shoulders.

- They increase weight and are involved in metabolism which is why removal of the testes leads to a lower metabolic rate.
- They stimulate red blood cell production; therefore, men have more red blood cells count than women (Guyton and Hall 2016; Bullock et al. 1991).

9.2.4.6 Recent Roles of Androgens in Female

Androgens have been found in recent years to be significant for regulation of female reproduction. The functioning of androgens is mediated through androgen receptor (AR). It has been proven that female fertility and follicle health, development, and ovulation is regulated by androgen functioning mediated by AR as suggested by global and cell-specific *AR*-knockout mouse models (Walters et al. 2019).

Numerous IVF clinics across the globe have implemented this concept on the basis of this information and the information obtained from clinics that depicted that androgens or androgen-modulating agents contribute in the enhancement of in vitro fertilization (IVF) stimulation in poor female responders. Moreover, it was also found from various human and animal researches that the onset of polycystic ovary syndrome (PCOS) may be attributed to the AR-mediated additional androgens. This implies that significant progress can be made in devising new therapies and treatments for PCOS by understanding the molecular processes behind the onset and advancement of PCOS as well as determining the target sites where AR is active (Azarchi et al. 2019).

The steroid-producing organs within human body including the adrenal glands and ovaries and tissues like skin are responsible for generation of androgens. The androgens commonly present in females are dehydroepiandrosterone, dehydroepiandrosterone sulfate, testosterone, dihydrotestosterone, and androstenedione. Many cutaneous conditions can arise as a consequence of such androgens in women. For instance, women may experience androgen-mediated cutaneous disorders like acne, hirsutism, and female pattern hair loss (FPHL) (Reisch et al. 2019). But the pathophysiology of the mentioned conditions does not render complete understanding and clarity of the contribution of androgens in this regard.

9.2.4.7 Regulation of Testicular Function

The pituitary gonadotropin hormones LH and FSH are heterodimeric glycoprotein circulating hormones play a key role in reproductive processes. Further paracrine modulation by local factors like activins, and follistatin released within the pituitary gland itself. The integration of the entire these mechanisms and signals lead to the coordinated control of several responses such as expression of the subunit gene, protein production, and gonadotropin release to boost sexual maturation and regulate normal reproduction functions. GnRH is defined as the gatekeeper of reproduction's development and functions. GnRH stimulates specific, high affinity external cell receptors on gonadotropes triggers signal transduction cascades to activate the coordinated synthesis and release of the pituitary FSH and LH (Stamatiades et al. 2019).

Hypothalamic–Pituitary–Seminiferous Tubule Axis

- The hypothalamus secretes GnRH in a pulsatile manner into the portal veins leading to the anterior pituitary and this stimulates the pituitary to secrete LH (interstitial cell–stimulating hormone [ICSH]) and, to a lesser extent, FSH into the circulation (Fig. 9.12). Conversely, gonadotropin-releasing inhibitory hormone (GnIH) influences GnRH neurons, and inhibits the release of gonadotropins from the pituitary.
- FSH and testosterone act directly on the Sertoli "nurse" cells which facilitate the process of gamete production.
- FSH and androgens regulate the tight junctions between Sertoli cells and their proteins.
- FSH acts on the Sertoli cells which then secrete testosterone-binding protein which makes it possible to maintain a high level of bound testosterone in the tubule.
- FSH is needed to initiate spermatogenesis, whereas full maturation of the spermatozoa requires not only FSH, but also testosterone.
- Sertoli cells secrete an inhibitory hormone called inhibin which inhibits FSH secretion by the pituitary gland (Guyton and Hall 2016; Bullock et al. 1991, 2001).

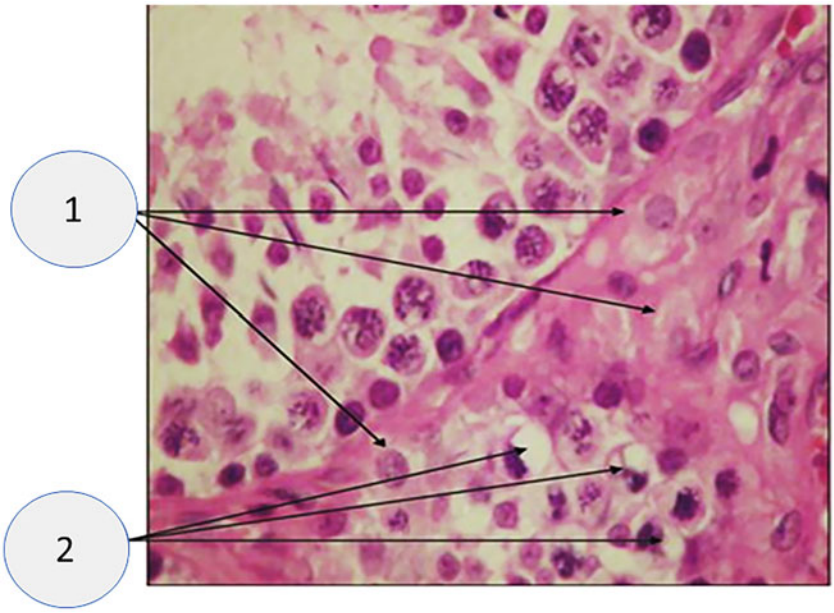

Fig. 9.12 Cross-section of the testis showing endocrine Leydig cells (testosterone secreting-cells) (1) with pale cytoplasm containing some spaces indicating secretory activity. It can also be seen that some of the other interstitial cells (2) contain cytoplasmic vacuoles due to fact collections (cholesterol as hormone's precursor pointing to lesser hormone production in them) (H&E magnification 200×)

The Domination Roles of Hypothalamic–Pituitary–Leydig Cell Axis

- The hypothalamus secretes pituitary-stimulating GnRH; these are hormones that stimulate the basal cells of the anterior pituitary to secrete the hormones ICSH (LH) and FSH.
- LH binds to its receptors on the Leydig cells. LH links with its G protein-coupled membrane receptors on Leydig cells and exerts its action through the protein kinase A signaling pathway.
- LH plays a key regulatory role in the steroidogenesis process at two levels:
  - By supporting cholesterol transfer into the mitochondria from the outer membrane to the inner membrane.
  - By activating the enzymatic pathways required for converting cholesterol to pregnenolone and then local testosterone production in enough amounts to meet the requirements for spermatogenesis, and to produce the hormone in the circulation.
- Increased testosterone levels in the blood inhibit LH secretion directly via the pituitary or the hypothalamus because both organs have androgen receptors which work by negative feedback mechanisms (Fig. 9.13).
- Many cytokines act at the hypothalamic level to inhibit significantly the release of LH but not FSH. Hypothalamic control of FSH and LH is influenced by cytokines, leptin, and nitric oxide. The adipocyte hormone, leptin, acts as a cytokine related to tumor necrosis factor (TNFα). In male rats, leptin shows a high tendency for the release of FSH and LH from the hemipituitaries in vitro. LHRH and leptin promote LH secretion by activation of neural nitric oxide synthase (nNOS) in the gonadotropes. The released nitric oxide induces guanylate cyclase that releases cyclic GMP leading to LH release (McCann et al. 1998).
- In addition to leptin, primate pituitaries express other adipokines such as adiponectin and resistin. It is thought that local production of adipokines/receptors integrates with circulating adipokine levels comprising of a relevant regulatory loop that participate to the precise regulation of pituitary functions (Sarmento-Cabral et al. 2017).
- The Leydig cells secrete peptide substances as paracrine signals which regulate testicular function.
- Prostaglandin acts as an autocrine signal. Dysfunction of its testicular synthesis may lead to idiopathic infertility in males (Rudolph et al. 2016a, b).
- Activins and inhibins are glycoprotein hormones that regulate the physiological function of the male and female reproductive systems. Inhibins antagonize activin and follistatin signaling in many aspects.
- Melatonin and serotonin from the pineal gland play physiological roles during puberty and the growth of the gonads. As described in Chap. 7, melatonin has important function on testis physiology, steroidogenesis and spermatogenesis in both somatic cells:
  - In Leydig cells, melatonin functions as a local endocrine modulator.
  - In Sertoli cells, melatonin affects cells growth, proliferation, energy metabolism and the oxidation state (Frungieri et al. 2017).

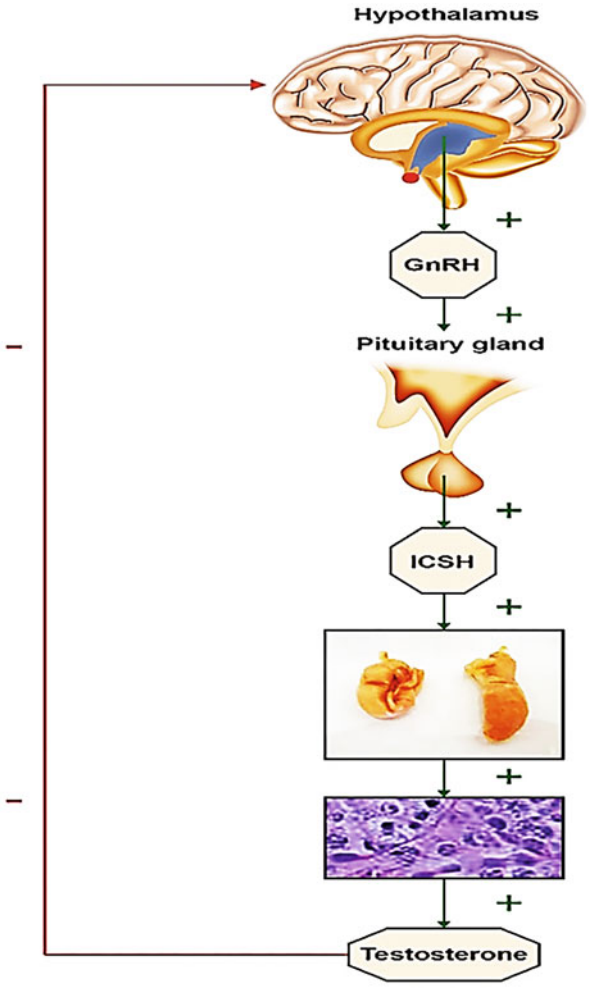

Fig. 9.13 Mechanism of testosterone secretion

  - Melatonin and corticotropin-releasing hormone (CRH) produced locally affect spermatogenesis through immune mechanisms in the testis (Rudolph et al. 2016a, b).
- **Calcium (Ca^{2+})** plays significant role in FSH & LH release. Calmodulin is calcium-modulated protein (CaM) which has four Ca^{2+} binding sites. Calcium influx plays a crucial function in GnRH regulation of rat LH DNA subunit gene transcription, Ca/CaMK II activation performs a significant role in the transit of GnRH signals from the plasma membrane to the LH subunit genes (Haisenleder et al. 2003). GnRH spends further effects on some gonadotroph functions, which clearly happen by CaM such as the proliferation of immature gonadotrophs, which is dependent on the enzyme called calcium and calmodulin-dependent

serine/threonine protein phosphatase (calcineurin) (Melamed et al. 2012). The Ca^{2+} molecular signal is essential pathway for testis steroidogenesis in Leydig cells (Abdou et al. 2013).
- Reproductive functions in male depend on cholesterol homeostasis, Sèdes et al. (2018) demonstrated cholesterol is a gatekeeper of fertility in male.
- There is an effect of bile acids on testis physiology and male reproduction, harmful impacts of bile acids on testis pathophysiology and fertility disturbances (Sèdes et al. 2017).

9.3 Ovarian Hormones and Reproduction

9.3.1 Ovaries: Tissue Zones of the Ovaries

These are the female reproductive glands and consist of a pair of endocrine and exocrine glands, whitish in color, each weighing up to 10 g. Their volume is about $4 \times 3 \times 2$ cm during the reproductive years. They are located in a low area called the ovarian fossa, on the anterolateral aspects of either side of the uterine wall and suspended super-posteriorly by the ovarian ligaments.

Tissue Zones of the Ovaries
- **Germline stem cell precursors** (ovarian surface epithelial cells).
- **Ovarian cortex** which is the part surrounding the medulla and is a connective tissue. The ovarian cortex contains ovarian follicles inside the stroma. It includes all follicular development stages: the oophorus pool, granulosa membrane with its cells, corona radiata, zona pellucida, primary oocyte, theca of follicle, antrum and follicular liquid, and the corpus luteum.
- **The inner layer is the ovarian medulla** which is in the center of the ovary and is devoid of follicles. It is made up of fibrous tissue, blood vessels and nerves.
- **Hilum**: This is the point of attachment of the ovary to the mesovarium (Gardner and Shoback 2007).

9.3.2 Puberty in Females

Puberty in females occurs when all the internal female organs are fully developed. This is clearly indicated by the start of menstruation (menarche) between the ages of 10 and 16 years. Puberty is basically regulated by the hypothalamus and pituitary glands. The hormones of the thymus gland, leptin, and pineal gland hormones are also involved. There is also a physiological relationship between melatonin levels and puberty in females. The decline in melatonin levels during puberty is not entirely accounted for by body mass or age (Crowley et al. 2012). The onset of puberty also varies according to geographical location, the environment, diet, and other regulatory hormones, in addition to psychological and other factors.

The period of minipuberty of infancy is characterized with greater activity of reproductive axis. The period is also characterized with variation in concentrations of reproductive hormone as well as variation in organ size; however, this period is not characterized with clear description of longitudinal changes. In short, the ovarian size of infant was at its peak at 16 weeks depending on the developed follicles' quantity and size. Hence, the current research may prove helpful in future to determine the reference range of postnatal development of ovary size in healthy term infants (Chin et al. 2021).

Puberty is unique in the sense that there has been continues changes may occur due to environmental influences and interaction with genetic determinants. These changes are suggested evolving pattern of the pubertal process. For example, some trends of early breast development have been observed during the two past decades in some countries (Bourguignon and Juul 2012).

9.3.3 Ovaries Functions

9.3.3.1 Exocrine Function

Production of mature ova during reproductive period from ovary epithelium stem cells. It has been reported that expression of pluripotent and oocyte-related genes in single putative stem cells obtained from human adult ovarian surface epithelium (Virant-Klun et al. 2013). In another study, differential expression of stem cell markers specific for pluripotent such as (nuclear OCT-4A, SSEA-4, CD133, cytoplasmic OCT-4 were reported in ovarian stem cells in vitro (Parte et al. 2014).

Oogenesis The embryo is initially developed on the basis of the genetic material supplied by female oocyte. Thousands of such oocytes are developed and incubated within the ovary. The first phase of ovary development or oogenesis involves the production of oogonia from the mitotic division of primordial germ cell. Consequently, oogonia act as primary oocytes that perform the first meiosis. This is followed by second meiotic division of the secondary oocyte which yields a haploid ovum as well as a second polar body (Rodrigues et al. 2008).

Folliculogenesis The main site of the onset of this process is core of ovarian cortex. There is a variation in the shape of membrane granulosa from flat to cuboidal shape with the development of primordial follicle inside the primary follicle. Moreover, the theca layer is developed inside the primary follicle; this layer contains the granulosa membrane. Due to mitotic division of Granulosa cells, there are 2–6 layers in secondary follicles. Follicular fluid is released by the granulosa cells when Graffian follicle is being developed; the form of this fluid and its constituents resemble those of blood. All the follicles found in a female are present in her body at the time of birth. The follicles do not develop afterwards. About 70–99% of these follicles are lost prior to ovulation age; this is because of atresia or apoptosis (Johnson and Everitt 2000).

9.3.3.2 Endocrine Function

- Production of most of the steroid sex hormones circulating in the blood.
- Production of local regulatory hormones.

The ovaries contain the following basic components (Fig. 9.14) which play a coordinated and regulated functional role under the control of the hypothalamus and pituitary:

- **Surface epithelium mesenchymal stem cells: Oogenic cells**.
- **Follicl**e: It performs both endocrine and exocrine functions simultaneously (synthesizes and releases the ovum, and releases estradiol). This is the functional unit in the pre-ovulatory phase and consists of theca cells and granulosa cells.
- **Ovum** which is surrounded by the same two layers.
- **Corpus luteum:** It functions as a temporary endocrine gland due its limited lifespan, releases progesterone. This is the main functional unit in the post-ovulatory phase.
- **The corpus albicans** is a white structure that results from luteolysis of the corpus luteum. The corpus albicans is the last component of the ovulatory cycle as it is absorbed completely.

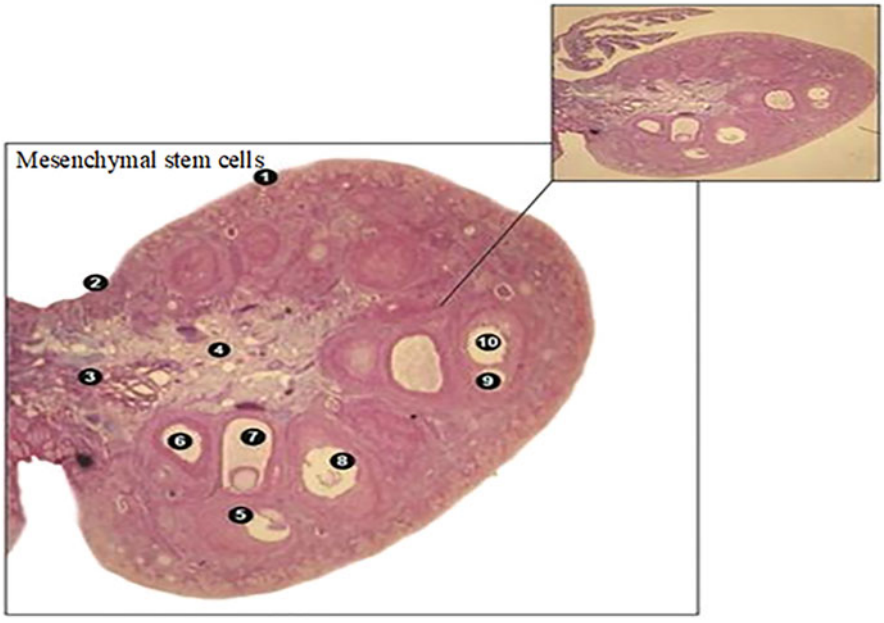

Fig. 9.14 Cross-section of ovarian tissue (H&E magnification 100×). Numbers (3–10) indicate the ten different stages of developing oocytes arising from mesenchymal/germline stem-cell precursors (oogenic-stem cells (1–2))

9.3.3.3 Main Sex Hormones and Sources

Sources of Androgens in the Male

- The Adrenal cortical layers of zona reticulata and zona fasciculata produce androgens namely the dehydroepiandrosterone (DHEA) and androstenedione.
- Leydig cells located near the seminiferous tubules in testes synthesize Testosterone which is acted upon by the 5α-reductase in target tissues to convert into dihydrotestosterone (DHT). Despite being about ten times scarce as compared to Testosterone, DHT performs almost all biological actions of testosterone.

Sources of Androgens in the Females

- The precursors circulating within the target tissues produce active androgens; these androgens act locally and are metabolized in the target tissues.
- In females, the following androgen precursors can be found (Burger 2002).
- DHEA sulfate (DHEAS) which is synthesized by zona reticularis within adrenal glands;
- DHEA which is synthesized by zona reticularis, theca cells in ovaries, and circulating DHEAS;
- Androstenedione which is synthesized by zona fasiculata within adrenal glands, ovarian stroma, and circulating DHEA.
- Circulating precursor molecules account for nearly 50% of testosterone production in females while the rest of the testosterone is produced in zona reticularis and ovarian stroma.
- Peripheral target tissues in females synthesize DHT but it has lower concentration within serum.
- Estrogens may be synthesized in postmenopausal women by conversion of Testosterone (not DHT) into estradiol in specific peripheral tissues in the presence of aromatase (P450aro).

Sources of Estrogen Synthesis

In the Females

- Estrogen is produced within the ovaries in granulosa and theca cells; it is also produced in corpus luteum.
- Considering the production of estrogen in extra-gonadal sites, it is produced by the conversion of androgens to estrone through peripheral aromatization in presence of aromatase.
- Fat cells: the conversion of androgen to estrone leads to rise in serum estrogens.
- Bone: estrogen is produced by the conversion of testosterone into local estrogen for assisting the development of epiphyses.

In the Males

- Aromatase is expressed in specific peripheral target tissues; it is an enzyme that plays a vital role in converting androstenedione to estrone and converting circulating testosterone into estradiol.

- It is believed that estrogens produced by the action of aromatase act locally and have limited systemic impact as they are metabolized in target tissues.
- Main expression of aromatase in males can be seen in bones (specifically osteoblasts and chondrocytes); in adipose tissues and in reproductive tract specifically in Leydig cells, Sertoli cells, and mature spermatocytes.

Sources of Progestin

- When 3β-HSD acts on pregnenolone in the corpus luteum, the progesterone is produced. It is also produced by the placenta during pregnancy. Adrenals also produce progesterone during the synthesis of androgen and mineralocorticoid.
- Two types of products are obtained during hormone synthesis depending on the menstrual cycle; one of these products is estradiol which is produced during follicular maturation, while the other is progesterone produced during the luteal phase after ovulation.
- The ovaries are not only the place where oocytes are produced and stored. It is also an endocrine gland that secretes, along with other tissues, several sex hormones essential for reproduction, the most important of which are:

  Pre-puberty: Estrogen is produced by peripheral and limited conversion of androgen to estrogen.

  Post-puberty: The ovary secretes estradiol, estrone, progesterone, and inhibin which circulate in the bloodstream. The ovary also secretes local hormones such as activin; the latter is a member of the TGF family (Burger 2002; Miller et al. 2006; Stocco 2001; Miller and Auchus 2011).

Steroidal Sex Hormones

The ovarian follicle and corpus luteum are the main units that secrete steroidal sex hormones.

- Follicle: This contains androgen-producing theca cells while the granulosa cells produce estrogen as well as inhibin A, B, and activin.
- The dominant follicle produces multiple intra-ovarian hormones and growth factors in its dominant follicular fluid (DFF) to serve as effective paracrine functions in both normal young and older ovulatory women (20–45 years) but in different concentrations. Several hormones are identified in DFF such as estradiol, progesterone, androstenedione, testosterone, inhibin A and B, activin A, and follistatin. DFF also, contains growth factors like Vascular Endothelial Growth Factor, Insulin-Like Growth Factor I (IGF-I), IGF-II, IGF-Binding Protein-2 (IGFBP-2), and IGFBP-3 (Klein et al. 2000).
- Corpus luteum which synthesizes progesterone.
- The zona reticularis of the adrenal cortex which produces androgens.
- Other tissues such as the liver, hair follicles and adipose tissue convert and produce estrone from precursor compounds (androgens).

- The placenta produces hormones such as estriol which is believed not produced by the ovary.
- The most important estrogens in the circulation are estrone, estrone sulfate, estradiol, and estriol, whose strengths of activity decrease from estradiol to the weakest, estriol.
- Woman produces greater amount of androgen than estrogen. The main blood steroids mostly categorized as androgens, comprise of the following compounds in ascending order of serum levels:
- Dihydrotestosterone, testosterone, androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulfate. Only the first two androgens bind their receptors, while the remaining steroids are working as pro-androgens.
- Dehydroepiandrosterone is mostly produced by adrenal, controlled by ACTH and acts as a precursor for the peripheral synthesis of effective androgens. The ovary is also synthesized dehydroepiandrosterone (Burger 2002).
- The most important androgens in the blood are dehydroepiandrosterone sulfate and testosterone.
- Steroids circulate in the blood in both free and bound forms. Most of steroids bind to sex-hormone binding globulin. A quarter is bound to albumin, apart from progesterone which binds to transcortin and albumin.

Chemical Structure of the Steroid Hormones

The estrogens and progesterone consist chemically of steroids synthesized by the cells from cholesterol, especially lipoproteins in the plasma, or from the small amount of cholesterol acetate produced in the cell (Fig. 2.4 in Chap. 2). After the synthesis of androgens, they are converted to estrogens by the process of "aromatization," during which androgens are converted to their corresponding estrogens by aromatases. The aromatase, estrogen synthetase, represents a key enzyme responsible for the biosynthesis of estrogens (Simpson et al. 1997).

9.3.4 Role of the Endometrium

During the reproductive period, the lining the uterus (endometrium) acts in synchrony with the ovulatory cycle functionally and periodically, but loses its regularity and continuity at the start of puberty and before the onset of menopause. The cycle is characterized by wide variability in its length, and normally ranges from 26 to 35 days, starting from the first day of the menses which takes about 5 days, followed by a fertile phase from 5 days before ovulation, and a lower fertility phase which depends on age and cycle length. The menstruation, endometrium phases and ovarian cycle with its hormonal regulation will be fully described in the following sections.

Proliferative Phase The endometrium becomes thicker, more vascular and richer in mucus-secreting glands. This phase coincides with the follicular phase in the ovary. It begins with menstruation and ends at ovulation.

Secretory Phase The uterine glands are characterized by aqueous mucous secretions. This coincides with the corpus luteum (luteal) phase in the ovary. It begins at ovulation and ends before menstruation.

Degenerative Phase and Menstruation The menstrual cycle in women is a physiological event and initiates between the ages of 12–16 years and continues throughout the reproductive period. It is an indication of puberty.

If the released ovum is not fertilized in the uterus, it is degraded and the endometrium—the continued existence of which is dependent on high progesterone and estrogen concentrations—begins to disintegrate and is eliminated with the blood. This is called **menstruation** and results from a decrease in these two hormones and other factors, after which the uterine cycle begins again.

Composition of the Menstruum This consists of secretions from the endometrial glands, uterine lining cells, blood from lysed capillaries and the unfertilized ovum.

Pregnancy When the ovum is fertilized during the ovulation phase, the hormones progesterone and estrogen continue to rise which maintains the corpus luteum throughout the pregnancy period and prevents the formation of an ovulatory follicle or new ovum. They also inhibit the pituitary from secreting FSH and LH. So long as the corpus luteum is present, the endometrium is not degraded, which means that menstruation does not take place.

9.3.5 Regulation of Ovarian Function

- The production of oogenic-stem cells is controlled by the endocrine and immune systems (Fig. 9.15).
- The main regulator of ovarian function is the hypothalamic–pituitary–ovarian axis. The arcuate nucleus of the hypothalamus contributes to reproductive function in females, so changes in its morphology influence female sexual receptivity in rats (Rudolph et al. 2016a, b). Ovarian function is linked to that of the hypothalamus which secretes GnRH (LHRH/FSHRH) in a repetitive and pulsatile manner, as the hypothalamus is the initiator of reproduction process.
- In contrast, Gonadotropin-Inhibiting hormone (GnIH) acts as an influential regulator of reproduction in human, mammalian, and birds. GnIH modulates the activity of GnRH neurons. GnIH inhibits the synthesis and release of pituitary gonadotropins by two pathways: decreasing the activity of GnRH neurons or/and directly by inhibiting the gonadotrophs (Ubuka et al. 2012; Aliaga-Guerrero et al. 2018).
- The hypothalamic hormones regulate the pituitary FSH and LH to synthesize and produce their gonad-regulating hormones which in turn regulate ovarian secretion.
- Kisspeptin (formerly known as metastin) in humans and non-human animal models was proven to be both vital and sufficient for activation of the

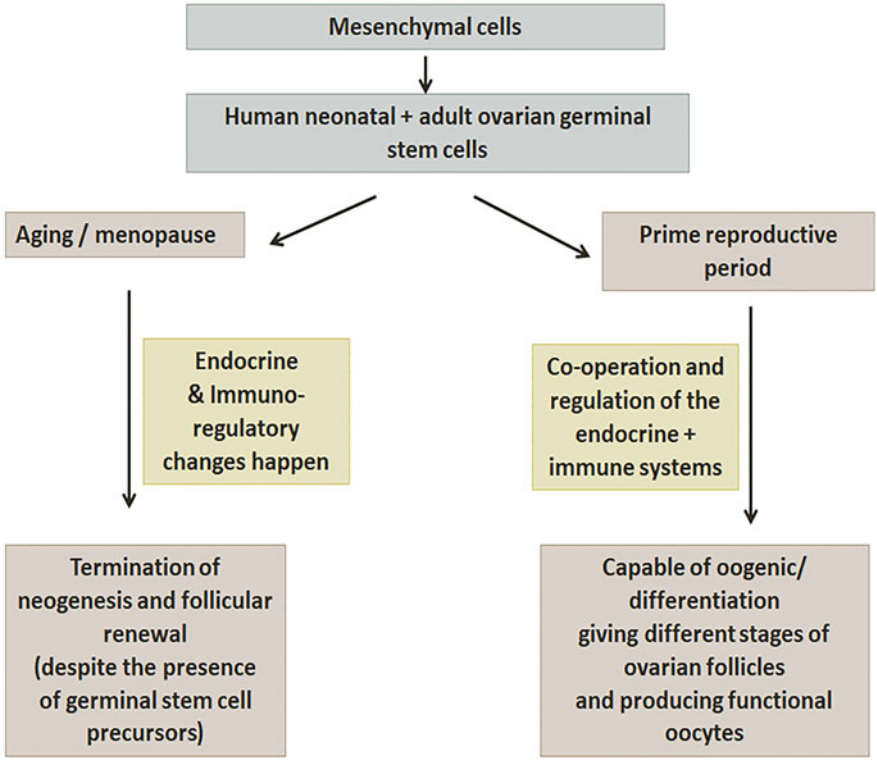

Fig. 9.15 Stem cell's role in oogenesis in ovary (Al-Suhaimi and Aljafary 2019)

reproductive system, during puberty and in adulthood, it is an essential component in the reproductive axis. Kisspeptin is the most potent activator of GnRH neurons. Importantly, kisspeptin neurons intermediate many of the regulatory actions of other signals such as metabolic, circadian, hormonal, or stress signals. This makes kisspeptin neurons as unique key and nodular points and ducts for transmitting many endogenous and exogenous signals to the reproductive axis (Kauffman and Smith 2013). Kisspeptin encoded by the *KISS-1* gene in humans acts as a modulator of puberty and fertility because of its stimulatory effect on hypothalamic GnRH. *KISS-1* transcripts are markedly elevated in both sexes 8 weeks after birth, and also maintains elevated levels in adults, indicating its reproductive functions at both the onset of puberty and maintenance of reproductive function (Chen et al. 2017a, b).

- The steroid hormones produced by the ovaries, especially estradiol and progesterone, are affected by negative and positive feedback, depending on the ovulation phase in question, and regulate the secretion of FSH with LH from the pituitary or through hypothalamus.
- Circadian rhythm cycles and photoperiod have a clear physiological effect on reproductive functions and regulation. Neuroendocrine hormones such as

gonadotropin-releasing hormones and gonadotropin-inhibitory hormones are influenced by melatonin in human and animals such as mammals, birds, and fishes.

- Important regulation is exerted by the hormones produced by the pineal gland, including melatonin and serotonin. During the prepubertal stage in females, melatonin and serotonin released from the pineal gland play modulatory roles in female puberty. Serotonin suppresses the hypothalamic signals to the gonads. In females, either intermediate decreases in hormone levels, or the decrease in melatonin peak may be considered as an indicator of pubertal progression (Crowley et al. 2012).
- In addition to the regulatory effect of GnRH on gonadotropins, there are important regulatory neurotransmitters, including acetylcholine, noradrenaline, dopamine, serotonin, melatonin, glutamic acid, oxytocin, and nitric oxide (NO). Neuropeptides such as noradrenaline, glutamic acid and oxytocin stimulate LHRH release through stimulation of nNOS, which follows the pathway described by McCann et al. (1998). Oxytocin and/or glutamic acid stimulate noradrenaline neurocytes in the hypothalamus that induces NOergic neurons by alpha-1 receptors. The secreted nitric oxide spreads into LHRH nerve terminals and stimulates LHRH secretion by activating guanylate cyclase and cyclooxygenase. Nitric oxide doesn't only regulate the secretion of LHRH to be directed to the pituitary, but also to stimulate mating by actions at the level of brain stem (McCann et al. 1998) (Fig. 9.16).
- Leptin. In females, leptin accelerates the onset of puberty. It also regulates LHRH secretion from the hypothalamus in adults. The leptin receptor gene is expressed in the hypothalamus and ovary. Leptin decreases estradiol production from the granulosa in vitro. Leptin as nutritional/ metabolic hormone gives rise a new tool on the tight correlation between leptin and reproductive functions. Its nutritional mechanisms are considered in several ovulatory disorders (Bringer et al. 1999).
- Other local or systemic ovarian hormones such as inhibin, activin, and follistatin have physiological effects will be fully described at the end of this chapter.
- Ovarian hormones such as activin and prostaglandin as well as oxytocin have a paracrine or autocrine action.
- The sympathetic innervation is required for the ovary as it influences the ovarian morphology, physiology and steroid synthesis. In contrast, prolonged post-natal denervation with an adrenergic blocking agent reduces more than 40% of the volume of ovarian follicles, granulosa cells and theca-interstitial cells. Also, denervation decreases the ovarian concentrations of pregnenolone and progesterone, leading to delayed puberty. The main cause of these disturbances is a blockade of the first steps of steroidogenesis, like levels of the cholesterol side chain cleavage enzyme P450, leading to reduction in pregnenolone levels (Rosa e Silva et al. 1997).
- The duration and intensity of regulatory hormones vary according to the phase of the ovarian cycle.
- Regulation varies depending on the life stage of the female: pre-puberty, reproductive age, and menopause.

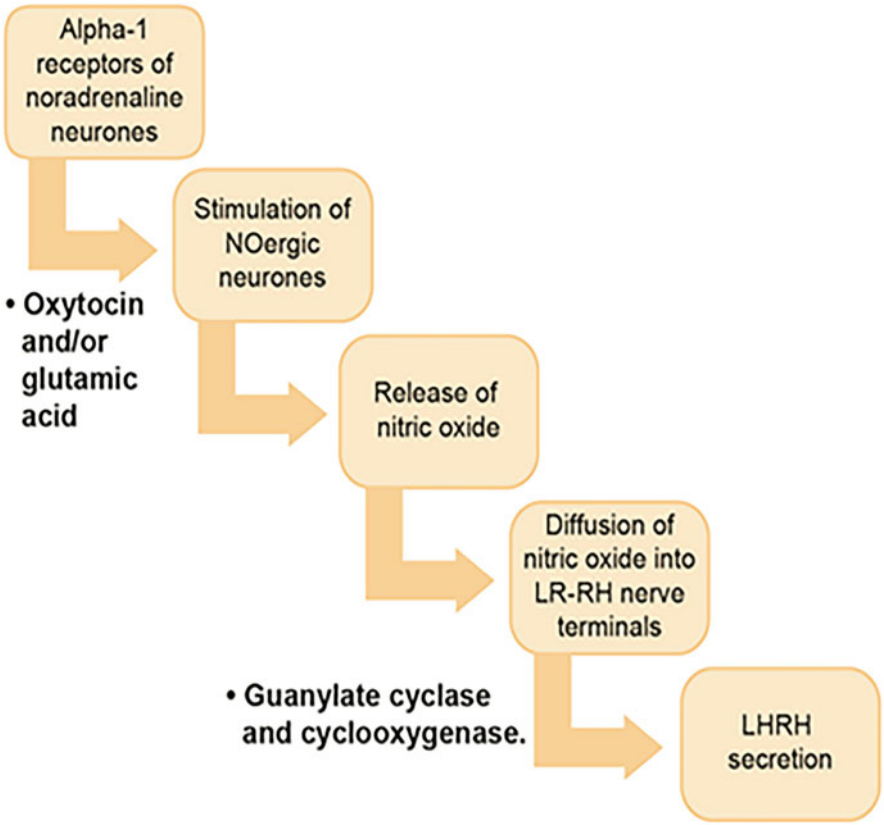

Fig. 9.16 Illustration of the pathway of the neurotransmitters on LHRH release

- During menopause, ovarian stem cells fail to maintain their oogenic property because of the changes in the endocrine and immunoregulation alteration (Fig. 9.15).
- Calcium (Ca^{2+})/ calmodulin plays significant roles in FSH & LH release in female.

9.3.6 Phases of the Ovarian Cycle

The ovary contains a network of blood vessels (for the endocrine functions) and local systems (for the paracrine and autocrine roles) to adjust the activity of gonadotropins FSH/LH and to coordinate follicular growth, steroid formation, and ovulation.

9.3.6.1 Pre-ovulatory Phase (Follicular Phase)

Gradual follicle growth takes place in the ovary until the follicle and ovum are fully mature. The beginning of follicular growth coincides with the release of menstrual blood from the uterus while the proliferative phase of the endometrium starts with the end of menstrual flow.

Duration The follicular phase takes about 10–16 days, its duration is variable, and it is therefore responsible for variations in the length of the ovarian cycle in women (Reed and Carr 2015), but the follicular phase can extend to 25 days.

A dominant follicle is selected in the mid period of this phase. By the end of this phase, only the dominant follicle generally reaches the final growth stage to be eligible for ovulation, after going through the following phases:

New concept on oogenesis

"Stem cells in the ovary and new concept on oocytes presence during the women life. In human and mammalian ovaries, surface epithelium mesenchymal stem cells are present not only in the fetal period but also in adulthood and old age".

New oocytes (with zona pellucida and granulosa cells) originate from the surface epithelium (somatic cells) arising from mesenchymal stem cells in the tunica albuginea. The ovary can form primary follicles during the reproductive period, so it was revealed that the term "germinal epithelium" could be reinstated (Nishida and Nishida 2006). It has become clear that both human neonatal and adult ovarian germline stem-cell precursors (ovarian surface cells) have the capability for oogenic/differentiating and producing functional oocytes, so it renews the oocyte pool (neo-oogenesis) and ensure follicular renewal during the prime reproductive period, with the co-operation and regulation of the endocrine and immune systems, and cellular support. After the prime reproductive period, aging starts, and menopause occurs because of the immunoregulatory changes that causes cessation and terminate neo-oogenesis and follicular renewal in vivo despite the existence of germline stem cell precursors. The rest of oocytes in the primordial follicles retain ovarian function but advancing age (aging oocytes) correlates positively with the occurrence of fetal chromosomal abnormality. Applications of ovarian stem cells can lead to a promising and advanced therapeutic approach to premature ovarian failure. For crossing over of chromosomes, new germ cells divide symmetrically, enter the cortical vasculature, and form new primordial follicles by connecting with cell nests of the granulosa deeply in the ovary cortex (Bukovsky 2011).

Primordial Follicles These are the essential reproductive units, composed of primary ovarian cells (oocytes) surrounded by a single layer of granulosa squamous cells and a basal membrane (Fig. 9.17). They haven't blood supply. The oocyte influences its own fate by releasing several factors include two specific growth factors related to Transforming growth factor (TGF-β); they are: Bone Morphogenic Protein (BMP)-15 and Growth Differentiation Factor (GDF)-9 that stimulate granulosa cell proliferation.

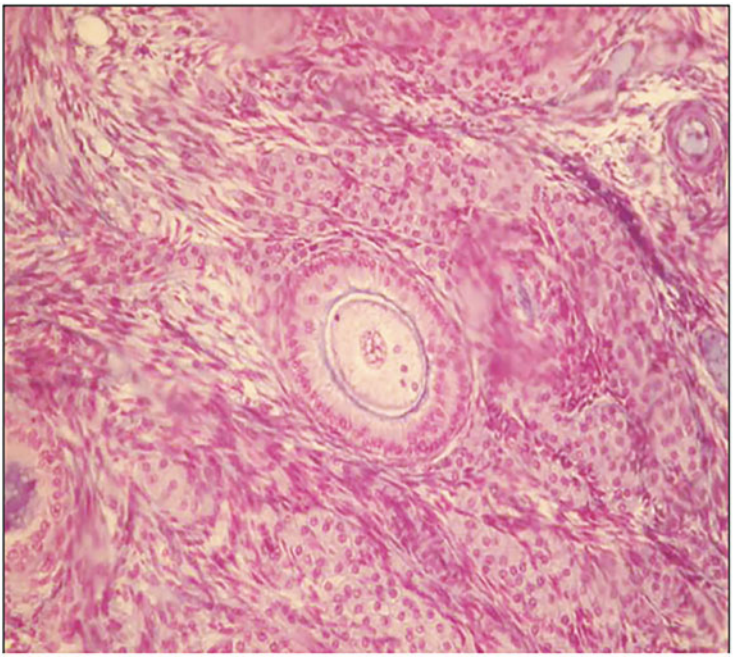

Fig. 9.17 Cross-section of ovarian tissue showing a primordial follicle (immature follicle) containing the oocyte (H&E magnification 100×)

The granulosa responds with local hormones such as follistatin, to decrease the inhibitory effects such as (activin A, Mullerian-inhibiting factor) and support stimulators of oocyte growth (Gardner and Shoback 2007).

Duration Develops from the sixth month of gestation.

Primary Follicle Its development indicates the beginning of follicular growth. Primary follicles differ from primordial follicles in multiple aspects. Primary follicle growth greatly leading to larger follicles called vesicular follicles. The oocyte begins to grow, the zona pellucida is formed, which is a thick glycoprotein layer synthesized by the oocyte and separates it from the granulosa layer, forming a barrier surrounds and protects the oocyte and the conception (Fig. 9.18). Finally, the granulosa transforms from squamous cells into cuboid cells.

Duration This stage mostly lasts for 150 days because of the doubling time (more than 250 h) of granulosa cells.

Secondary Follicle The developing follicle. The ovum develops inside the follicle to a maximal oocyte growth (120 μm in diameter), proliferation and increase in number of the granulosa cells and acquisition of theca cells (Fig. 9.19). During this phase, receptors of FSH, estrogen, and androgen develop.

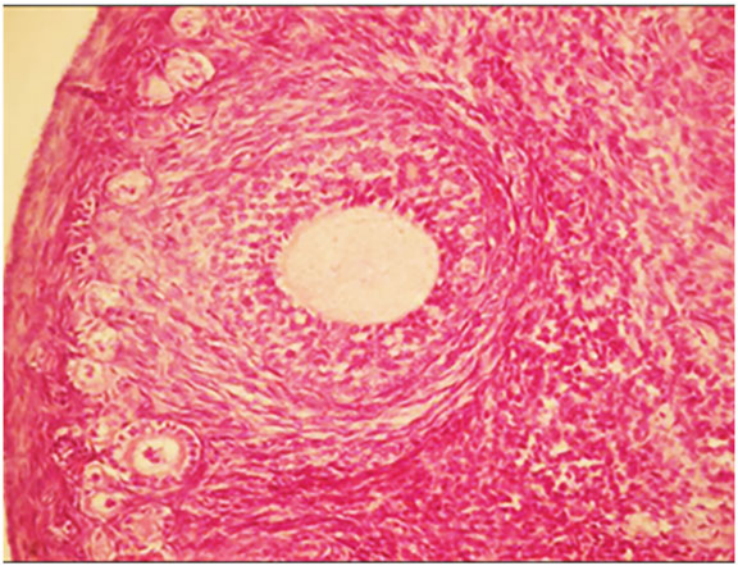

Fig. 9.18 Cross-section of ovarian tissue showing a primary follicle (immature follicle) containing the oocyte (H&E magnification 400×)

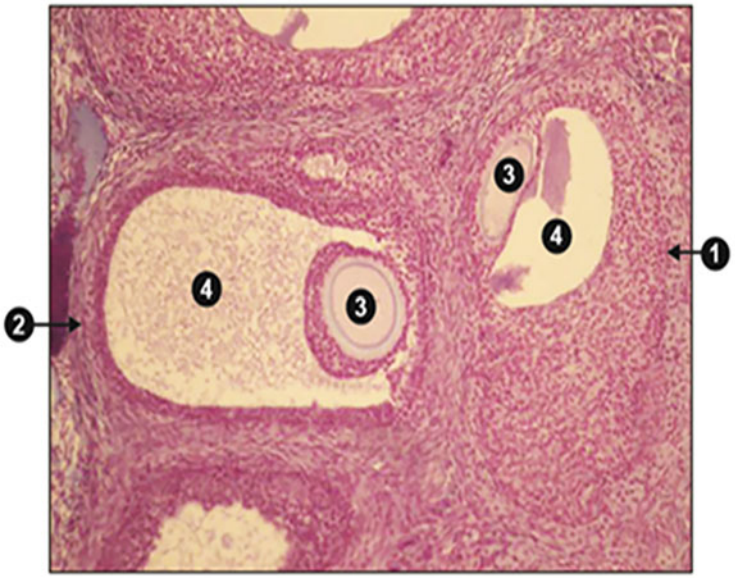

Fig. 9.19 Cross-section of ovarian tissue showing a secondary follicle (1) and mature follicle (2) containing the oocyte in a peripheral position (3) Note the difference in the amount of follicular fluid (4) Note the corona radiate surrounding the oocyte and the cellular connections with the granulosa zone (H&E magnification 400×)

Duration This stage takes about 120 days for the doubling time of granulosa cells.

Tertiary Follicle The follicle develops further (called the early antral phase). This is characterized by the formation of an antrum/ cavity containing a fluid made up of proteins, minerals, progesterone, steroid hormones and other permeable substances. During this phase the granulosa cells differentiate into several distinct layers by the control of FSH, the oocyte derived growth factor GDF-9 is an important in this process. The thecal cells become more differentiated and some of its subpopulations acquire LH receptors and are eligible for steroidogenesis, theca cells act as templates for the secretion of pre-estrogens as well as other hormones. The follicles develop receptors for FSH and can also secrete **activins** which generally function in an autocrine manner; there is negligible level in the blood and doesn't change throughout the menstrual cycle, but it acts in the ovary to stimulate FSH receptors expression in the granulosa to accelerate the folliculogenesis and forms the follicle with diameter of up to 400 μm. In addition to activin that regulates folliculogenesis, the granulosa cells synthesize inhibin A and inhibin B which have indirect roles in folliculogenesis and steroidogenesis.

In this stage, the ovum grows to its maximum size and is surrounded by cellular cumulus oophorus. In the cases of prepubertal females and those taking oral contraceptives, the follicles may arrest at different stages up until this point as in this phase of follicular development, FSH is critical for growth and survival, so if FSH doesn't rescue these follicles, they undergo atresia.

Mature Follicle It was called formerly a Graafian follicle. When the follicle nears maturation, additional follicles stop developing and the antrum filled with the follicular fluid increases in size. Highly differentiated granulosa cell layers begin to surround it. These can secrete steroids, particularly estrogen, at a maximum activity as can the inner thecal layer consisting of large clusters of steroid-producing cells. Immediately before ovulation, the cumulus oophorus surrounding the ovum ruptures, leaving the ovum surrounded by the corona radiata which are attached to the granulosa by cell connections which break before ovulation to allow the ovum surrounded by the corona radiata to float freely inside the follicle. At this point, the follicular diameter is 1.5–2.5 cm (Fig. 9.19) and can be seen below the surface of the ovary so that these cells no longer appear at the surface and become avascular and lysed (Al-Motabagani 2008; Burkitt et al. 1996; Young and Heath 2000; Bullock et al. 1991; Gardner and Shoback 2007; Greenspan and Forsham 1986). The ovarian follicle includes oocyte, cumulus oophorus, membrana granulosa & granulosa cell, and theca of follicle.

9.3.6.2 Ovulation Phase

The ovulation occurs 14 days after the onset of menstruation. Immediately before ovulation, the protruding outer wall of the follicle swells speedily, the *stigma*

(a small area in the center of the follicle) protrudes forming like a nipple, in about 30 min fluid oozes through the stigma, 2 subsequent minutes, the stigma ruptures allowing moving of viscous fluid carrying with it the ovum surrounded with a mass of thousands of granulosa cells called *corona radiata* to be released into the peritoneal cavity near the uterine opening.

Duration The ovulation period lasts for only 1 day, and the ovum survives for a short time after ovulation, usually 8 h. Sperms only have fertilization capability for 24 h even though they can survive for several days.

9.3.6.3 Post-ovulatory Phase

Corpus Luteum Phase

This begins after ovulation phase when the ovary secretes the basic hormones needed for implantation of the ovum. The mature follicle which secreted the ovum collapses and fills up with clotted blood. The cells re-regulate themselves with a well-developed blood supply to allow the follicle to form another, temporary yellow endocrine structure called the *corpus luteum*, which is similar in size to the follicle (Fig. 9.20). Vascular endothelial growth factor-A is involved in the angiogenesis for developing the corpus luteum (Rudolph et al. 2016a, b).

The theca cells continue to grow and secrete estrogen along with the zona reticularis cells which grow considerably more and start to secrete progesterone in addition to estrogen; their cytoplasm is distinguished by a bright yellow color due to its concentration of carotenoids pigments particularly (*lutein*), led to the name of

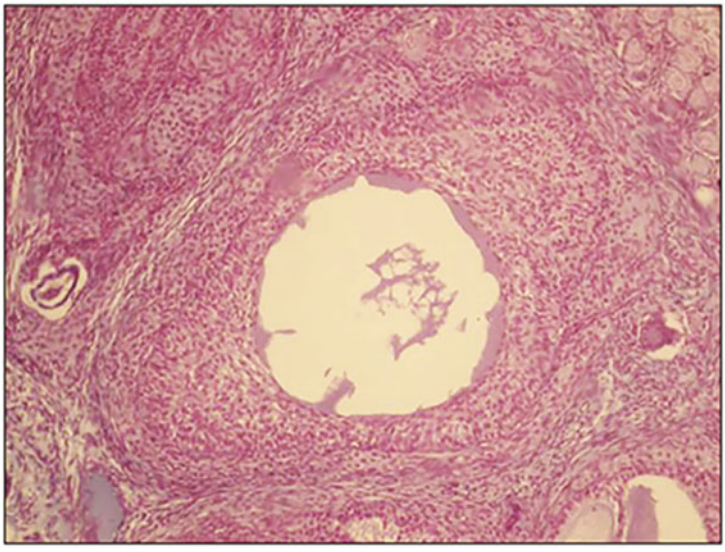

Fig. 9.20 Cross-section of ovarian tissue showing a corpus luteum (H&E magnification 400×)

granulosa lutein cells. The name of *corpus luteum* also came from a similar origin. (Czeczuga-Semeniuk and Wolczynski 2005, 2008) analyzed ovarian tissue of both normal and pathological lesions groups for identifying 14 carotenoids and revealed the presence of provitamin A carotenoids; beta-carotene, beta-cryptoxanthin, echinenone, and hydroxyechinenone but alpha-carotene was not detected. The results were similar in both groups; the overall carotenoid content was relatively low, while the mean content of provitamin A carotenoids was 17.28%. In 2008, the same authors analyzed physiological and pathological tissues of ovary, uterus and breast for identifying 16 carotenoids including the carotenoids belong to provitamin A group, the common carotenoids are: beta-carotene, beta-cryptoxanthin, lutein, mutatoxanthin, violaxanthin, lutein epoxide, and zeaxanthin. The corpus luteum relatively produce high levels of progesterone, moderate levels of estradiol and inhibin A, and small to moderate levels of estrogen inhibin B (Yamoto et al. 1997).

Duration The post-ovulatory phase has a set relatively length of time 12–15 mostly 14 days following ovulation in all women, the lifespan of the corpus luteum.

9.3.6.4 Corpus Albicans

If the ovum is not fertilized and implanted, the corpus luteum atrophies and turns into a non-functional **corpus albicans**. This means that the hormones responsible for continued growth of the endometrium disappear, leading to the appearance of menstruation signs. On the other hand, if fertilization happened, the role of the corpus luteum continues, and occupies a large space in the ovary, and estradiol and progesterone continue to be secreted gradually, especially at the beginning of pregnancy.

Additional Notes

- The ovulatory cycle can be counted by many ways: (a) Taking the first day of menstruation as day zero and the last day as 28 days after that, (b). A menstruation cycle duration is the count of days from the first day of menstruation (bleeding) of one cycle to the starting of menstruation of the next cycle, and (c) The occurrence of the peak LH level can be taken as day zero and the premenstrual period is indicated with a negative sign and the period after ovulation with a positive sign.
- The average length of the menstrual cycle is 28 days with common cycle durations of 25–30 days.
- The ideal volume of blood missing during menstruation is approximately 30 mL, any amount greater than 80 mL is believed abnormal.
- The menstruation cycle is most irregular and unequal at parties of the reproductive age (menarche and menopause) because of anovulation and inappropriate follicle growth.
- Carotenoids act as chemoprotective structures, irrespective of whether they are converted finally into vitamin A, and act a potential potent alternative to current chemotherapeutic methods to treating the ovarian cancer (Reed and Carr 2015; Czeczuga-Semeniuk and Wołczyński 2008; Yamoto et al. 1997).

9.3.7 Regulation of the Ovulatory Cycle (Figs. 9.21 and 9.22)

1. **Pre-ovulatory phase:**
   A stage of the primary follicle growth up to the antral stage is mainly stimulated by FSH alone. Then several mechanisms follow lead to significant accelerated growth to give rise larger follicular. Varying degrees of frequency for the synthesis and secretion of FSH which acts in harmony with a lower LH concentration to synthesize and secrete estrogens (for example, slow pulsed secretion stimulates activin which in turn stimulates FSH production). FSH is the main gonadotropic stimulating hormone secreted by the pituitary during this phase which leads to a slight increase followed by a gradual rise in the level of the main estrogen in the blood (estradiol) secreted by the ovary. Therefore, it is called the *follicular phase or the estrogenic phase*.

   Also, inhibin B is released in the early follicular phase. In the mid-follicular phase, one of the ovulatory follicles will be defined as a dominant follicle which grows and increasingly releases estradiol and inhibin A for 1-week prior to ovulation. Related actions are also identified for the dominant follicle which progresses to be the only follicle able to respond to the reduced levels of FSH, actions such as gonadotrophin responsiveness, vascularization, insulin-like growth factor binding protein expression and degradation.

   During the follicular phase, estrogen has a positive feedback effect on GnRH which increases its repeated pulsatile secretion from slow (every 1.5–2 h) to fast (every hour). Estrogen also acts directly on the pituitary to stimulate LH as well as FSH secretion to stimulate more estradiol production. This is further boosted by the local hormone activin, which encourages the expression of FSH receptors in the granulosa cells of the follicle. During this phase, estradiol increases positively with FSH. The estradiol concentration increases and peaks on day 13 as a function of the increase in inhibin A, which induces the pituitary-hypothalamus to secrete GnRH. This leads to an LH surge which peaks, if there is a sufficient level of FSH, 24 h after the estradiol peak on day 14 of the ovulation cycle.

   This is accompanied by negative feedback leading to a gradual decrease in FSH from the pituitary or indirectly from the hypothalamus (via the gamma-aminobutyric acid-containing neurons). In addition, inhibin B, whose levels align to those of FSH, has a negative feedback effect so that its concentration in the blood also rises in the pre-ovulatory phase to inhibit the secretion of FSH from the pituitary; progesterone on the other hand is at its lowest level during this pre-ovulatory phase.

   Per cycle, two-thirds of women show two follicular waves while one-third shows three follicular waves and have longer cycles, and a later estradiol increase and LH surge (Guyton and Hall 2016; Bullock et al. 1991, 2001; Mihm et al. 2011; Ursula 2017).
2. **Ovulation phase**
   Progesterone, LH, and FSH act in synchrony to stimulate the expression of lytic enzymes which degrade collagen in the follicular wall in preparation for its rupture. Prostaglandin production also increases as it is needed for contraction

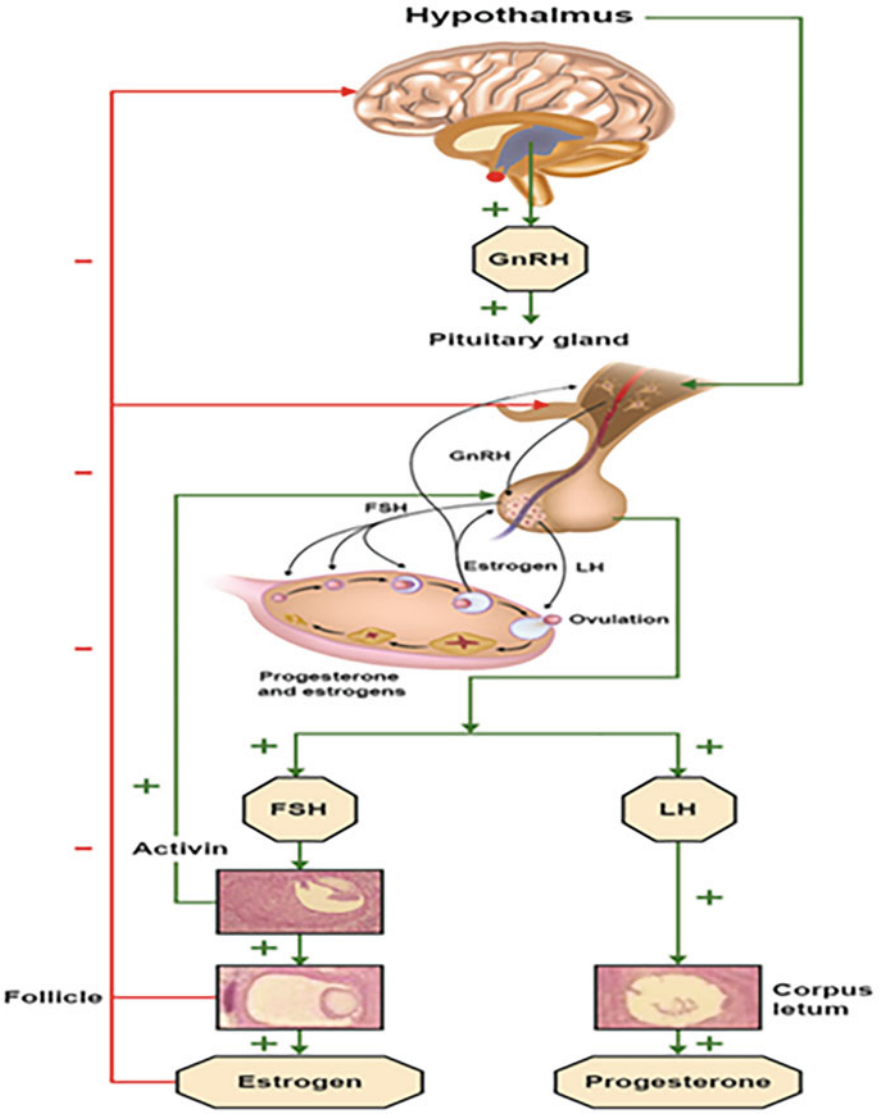

Fig. 9.21 Shows the hypothalamic and pituitary regulation of the ovarian cycle phases and the role of activin which is secreted by the granulosa cells of the ovary with a negative feedback effect on FSH secretion. Estrogen has a positive feedback effect on FSH during the follicular phase and at the end of this phase (on ovulation) estrogen has a negative feedback effect on FSH

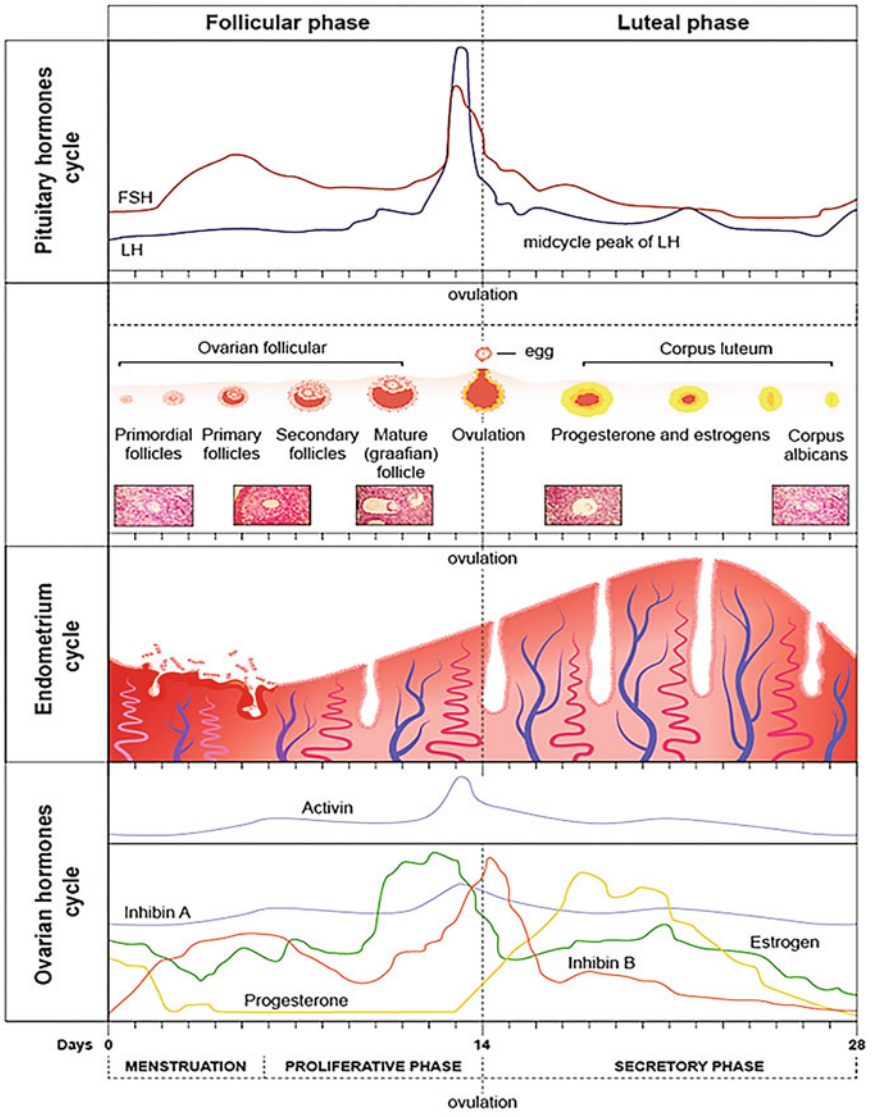

Fig. 9.22 Shows complementarity between the pituitary and ovarian hormones and the ovary and uterus during a single ovarian cycle. (1) Pituitary hormone cycle: FSH and LH levels. (2) Ovarian cycle: follicular maturation, ovulation, and corpus luteum growth. (3) Endometrial cycle: menstruation, reproductive phase, secretion phase. (4) Ovarian hormone cycle: estrange, progesterone, inhibin, and activin levels. All these events take place in the pituitary, ovary, endometrium, and the ovarian circulatory hormones simultaneously

of the smooth muscles in the ovary to release the ovum. The ovum is released 36 h after the LH surge, high levels of which continue for 48–50 h and this usually occurs on day 15 of the cycle (mid-cycle) just after LH peaks on day 14 in the presence of a significant lower FSH peak, a specific concentration of which is needed for LH to be effective. The increase in progesterone leads to sensitization of GnRH to lower the LH level.

3. **Post-ovulatory phase**
   After ovulation, the hypothalamus continues its pulsatile secretion of GnRH to stimulate the pituitary to secrete LH in sufficient quantities to maintain the function of the corpus luteum during this phase so, it secretes more progesterone, estradiol, and inhibin A as a response to LH pulses, and achieves its top size, secretions, and vascularization 6–7 days post-ovulation. Therefore, this phase is called ***the luteal phase or progesterone phase***. It has also been shown that inhibin A starts to increase at the end of the pre-ovulatory phase and remains high in the post-ovulatory phase.

During the **interim phase** from follicle to corpus luteum, progesterone acts via negative feedback on the hypothalamus to slow down pulsatile secretion of GnRH to once every 3–5 h. Luteal degradation is independent of the endometrium, but it is blocked by gestation as the placenta releases hCG (it has an effect similar to that of LH on maintaining the corpus luteum), the luteotropic signal from the trophoblasts from 8 days after conception.

The endometrial functional layer (proliferation, differentiation) has a steroid hormone-dependent effect. Thus, once steroids are reduced, or in the absence of trophoblasts, shedding occurs. With the degradation of the corpus luteum, progesterone levels begin to fall, and GnRH secretion increases to stimulate FSH to restart the cycle (Bullock et al. 1991, 2001; Mihm et al. 2011; Ursula 2017).

9.3.8 Reasons for Corpus Luteum Degradation and Reduced Progesterone

Endocrine, paracrine/autocrine molecular signals are significantly involved with progesterone release during the luteal phase for endometrial receptivity, corpus luteum formation, maintenance, and regression.

Menstruation is initiated by reduction in progesterone-responsive decidual cells. Several factors result in menstruation, including:

- Estradiol metabolites: The human corpus luteum produces many estradiol metabolites which have a significant physiological role through autocrine/paracrine signals, and influence angiogenesis or LH-mediated mechanisms on corpus luteum functions and regression in non-conception ovarian cycles. One of these metabolites is 2-methoxyestradiol (2-ME2), which plays a role in corpus luteolysis through its anti-angiogenic and anti-proliferative effects, while

4-hydroxyestrone (4-OHE1) and 16-keto-E_2 promote angiogenesis in the early and midluteal phases of the normal ovarian cycles (Devoto et al. 2017).

- Increasing the activity of the enzyme aromatase: Rapid actions of estradiol are mediated through changes in aromatase activity, which accurately regulates the temporal and spatial availability of estrogene (Rudolph et al. 2016b).
- Local hormones, such as oxytocin which is secreted from the luteal cells and reduces the secretion of progesterone (Salonia et al. 2005). Also, autocrine signals like prostaglandins released from uterus or ovary during the post-ovulatory phase (such as prostaglandin E2 and prostaglandin F2-alpha.
- Matrix metalloprotease released from leukocytes (Mihm et al. 2011).
- Cytokines which cause necrosis and apoptosis.
- Vasoconstriction.

9.3.9 Functions of the Ovarian Hormones After Puberty

9.3.9.1 Functions of Estrogens

- There are only three estrogens are present in significant amounts in the plasma of nonpregnant human female: **β-esradiol, estrone, and *estriol***.
- **The ovarian cycle is regulated by estrogen feedback** with the help of the pituitary and hypothalamic hormones in general and the pre-ovulatory phase in particular.
- **In the hypothalamus of rats, estradiol and neuroprogesterone interact to stimulate kisspeptin** release in the rostral periventricular nucleus of the third ventricle to exert the LH surge; this is the most critical event in the reproduction cycle. In female rats, morphological alterations in the hypothalamic arcuate nucleus affects sexual receptivity. This is mediated by the rapid control exerted by estradiol-2 on synaptogenesis in that nucleus (Rudolph et al. 2016b).
- **Estrogens activate the cellular response**. Steroid hormones do not only act by binding to their classic mobile/nuclear receptors, but also bind to the target cell membrane. Thus, steroid hormones can initiate cascades of intracellular signaling which in turn elicit rapid actions such as releasing internal calcium ions from stores and the activation of kinases (Mittelman-Smith et al. 2017).
- **Effect of estrogen deficiency**. Several mutations in the aromatase gene (estrogen synthetase gene) lead to complete estrogen deficiency. Thus;
  - **In women**, at the time of puberty, this case leads to virilization of the uterus, primary amenorrhea, hypergonadotropic hypogonadism.
  - **In men**, the most notable characteristic is continued longitudinal bone growth after puberty phase, delayed bone age, and failure of epiphyseal closure; this clearly indicates the significant role of estrogens in bone metabolism in men.
  - **In both sexes**, these symptoms can be treated by estrogen administration (Simpson et al. 1997).
- **In normal humans, estrogen is involved in numerous post-pubertal functions**. Its concentration increases by about 20 times or more from its pre-puberty limited level.

- **Estrogens increase total proteins** slightly in the body and this is seen by a positive nitrogen balance, this is due to the growth promoting action of estrogen on the gonads, the bones and other tissues, but this effect is lesser than the powerful effect caused by testosterone.
- **The metabolic rate** slightly increases equivalent to one-third of what obtained by the effect of testosterone.
- **Effect on body weight and fat deposition**. Total weight of women is less than that of men who have more protein and less fat which can be seen by men floating less than women in water. Women mostly have a larger ratio of body mass as fat, and are more likely to deposit fat subcutaneously, in the breasts, buttocks, thighs (lower extremities) while men are more likely to deposit fat in the abdominal area. Estrogen seems to underlie many of these differences as women tolerate higher nutrient cost during reproductive period. Fat is linked with fertility in women via leptin, low leptin concentrations reduce fertility. Ovarian functions of adult women are associated with their fatness at childbirth. Women have benefited from an increased ability to store fat in easily metabolizable depots, while the style of trunk obesity, more usually seen in men, is not adaptive, but to some extent reflects the genetic trend hypothesis of human susceptibility to obesity. Female obesity, with extreme adiposity in the lower extremities reflects an excess of an adaptation for female reproductive success (Power and Schulkin 2008).
- **Effect on skin**. Estrogens result in skin softness and vascular which associates with elevated warmth and boost greater bleeding of injured surfaces than is seen in men. Estrogens have slight effect on hair distribution.
- Estrogen-like aldosterone and some adrenal cortex hormones **cause sodium** and water retention.
- **They have an anabolic effect on the organs of the female reproductive system**. Estrogens increase the glandular tissue of the fallopian tube, triple uterine size compared to its prepubertal size, changes the vaginal epithelial layer from cuboid to stratified cells, and boosts the proliferation of the mucous cells lining these organs.
- Under the influence of estrogens, the **cervix** secretes a water-based mucous substance.
- Estrogens stimulate proliferation of the **endometrial layer of the uterus** by increasing the flow of water and blood to it and this rapidly increases blood flow to the spiral arteries which become sensitive to estrogen and the subsequent processes needed to feed the implanted ovum.
- Estrogens increase the amount of contractile proteins in the **myometrium** and this effect is very evident during birth which makes this layer contracts by itself with the increase in estrogen. It also increases the sensitivity and number of oxytocin receptors in the area as this helps to expel the fetus, and this is accompanied by a decrease in progesterone and an increase in oxytocin.
- Estrogens stimulate the synthesis of **thyroxine-binding globulins**.
- **Effect on breasts**. Estrogens have receptors on breast tissues, they increase breast connective and develop stromal tissues, as well as growth of the mammary ducts, and deposit fat in the breasts. Estrogen alone develops the lobules and alveoli of

the breast to a little extent lesser than progesterone and prolactin that exert the typical growth and functions of these structures.

- **Estrogens initiate the milk-producing apparatus** and are responsible for the distinctive growth and external look of breasts in the mature female. But they don't complete the task of converting the breasts to complete milk-producing glands.
- **Effect on bones**. In the bones, estrogens act similarly to the androgens
  - Increase bone growth activity.
  - Estrogen plays critical functional function in the development, maturation of bones and in the bone turnover regulation in adult bone. During bones growth, estrogen is required for timely and adequate closing of epiphyseal growth plates. The sex steroids exert this effect in both sexes, but the bone epiphysis closures earlier in female due to the faster and intensive action of estrogen on skeletal growth leading to smaller skeleton volume in female than male that affected by slower action of androgens.
  - To a lesser degree than androgens, estrogens are involved in matrix deposition process in the bones and in calcium and phosphate retention.
  - **In young bone**, estrogen deficiency leads to increased osteoclasts synthesis and stimulated reabsorption of the bone.
  - **In menopause and old age**, estrogen deficiency stimulates osteoporosis and cortical bone derivation.
  - Estrogen is responsible for the distinctive oval shape of the pelvic area in women.
  - (Guyton 1986; Guyton and Hall 2006, 2016; Bullock et al. 1991, 2001; Gardner and Shoback 2007; Greenspan and Forsham 1986; Väänänen and Härkönen 1996).

9.3.9.2 Functions of Progesterone

The most important progestins is progesterone, some amounts of another progestin, 17-α-hydroxyprogesterone is released along with progesterone and have the same actions.

- Progesterone regulates the ovarian cycle in general and the post-ovulatory phase in particular by feedback mechanism with the pituitary and hypothalamus hormones.
- Like estradiol, progesterone can initiate signaling cascades at the cell membrane through classic and non-classic progesterone receptors (progesterone membrane receptors). Progesterone can then also elicit target cellular actions (Mittelman-Smith et al. 2017).
- Progesterone supports the secretory glands in the mucosa lining the fallopian tube.
- Under the effect of progesterone, mucous secretions of the cervix decrease and become thicker. These signs indicate that ovulation has done.

- Estrogen helps proliferation of the uterine endometrium (Fig. 9.18) whereas progesterone supports its secretory glands so that during the post-ovulatory phase, the glands increase in length and secrete a glycogen-rich fluid that encourages implantation of the ovum.
- Progesterone reduces the intensity and sequential contractions in the myometrium. So, it protects the fetus inside the uterus and is therefore the dominant hormone during pregnancy up until birth.
- Progesterone boosts the growth and development of the mammary lobules and alveoli, increasing the alveoli cellular proliferation, enlarge volume and become secretory. Progesterone doesn't cause milk secretion from the alveoli without further preparation of the breasts by the stimulatory effect of prolactin.
- It increases body temperature by 0.2–0.5 °C during most of the luteal phase; this serves as an indicator of ovulation.
- It has a moderate effect on protein catabolism in the body, similar to adrenal cortex hormones which regulate sugar levels especially during pregnancy.
- Similar to estrogen, testosterone, and adrenal cortex hormones, progesterone in large quantities increases the reabsorption of chloride and water from the distal kidney tubules and increase ionic sodium excretion (Guyton 1986; Guyton and Hall 2006, 2016; Bullock et al. 1991, 2001; Gardner and Shoback 2007).

New Novel Functions of Progesterone

Over the years, an interesting observation was made: the rapid effect of the non-genomic progesterone-R interaction in various tissues activates a wide variety of secondary messengers. These include Immunoregulatory function in Human T-lymphocytes via G-protein activation (Sartor and Cutler Jr 1996), platelet aggregation in human T-lymphocytes via Ca^{2+} influx (Scarpin et al. 2009), anti-apoptotic effects in rat (granulosa cells via Ca^{2+} homeostasis (Schlehofer et al. 1999), human intestinal smooth muscle cells contraction via Ca^{2+} currents reduction (Shao 2013) and vasoreactivity in rat vascular smooth muscle cells via Ca^{2+} influx regulation (Shinomiya et al. 1986). In addition progesterone has multiple functions on osteoporosis (Sica et al. 1989) and calcitonin secretion (Silvera et al. 2006). It increases insulin release resulting in promotion of metabolism (Sitruk-Ware 2006). It also reduces brain edema and restores the blood brain barrier (Snow et al. 2011).

Reproductive epidemiology of glial tumors may reveal novel treatments: high-dose progestins or progesterone antagonists as endocrino-immune modifiers against glioma. Progesterone receptor (PR) expression and mifepristone treatment was highly discussed for meningiomas. However, much less is known in regard to progesterone actions in gliomas despite PR expression strongly correlates with their grade (Altinoz et al. 2019). It has been found that systemic treatment of progesterone is neuroprotective as shown in multiple animal models of brain injury including traumatic brain injury. Moreover, progesterone has poor aqueous solubility which has limits its potential for use as a therapeutic agent (Sayeed et al. 2019). It has been reported that treatment with *Coriandrum sativum* has no negative impact on endocrine and reproductive organ structure and function (Al-Suhaimi 2008).

9.3.9.3 Extra-Regulating Roles of Local Hormones Inhibin, Activin, and Follistatin on the Physiology of the Gonads

The gonadotrophs and other cells types within the pituitary gland, produce both inhibin α- and β-subunits as well as follistatin. Activin B is also synthesized locally and promotes FSH release from the pituitary, as proven by a reduction in FSH release following treating cultures of pituitary with an activin-inhibiting antibody (Gregory et al. 2005). In addition to their production from the gonads.

Inhibin Inhibin is a dimeric glycoprotein hormone. It has two biologically active structures; inhibin A and B. Inhibin B is the circulating form. Inhibin is produced mainly by the Sertoli cells and Leydig cells in the testis and, granulosa and theca cells of the ovary.

Activins They are glycoproteins and members of the pleiotropic family of the TGF-beta superfamily of cytokines. They are isolated from pituitary extracts as activin acts as a stimulating factor for FSH.

Follistatin It is also a monomeric glycoprotein, and has multiple biological functions and pathological processes. It plays a key role in reproduction and adipocyte differentiation in vertebrates. It has muscle stimulatory, anti-inflammatory and energy homeostatic effects. Mostly, activin antagonizes the physiological functions of both inhibin and follistatin on the reproductive system.

9.3.9.4 Physiological Functions and Regulation

- In adults, serum inhibin B concentration is positively correlated with Sertoli cell physiological function, testicular volume and number of sperms, and spermatogenesis phase, while it is negatively correlated with FSH level. Thus, inhibin suppresses FSH secretion from the pituitary and regulates steroidogenesis through a negative feedback mechanism.
- Inhibin B aligns FSH levels patterns during the ovulatory cycle.
- Inhibins, which are produced mainly by Leydig cells, support testosterone release.
- The production of inhibin B is regulated by an interaction between FSH, Sertoli cells, germ cells, and Leydig cells.
- It acts as an autocrine or paracrine signal to modulate the activin activity.
- Inhibin A and activin A act inversely. Inhibin A has a perinatal peak in rats, while activin A reaches its peak level in the immediate post-natal duration as it is essential for the growth and regulation of both Sertoli cells and germ cells to be modulated by follistatin.
- The inhibin A concentrations in serum increase toward the late follicular stage. The levels reach a peak during the midluteal stage, followed by a decrease during the late luteal stage. The inhibin B levels in serum are high during the follicular stages and the early luteal stage. Then, decrease during the midluteal and late luteal stages (Yamoto et al. 1997).

- Follistatin suppresses FSH which is responsible for ovarian follicle and oocyte growth, while it has no significant effect on testicular growth,
- Follistatin is a key regulator of activin's biological action, and should be evaluated as a therapeutic polypeptide agent in conditions where activin A overexpression is known essentially as a contributing factor.
- Hypergravity influences follistatin concentration in muscle through the vestibular system in mice. Follistatin plays roles in the metabolic interactions between muscle and bone as response to gravity change.
- Follistatin acts as a stress responsive protein plays a protective function under a several stresses.
- Follistatin like-1 (FSTL1) inhibits proliferation of tumor cell, invasion and survival in non-small cell lung cancer, the overexpression of FSTL1 in the cell line (H446) with low endogenous levels of FSTL1 suppresses cell proliferation, migration, and invasion lead to increase cell apoptosis.
- Activin, is a well-known cytokine belonging to a transforming growth factor (TGF) class, and regulates many reproductive biological functions, inflammation and immunological pathways.
- Activin is a major regulator of testicular and ovarian development. In canines, both glycoproteins activin and inhibin are expressed by developing follicles and corpora lutea in the ovary.
- Activin group is a powerful morphogenetic factor in the fetal testis. While in the adult, it exerts a modulatory effect on Sertoli cell function and spermatogenesis.
- Activins have a promoting role on FSH and ovarian follicular development through the function of aromatase; in contrast, inhibin suppresses hyperplastic/ neoplastic activity.
- Activins are involved in both phases of ovulation and corpus luteum development, but inhibin A is elevated at the end of the follicular phase and continue during the luteal phase to stimulate progesterone production.
- Activin A is increased in both the circulation as well as locally in most reproductive disorders, which makes it an important biomarker. Thus, activin A has a role both as a pro-inflammatory and pro-fibrotic factor.
- Activin A concentrations are much lower in the testis of adult, but Sertoli cell production is activated by interleukin-1 and suppressed by FSH.
- There is limited information on the production of activin B, as a suitable assay method has not yet been identified. However, inhibin beta B-subunit mRNA is expressed in testicular Sertoli and germ cells and is phase-dependent; this expression indicates its importance as an autocrine/ paracrine factor in the seminiferous epithelium.

Understanding the physiological and pathological functions of inhibins, follistatin, and activins in humans make them of significance as biomarkers for conditions of infertility in men, and as prognostic markers in diseases of the reproductive system, or in women undergoing ovulation induction therapy (de Kretser et al. 2004; O'Connor and De Kretser 2004; Kumanov et al. 2005; Marino and Zanghì 2013;

Wen et al. 2015a, b; Tseng et al. 2016; Wijayarathna and de Kretser 2016; Kawao et al. 2018; Ni et al. 2018; Zhang et al. 2018).

9.3.10 Update on Minipuberty: The Fetal Hypothalamus–Pituitary Gonadal Axis

The period of minipuberty of infancy is characterized with greater activity of hypothalamic–pituitary–reproductive axis (Becker and Hesse 2020). The period is also characterized with variation in concentrations of reproductive hormone as well as variation in organ size; however, this period is not characterized with clear description of longitudinal changes. In short, the ovarian size of infant was at its peak at 16 weeks depending on the developed follicles' quantity and size. Hence, the current research may prove helpful in future to determine the reference range of postnatal development of ovary size in healthy term infants (Lanciotti et al. 2018).

The adrenal sex steroid precursor dehydroepiandrosterone initiates the biosynthesis of androgens within the human fetus. The testosterone is produced in the gonads by the conversion of dehydroepiandrosterone. Moreover, the dehydroepiandrosterone in genital skin is stimulated to 5α-dihydrotestosterone which allows external genital differentiation in males. The condition of under-virilization in males is caused by the disruption in dehydroepiandrosterone biosynthesis caused by congenital adrenal hyperplasia because of deficiency of P450 oxidoreductase. Moreover, females may suffer from virilization at the time of birth even in the presence of limited circulating agents. It is postulated that the prenatally active androgen biosynthesis pathway from 17α-hydroxyprogesterone to 5α-dihydrotestosterone may cause virilization in females and it also outperforms the dehydroepiandrosterone and testosterone due to its greater activity in congenital adrenal hyperplasia variants linked with accumulation of 17α-hydroxyprogesterone. The study was performed to investigate the explant cultures of human adrenals, gonads, and genital skin during sexual differentiation. The study analyzed cultures with the help of the liquid chromatography-tandem mass spectrometry which showed the activity of alternative pathway androgen biosynthesis in the fetus. The androgen receptor expression in male and female genital skin were observed in the study with the help of immunohistochemistry which revealed that nuclear translocation of the androgen receptor was stimulated by 5α-dihydrotestosterone as well as adrenal explant culture supernatant in primary cultures of female genital skin. The urinary steroid excretion was evaluated with the help of gas chromatography-mass spectrometry which revealed that androgens are synthesized in the first month of life in P450 oxidoreductase-deficient neonates with the help of alternative androgen pathway (Bizzarri and Cappa 2020).

9.3.11 New Concepts in Gametogenesis

9.3.11.1 Update on Spermatogenesis: Quantitative and Qualitative Factors

Temperature

The spermatogenesis may be adversely affected due to rise in scrotal temperature. It may also result in male infertility. The rise in scrotal temperature causes a sharp decline in the quantity and motility of spermatozoa besides affecting the capability of the sperm to undergo fertilization and it may also lead to poor fertilization-embryo. Mice subjected to 43 °C of scrotal temperature depicted changes in the structure of seminiferous tubule and spermatogenesis. The group of mice subjected to high temperature showed decline in their high Johnsen scores. On the other hand, there was a rise in their ratio of low Johnsen score points. This shows that a scrotal temperature of 433 °C disturbed the Spermatogenesis in male mice. From this experiment, it is also evident that histopathological alterations and spermatogenesis arrest are adversely affected by chronic scrotal heat stress (Thanh et al. 2020).

Obesity

Obesity is the key factor that affects the semen parameters and leads to poor fertility in males. But, there is still some uncertainty regarding the causal association of obesity with infertility in males. This uncertainty is specifically prevalent on a molecular level. This showed that reproductive system dysfunction in males with obesity is closely linked with oxidative stress and inflammation which ultimately affect their sperm function and may cause subfertility due to the negative impact of these processes on spermatogenesis specifically during the protein translation and folding phases (Pini et al. 2020).

The Role of Retinoid-Related Orphan Receptor (ROR)

Morphological testicular defects were detected in retinoid-related orphan nuclear receptor alpha-deficient (ROR) mice (Sayed et al. 2019). Irregular Sertoli cells and hypertrophied spermatogonia were observed during the Transmission electron microscopy examination conducted on mutant mice. The examination also showed spermatocytes with degenerated mitochondria along with partially developed sperms. This implies the significance of ROR alpha protein for regulating the functioning of testicles (Sayed et al. 2019).

9.3.11.2 Emerging Concept of Oogenesis

It was believed for long time that all ova were formed during the fetal period and remained inactive till the female matures. However, this belief has been debunked, as all mammalian ova are continuously formed, produced, and degraded during the reproductive phase and ovaries contain stem cells during the fetal and reproductive periods in human females.

Lately, there has been a development that suggested the presence of mitotically active germ cells in the ovaries of young and adult mice which challenged the earlier

belief regarding the loss of oocyte production ability in majority of female mammals at the time of birth. Consequently, it is confirmed that immunomagnetic separation and consequently culture for at least 15 months may help the neonatal mouse female germline stem cells (FGSCs) line to depict normal karyotype and high telomerase activity. The separation and the consequent culture were performed for FGSCs of adult mice for over 6 months. The process of oogenesis took place within the transplanted cells producing mice with GFP transgene. These outcomes support the performance of fundamental research besides providing foundations for further research in oogenesis as well as stem cell self-renewal; it also allows exploration of new prospects for effective application of FGSCs in the field of biotechnology and medicine (Zou et al. 2009).

In light of the existing evidence, it is clear that mitotically active germ cells are present in postnatal ovaries of various mammals specifically humans. As a result, there are high prospects of making the best use of germline stem cells in adults for generating female gametes externally. Extensive experimentation has been conducted to study the functional attributes of germline stem cells in women ovaries; (these stem cells are also called female germline or oogonial stem cells (OSCs)). One of these experiments includes the study about differentiation capacity of OSCs in humans at cellular level (Virant-Klun 2015). It has become possible to conduct further discussion on this novel concept and study the significance of these cells and management of female fertility in humans by considering the data obtained from these experiments and other data obtained from analysis of intra-ovarian transplantation and genetic tracing through animal models; the models indicated that OSCs are able to produce healthy eggs, embryos and offspring.

Different hormonal signals can critically impact stem cells functions and utilities in different stages of human life; fetal, postnatal, and adult tissues (Ghorbani and Naderi-Meshkin 2016). Growth hormone, insulin, thyroid hormone, parathormone, adrenocorticotropin, glucocorticoids, erythropoietin, and gastrointestinal hormones control stem cells behavior through influencing survival, proliferation, migration, homing, and differentiation of these cells (Fig. 9.23).

9.3.12 Physiology of Menopause and Andropause

Dehydroepiandrosterone (DHEA) is an important source for steroid sex hormone synthesis in men, as well as in women. A high secretion rate of DHEA by the human adrenal glands is associated with the premenopausal age, which suppresses ovarian estrogen release. Cessation of estrogen release at menopause abolishes risks of endometrial hyperplasia and cancer which could result from the non-negative back mechanism of estrogen release during the postmenopausal age. At the start of menopause and with the aging process, DHEA is the only, exclusive and particular sex steroid received by all tissues, except the uterus (Labrie 2010).

Dihydrotestosterone (DHT) is mostly a peripheral production of testosterone metabolism. The postmenopausal ovary is an androgen-releasing endocrine organ, the testosterone concentrations are not straightly influenced by the menopausal

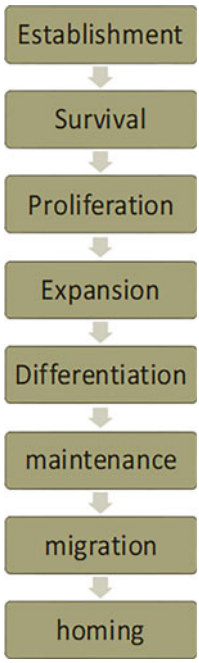

Fig. 9.23 Hormones control Stem cell stages and activities (Al-Suhaimi and Aljafary 2019)

transmission or the happening of menopause (Burger 2002). DHEA, the sex steroid is involved in the improvement of postmenopausal symptoms which appear in women at menopause, including some/most of the following: osteoporosis, muscle weakness, hot flushes, skin and vaginal atrophy, depression, impaired memory and cognition, and some metabolic issues like fat accumulation, type 2 diabetes, and reproductive dysfunction, and other changes. Similar symptoms are associated with aging in men: impaired concentration, insomnia, easy fatigability, nervousness, impatience, depression, impaired memory, hot flushes, periodic sweating, skin atrophy, reduction in muscle mass and strength, bone pain, and sexual dysfunction. Medically, DHEA could be administered without systemic exposure to estrogens for improving the life. In men, adrenal DHEA also contributes to the total androgen. Blocking both testicular and adrenal androgen sources is required for in the typical prostate cancer therapy (Labrie 2010; Tenover 1992).

Reductions in systemic steroid hormones levels may be responsible for the characteristic FSH increase in the premenopausal period in women (Mihm et al. 2011). Menopause starts around the age of 45–50 years in women and andropause at the age of 75 years or more in men due to lack of steroid hormone secretion owing to diminishing gonadotropic function of the testis/ovary and their non-responsiveness to the pituitary hormones' LH and FSH. Menstruation stops suddenly over a short period of time or after a gradual decrease in the number of cycles or irregular ovulation and menstruation. This can take up to 10 years.

As mentioned earlier, although ovarian stem cells are available during the aging period, stem cells cannot prevent menopause because with advancing age, there is a

regression in the contribution of the immune system to the process of neo-oogenesis and follicular renewal in vivo from germline stem-cell precursors. The reduction in fertility induced by advanced age involves ovarian kisspeptin regulation for the sympathetic innervation (Rudolph et al. 2016b). It has been reported that treatment of cerium oxide nanoparticles caused amelioration of diabetes-induced testicular and sperm in rats (Artimani et al. 2018).

9.3.13 Stem Cells and Bi-Potential Progenitor Structure Genital Ridge

The significance of sex determination in context of sexual reproduction cannot be denied for reproductive functions since it develops functional male or female reproductive cells or gametes essential for the reproduction. The sex is initially determined in mammals depending on whether the Y-chromosome is present or not; the Y-chromosome regulates gonadal ridges or primordium. The reproductive organs that distinguish between male and female reproductive systems (i.e., ovary or testis) are developed at the mid-gestation stage by the genital ridges which are somatic precursor of gonads (Burkitt et al. 1996). An individual's gender and his germ cells are identified on the basis of genital ridge which in turn is dependent on somatic cell differentiation; in case of testes, the somatic cells differentiate into Sertoli cells while in case of ovary, there is differentiation of somatic cells into granulosa cells. Various factors that help in the determination of sex in mammals were identified in mouse models and human mutation studies performed on individuals with disorders of sex development. The testicular differentiation in majority of mammals is regulated by the genetic indicator of the gene Sry present in Y-chromosome (Tanaka and Nishinakamura 2014).

Anatomy, physiology, genetics, bio and regenerative medicine, should be integrated to know a comprehensive knowledge of the later stages of development of the reproductive system in human and animal models. The ovary and testis arise from bi-potential progenitor structure called as the genital ridge (GR). This structure forms in a late stage in embryonic growth to potential formation either the ovary or testis, and then their hormones required for the growth of the reproductive system. Studying the genetic networks for GR formation leads not only to understand of the genetic regulation of reproductive development but gives new approaches for managing reproductive abnormalities and infertility (Yang et al. 2018).

Oncostatin M (OSM) acts as an inhibitory factor of rat stem Leydig cell growth. In vivo seminiferous tubule culture system, stem Leydig cell growth was reduced along with the differentiation due to action of inhibitor Oncostatin M (OSM) (Wang et al. 2019). Mainly the testosterone level and downstream effect of the Leydig cell-specific genes expression and their proteins (Lhcgr, Star, Hsd3b1, Cyp17a1 Cyp11a1, and Hsd11b1). OSM related function was downregulated due to the influence of S3I-201 (a STAT3 antagonist) or filgotinib (a JAK1 inhibitor). Clinically, exogenous testosterone could be administrated for treating testosterone deficiency; but it has several contrary effects include infertility because of negative

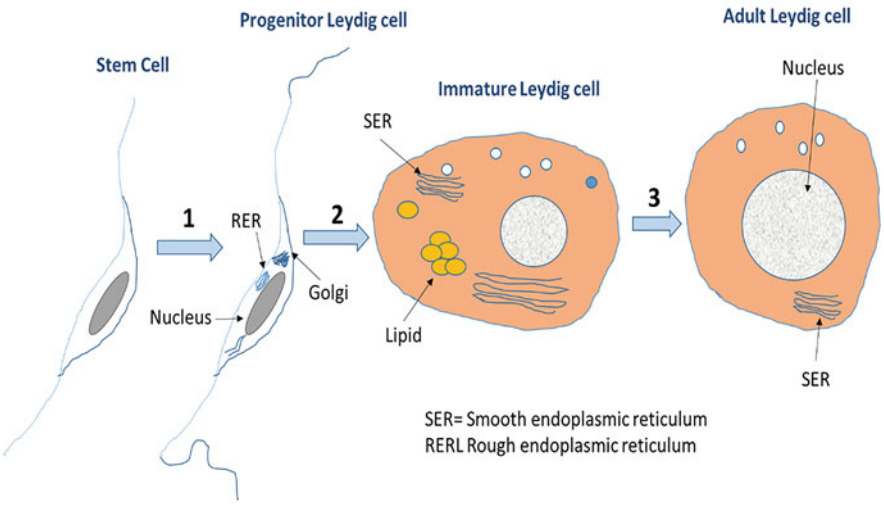

Fig. 9.24 Differentiation of stem cells into Leydig cell (1) stem cell differentiates into progenitor cell (2) progenitor cell differentiates into immature Leydig cell and (3) immature Leydig cell grows into adult Leydig cell

feedback on the hypothalamus-pituitary-gonads (HPG) axis. Arora et al. (2019) for the first time demonstrated that autografting of Leydig stem cell (LSC) subcutaneously together with Sertoli cells and myoid cells, stimulate testosterone synthesis. Therefore, autograft of LSC may provide a new strategy for treating testosterone deficiency while simultaneously stabilizing HPG. The differentiation of stem cells into Leydig cell with three stages is shown in Fig. 9.24.

Presently, the effective de novo synthesis of testicular tissues and consequently the complete spermatogenesis in vitro has been observed only in rodents. It is also indicated in some outcomes that human testicular organoids (TOs) or multi-cellular tissue surrogates may be produced as primary human testicular cells undergo self-organization both in the presence and absence of biological support (Baert et al. 2017). Although these mini-tissues thus produced do not have the testis-specific topography, they are successful in facilitating spermatogonia. Moreover, in long-term culture, the niche cells inside these tissues maintained their particular functionalities. Hence, it is evident that in vitro re-engineering of human testicular microenvironment can be done from primary cells. It is possible to develop a biomimetic testicular model from human TOs; such a model would facilitate research and development in this field as well as clinical treatment and screening of infertility; it also supports drug discovery and toxicology (Baert et al. 2017). Functional assessment of spermatogonial stem cell has been done and this technique will not only be useful to link functional relevance to novel markers that will be identified in the future, but also for providing validation of purity for marker-selected populations of spermatogonia that are commonly considered to be spermatogonial stem cell by many researchers (Lord and Oatley 2018).

9.4 Conclusion

Pituitary gland in endocrine system in coordination with hypothalamus plays a vital role in reproductive system, differentiation and different physiological functions in the entire stages of life and its circadian rhythm in both male and female. Description about male gonads, location, physiological functions of testicles, interstitial tissue (Leydig cells) and peritubular myoid cells. The Sertoli cells act as "nurse & stem" cells, spermatogenesis, spermiogenesis. Gonad's steroid hormones such as androgens are involved in male reproductive activity. Ovaries are female reproductive glands and there are two main functions (exocrine and endocrine) controlled and coordinated by the hypothalamus and the pituitary. Female sex hormones in pre-puberty (Estrogen) and post-puberty (estradiol, estrone, progesterone, and inhibin), and sources (ovarian follicle and corpus luteum) were discussed in detail. Structure of steroid hormones discussed with role of endometrium, regulation of ovarian functions, and puberty by endocrine and immune system. The different phases of ovarian cycle explained to regulate gonadotropins, follicular growth, steroid synthesis, non-functional corpus albicans (infertile ovum), and regulation of ovarian cycle (pre-ovulatory phase, ovulation phase, and post-ovulatory phase). The involvement of estradiol metabolites, enzyme aromatase, hormone oxytocin, matrix metalloprotease, cytokines and vasoconstriction in corpus luteum formation related along with maintenance and regression. Synthesis of ovarian hormones (β-esradiol, estrone, and estriol) after puberty discussed with important functions of progesterone, regulatory roles of inhibin, activin, and follistatin in physiology of testis and ovary. Dehydroepiandrosterone (DHEA) involvement discussed for menopause in elderly women and andropause in men. The chapter declares that the classic theory of cessation of oocytes production after birth was cancelled. Both human neonatal and adult ovarian germline stem-cell precursors (ovarian surface cells) have the capability for oogenic or differentiating and producing functional oocytes, so it renews the oocyte pool (neo-oogenesis) and ensure renewal during the prime reproductive period, with follicular co-operation under regulation of the endocrine, immune systems, and cellular support. After the prime reproductive period, aging starts, and menopause occurs because of the immunoregulatory changes that causes cessation and terminate neo-oogenesis and follicular renewal in vivo despite the existence of germline stem cell precursors. The rest of oocytes in the primordial follicles retain ovarian function but advancing age (aging oocytes) correlates positively with the occurrence of fetal chromosomal abnormality.

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