Chapter 3 Physiology

CHAPTER OUTLINE

Neuroendocrinology with vast hormonal interactions is responsible for menstrual cycle and reproductive functions in a woman.

It is now well established that a normal menstrual cycle depends on cyclical ovarian steroid secretions, which in turn are controlled by the pituitary and the hypothalamus and, to some extent, are influenced by the thyroid and adrenal glands. It is therefore essential to understand the hypothalamus–pituitary–ovarian axis in normal women (H–P–O) and apply this knowledge in therapeutic management in infertility, family planning and various gynaecological disorders.

Hypothalamus

Hypothalamus with its several nuclei and extrinsic connections is now considered the main neuroendocrine gland and the regulatory factor in the chain of hypothalamic–pituitary–ovarian–uterine axis. Hypothalamus regulates the functions of the anterior pituitary gland through portal vessels by releasing both the stimulatory and the inhibitory hormones that in turn influence the functions of the target tissues through the systemic circulation (Figure 3.1A and B). These hormones in turn are controlled by positive and negative feedback loops from ovarian hormones. External and internal stimuli further modify or influence hypothalamic functions.

Hypothalamus is located at the base of the brain behind optic chiasma and below the thalamus above the pituitary and forms the base of the third ventricle. The base of the hypothalamus forms tuber cinereum, which merges to form the pituitary stalk. The origin of this stalk is known as median eminence, which is rich in capillary loops as well as nerve endings. Median eminence is an important site of storage of chemical signals, which get transferred into portal circulation to reach the anterior pituitary gland. Schally and Guillemin were the first to discover a decapeptide called gonadotropin-releasing hormone (GnRH) in 1971. GnRH is secreted by the median eminence and the arcuate nucleus, which modulates the neural control of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary gland. It (arcuate nucleus) also secretes prolactin-inhibiting factor (PIF), which is dopamine that inhibits the release of prolactin. During late pregnancy and lactation, a low or absent inhibitory factor leads to a high secretion of prolactin that initiates and maintains lactation.

Hypothalamus is also responsible for secretion of thyrotropin releasing factor, corticotropin releasing factor, insulin-like growth factor and melanocyte releasing factor.

Hypothalamus is connected to the anterior pituitary gland through special hypophysis pituitary portal system of vessels but connected directly to the posterior pituitary gland (neurohypophysis) by the supraoptic and paraventricular nuclei (Figure 3.2).

GnRH (decapeptide) is synthesized in arcuate nucleus and is released at the nerve endings near tuber cinereum. GnRH has a half-life of 2–4 min and is therefore difficult to assay. Its level is assessed through the LH level. It is released in a pulsatile manner into the portal vessels and reaches the anterior pituitary gland. *The pulsatility and amplitude of its release vary with the various phases of the menstrual cycle*. In the preovulatory phase (follicular phase), it pulses once in

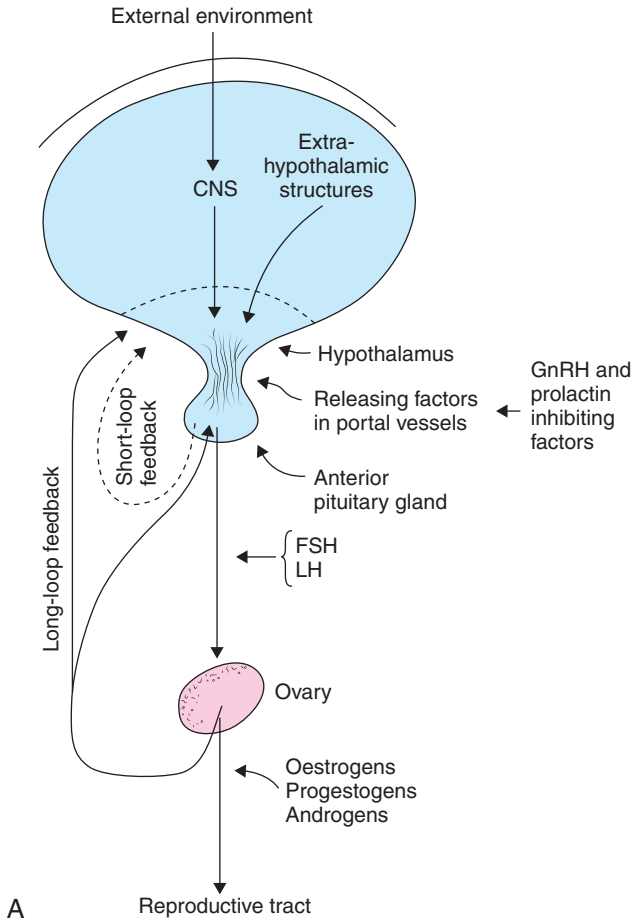

Figure 3.1 **(A)** Hypothalamic–pituitary–ovarian axis.

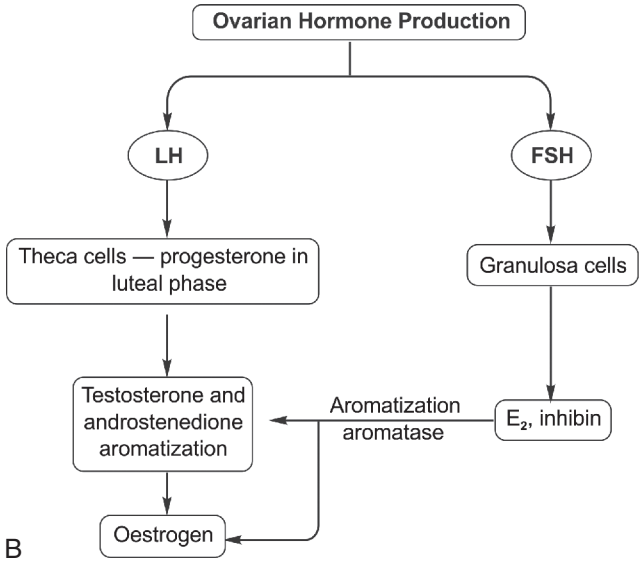

Figure 3.1 **(B)** Ovarian hormone production.

every 60 min, but it slows down to once in 3 h in the luteal phase, with increased amplitude of each pulse.

GnRH exhibits different actions depending on the manner in which it is released. Its continuous release causes suppression of gonadotropins and thereby the ovarian functions through the process of 'down-regulation' or desensitization

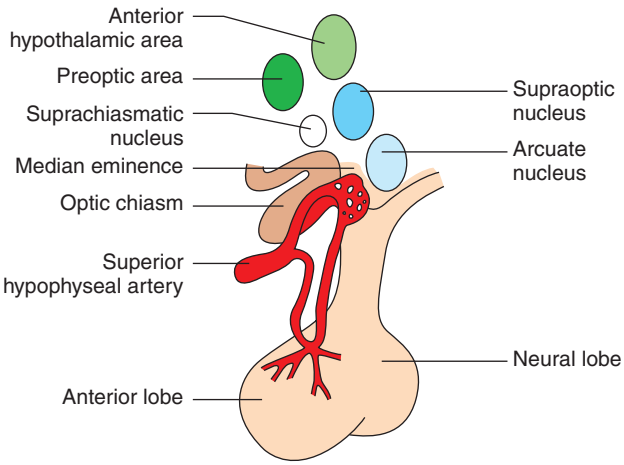

Figure 3.2 Hypothalamic nuclei.

of pituitary hormones. This mode of administration is now employed in therapy using synthetic analogues of GnRH in regulating ovulation in in vitro fertilization and suppressing menstruation in precocious puberty, in reducing the size of the uterine fibroids and in causing shrinkage of endometriosis. Its suppressive effect on ovulation is also being tried as a contraceptive, but the drug has proved expensive as of today. The pulsatile administration, on the other hand, causes cyclical release of gonadotropins, FSH first and later LH which induces ovulation and the possibility of a pregnancy. This therapy is applied in women with anovulatory infertility.

Hypothalamus can be influenced by the higher cortical centres, especially the temporal lobe. Emotional upsets are known to stimulate or depress the H–P–O axis and disturb the menstrual cycles. Neuro-endocrine system works through several loops, both positive and negative.

- Long loops through oestrogen and progesterone
- Short loop through anterior pituitary gland
- Ultrashort loop within the hypothalamus

Epinephrine and oestrogen stimulate whereas dopamine, serotonin and opioides inhibit the release of GnRH by the hypothalamus. Gonadotropins also inhibit GnRH secretion.

Until puberty, the hypothalamus is in a dormant state under the inhibitory influence of adrenal cortex, and the higher cortical centres, or it may be insensitive and nonresponsive to these stimuli. It becomes gradually sensitive around 8–12 years and starts its hormonal functions, fully establishing the H–P–O axis by the age of 13–14 years. What triggers GnRH to start functioning is not clear, but perhaps leptin produced by the adipose tissue that initiates the response. Initially, GnRH is released in a pulsatile manner during sleep, but later throughout 24 h. In the follicular phase, with low oestrogen (E_2) level, pulsatility is every 90 min, and with rise in E_2 level, the frequency rises to every 60 min. In the luteal phase, the frequency slows down to 1 in 3 h. Hypothalamus is sexually differentiated at birth. GnRH secretion is continuous in males, but pulsatile in females. Administration of testosterone to a female rat at birth is shown to cause a continuous secretion of GnRH in later life and alter the hormonal function to a male type.

Synthetic analogues of GnRH are nanopeptides and are now available and are used in the following:

- Preoperative shrinkage of uterine fibroids
- Shrinkage of endometriosis
- Shrinkage of endometrium prior to endometrial ablation
- Hirsutism
- Precocious puberty
- In vitro fertilization
- Prostatic cancer

Prolonged administration over 6 months can cause oestrogen deficiency and osteoporosis, and therefore the therapy should be used on a short-term basis. This peptide is degraded in the gastrointestinal tract and is therefore given intravenously, subcutaneously or intranasally. Its short life mandates repeated administration at short intervals. However, depot monthly injections are available.

Side effects of GnRH are as follows:

- Insomnia
- Nausea
- Osteoporosis caused by oestrogen deficiency, but reverts to normal after stoppage of the drug
- Decrease in breast size—reversible
- Myalgia, oedema
- Dizziness
- Decreased libido
- Decrease in high density lipoprotein (HDL) and increase in cholesterol by 10% each

The drugs and their administration are as follows:

- Nafarelin 200 mcg intranasally daily for 6 months.
- Buserelin 300 mcg TID subcutaneously daily × 5 days.
- Depot injection of goserelin IM or implant 3.6 mg monthly.
- Leuperide 3.75 mg IM monthly × 5 months.
- Triptorelin 3.7 mg IM 4 weekly.
- Antagon is GnRH antagonist used in down-regulation in in vitro fertilization.

Pituitary Gland (Adenohypophysis)

Pituitary gland lies in the sella turcica. It measures 1.2 × 1 × 0.6 cm and weighs 500–900 mg. It comprises the anterior pituitary gland (adenohypophysis) and the posterior pituitary gland (neurohypophysis). The anterior pituitary gland originates at the roof of the embryonic pharynx called Rathke's pouch and contains chromophil and chromophobe cells. The posterior lobe develops from the floor of the brain. The two lobes of the pituitary gland develop independently of each other. The anterior lobe is ectodermal in origin.

The anterior pituitary gland measuring 30 × 6 × 9 mm in size is located at the base of the brain in a bony cavity called sella turcica below the hypothalamus. It consists of three histologically distinguishable cells: (i) the chromophobe or parent cell, (ii) the chromophil cells described as eosinophil or alpha (α) cells and (iii) basophil or beta (β) cells. The β-cells secrete the gonadotropins that control the ovarian function and menstrual cycles. These gonadotropins are FSH, LH, thyroid-stimulating hormone (TSH) and corticosteroid hormone. Each of these hormones has α- and β-fractions. Whereas α-fraction is identical in all (contains 92 amino acids), β-fraction is specific in its action.

Follicle-Stimulating Hormone

FSH is a water-soluble glycoprotein of high molecular weight and is secreted by the β-cells; it contains 115 amino acids in β-fraction. The carbohydrate fraction is mannose. FSH controls the ripening of the primordial follicles, and in conjunction with the LH, it activates the secretion of oestrogen. Its activity builds up as the bleeding starts to cease reaches a peak around the seventh day of the cycle (40 ng/mL) and then declines to disappear around the 18th day. Another small peak occurs after ovulation, perhaps as a result of a fall in the level of oestrogen in the premenstrual phase. The half-life of FSH is 4 h. Low FSH causes defective folliculogenesis and short or defective corpus luteal phase. Oestrogen suppresses FSH secretion through negative feedback mechanism. It develops LH receptors in the granulosa cells.

Gemzell initially isolated FSH from the pituitary of human cadavers at autopsy, but it required 10 pituitaries to produce enough FSH for one ovulation. FSH is now commercially obtained from the urine of menopausal women. The preparation contains both FSH and LH. Pure FSH is now available on the market but is very expensive.

Luteinizing Hormone

LH is a water-soluble glycoprotein of high molecular weight secreted by β-cells; it also contains 115 amino acids. The carbohydrate fraction is mannose. LH pulse occurs only during sleep initially, but later extends throughout the day. LH surge initiated by oestrogen lasts for 48 h and is preceded by a small amount of progesterone 2 h earlier. LH level doubles in 2 h and the peak plateaus for 14 h before declining. Progesterone secretion begins 34 h after LH peak. In conjunction with FSH, it activates the secretion of oestrogen, brings about the maturation of the ovum and causes ovulation. LH stimulates the completion of the reduction division of the oocyte. Following ovulation, it produces luteinization of the granulosa and the theca cells and initiates progesterone secretion. The LH surge precedes ovulation by 24–36 h (mean 30 h) and a minimum of 75 ng/mL is required for ovulation. This time relationship of LH peak to ovulation is helpful in predicting the exact time of ovulation in infertile women on gonadotropin therapy, making it possible to retrieve ova in in vitro fertilization and to arrange for timely artificial insemination to enhance chances of conception. LH stimulates the secretion of testosterone and androstenedione in the ovarian stroma (theca cells), which diffuse into the follicular fluid and are aromatized into oestradiol.

Today, for diagnostic and therapeutic purposes, a rapid, visual semiquantitative enzyme immunoassay dipstick test,

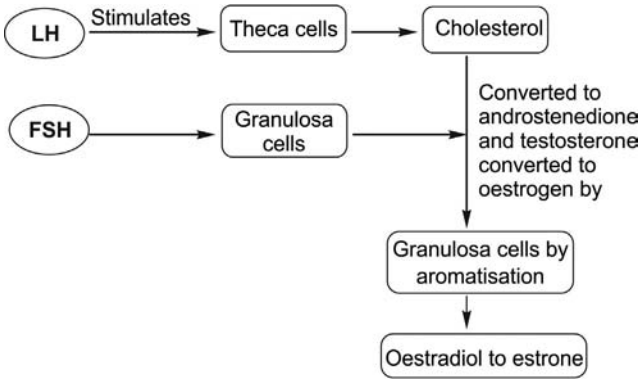

Figure 3.3 Two-cell two-gonadotropin theory of ovarian steroidogenesis.

called OvuSTICK, is available for testing urine to detect the LH surge by undertaking daily LH estimations around the period of ovulation. These kits are expensive. The half-life of LH is 30 min (Figure 3.3).

Human Chorionic Gonadotropin (hCG)

Secreted by the trophoblastic tissue in pregnancy, human chorionic gonadotropin (hCG) has a luteinizing action and is available in injectable form for use in cases of anovulatory infertility, in vitro fertilization, corpus luteal insufficiency and habitual abortions. hCG contains α- and β-fractions. The α-fraction resembles LH and TSH, but the β-fraction is exclusively specific to chorionic tissue. It is commercially obtained from the urine of pregnant women. The level is increased in trophoblastic tumours and some ovarian tumours. Recombinant hCG is now available, which has less side effects at the site of injection.

Prolactin

Prolactin is an alcohol-soluble protein (polypeptide) (198 amino acids) without a carbohydrate fraction and with a half-life of 30 min. It is secreted by α-cells. Its main action is on lactation. It has a suppressive effect on the pituitary–ovarian axis, and therefore the patient who suffers from hyperprolactinaemia may develop amenorrhoea or oligomenorrhoea due to anovulatory cycles, with or without galactorrhoea. Normal prolactin level is 25 ng/mL. Up to 100 ng/mL occurs in hyperprolactinaemia but over 100 ng/mL is seen in pituitary tumours. The prepubertal level of 7 ng/mL rises to 13 ng/mL at puberty and 25 ng/mL in an adult woman. Active prolactin is present in the form of monomer or 'little prolactin' (50%), whereas dimeric and multimetric (big prolactin) forms have negligible biological activity. Normally, the prolactin is under tonic hypothalamic inhibitory factor (PIF), which is probably dopamine and is released into the portal system. The level of prolactin is raised during sleep, nipple stimulation and the secretion of thyroid-releasing hormone, β-endorphin, serotonin and oestrogen.

Prolactin level does not fluctuate much during the menstrual cycle. It suppresses LH but not FSH, so hyperprolactinaemia decreases the LH/FSH ratio.

Growth hormone, insulin-like growth factor, epidermal growth factor, adrenal cortex and *TSH* also participate in the endocrinological functions in a woman, through their action on the hypothalamus and anterior pituitary gland. A high level of TSH stimulates prolactin secretion and causes ovulatory and menstrual dysfunction. Interleukin-1 is a cytokine with antigonadotrophic activity and it prevents luteinization of granulose cells.

Posterior Pituitary Gland (Neurohypophysis)

Oxytocin and vasopressin are nonapeptides formed in the hypothalamus and released directly into the posterior pituitary gland. Oxytocin is produced by the paraventricular nucleus and vasopressin by the supraoptic nucleus of the hypothalamus.

Oxytocin

Oxytocin acts mainly on the smooth muscle of the uterus, causing contraction of the muscles and controlling the bleeding in the third stage of labour. By intermittent uterine contractions and relaxation, it induces and enhances the labour pains, in the first and second stage of labour. It causes contraction of the myoepithelial cells lining the mammary ducts and ejects milk during suckling.

Vasopressin

Vasopressin maintains the blood volume and blood pressure. Both have antidiuretic action when given in large quantities (over 20 units of oxytocin in 24 h). The therapeutic applications of these hormones are described in Chapter 43.

Ovarian Steroidogenesis

The active hormones of the ovary are the steroids derived from cholesterol. These include oestrogens, progesterone, testosterone and androstenedione (Figure 3.1A).

Oestrogen

Natural oestrogens are C18 steroids, the main source of which are the theca and granulosa cells of the Graafian follicles and corpus luteum, while the adrenal cortex is the secondary source of supply. Oestrogen is secreted as oestradiol. It is bound to albumin (30%) and sex-hormone-binding globulin (SHBG, 69%), and only 1% is biologically active. It acts by binding to cytoplasmic receptors in the cells. It is inactivated by the liver and excreted as conjugates of oestrone, oestradiol and oestriol

in the urine and bile (85% in urine, 10% in faeces). The plasma oestradiol level rises approximately 6–7 days before ovulation from 50 mcg daily to the peak level of 300–600 mcg about 2 days before ovulation and approximately 24 h before the LH peak (level up to 350 pg/mL). Thereafter, the oestradiol concentration falls to 150–200 mcg daily, but a small rise is seen again in the mid-luteal phase. The urinary excretory level follows the pattern seen in the plasma. The oestradiol peak seen before ovulation is not as a good marker for indicating ovulation as LH, because follicular maturation does not always end in ovulation. A serum level of oestrogen with ultrasonic monitoring is used to monitor the optimal time to administer hCG for the therapeutic induction of ovulation. Whereas oestradiol, which is 10 times as potent as oestrone, is present during reproductive period, it is oestrone derived from peripheral aromatization of androstenedione that is predominant in menopausal women. The placenta is the main source of oestriol. Each cycle produces 10 mg of oestradiol.

Synthetic oestrogens are readily available in the market and are used in various gynaecological disorders. They are absorbed orally and through vagina and skin.

Actions of Oestrogens (Figure 3.4)

1. **Feminization and secondary sex characteristics.** The texture of the female skin and hair and the shape of the female form are considerably influenced by oestrogen.
2. **Specific action on the genital tract.**

Vulva and vagina

- Development of the vulva.
- Vascular stimulation of the vulva and vagina.
- Epithelial stimulation of the vulva and vagina.
- Cornification of the superficial layers of the vagina, which appear as acidophilic polyhedral cells with a small pyknotic nucleus. Oestrogen raises the karyopyknotic index in vaginal cytology (Ch. 6).
- Deposition and metabolism of intracellular glycogen in the vaginal epithelium.

Uterus

- Causes myohyperplasia of the myometrium and cervix.
- Increases uterine vascularity.
- Regenerates the endometrium after menstruation and is responsible for the proliferative (preovulatory) growth of the endometrium. Oestrogen causes proliferation of epithelial lining, glandular cells and stroma and mitosis. Spiral vessels elongate and stretch the entire length of endometrium, and dilate.
- Stimulant effect on the glands of the endocervix and their mucous secretion.

Fallopian tubes

Oestrogen stimulates the tubal musculature, which is, in fact, morphologically specialized myometrium.

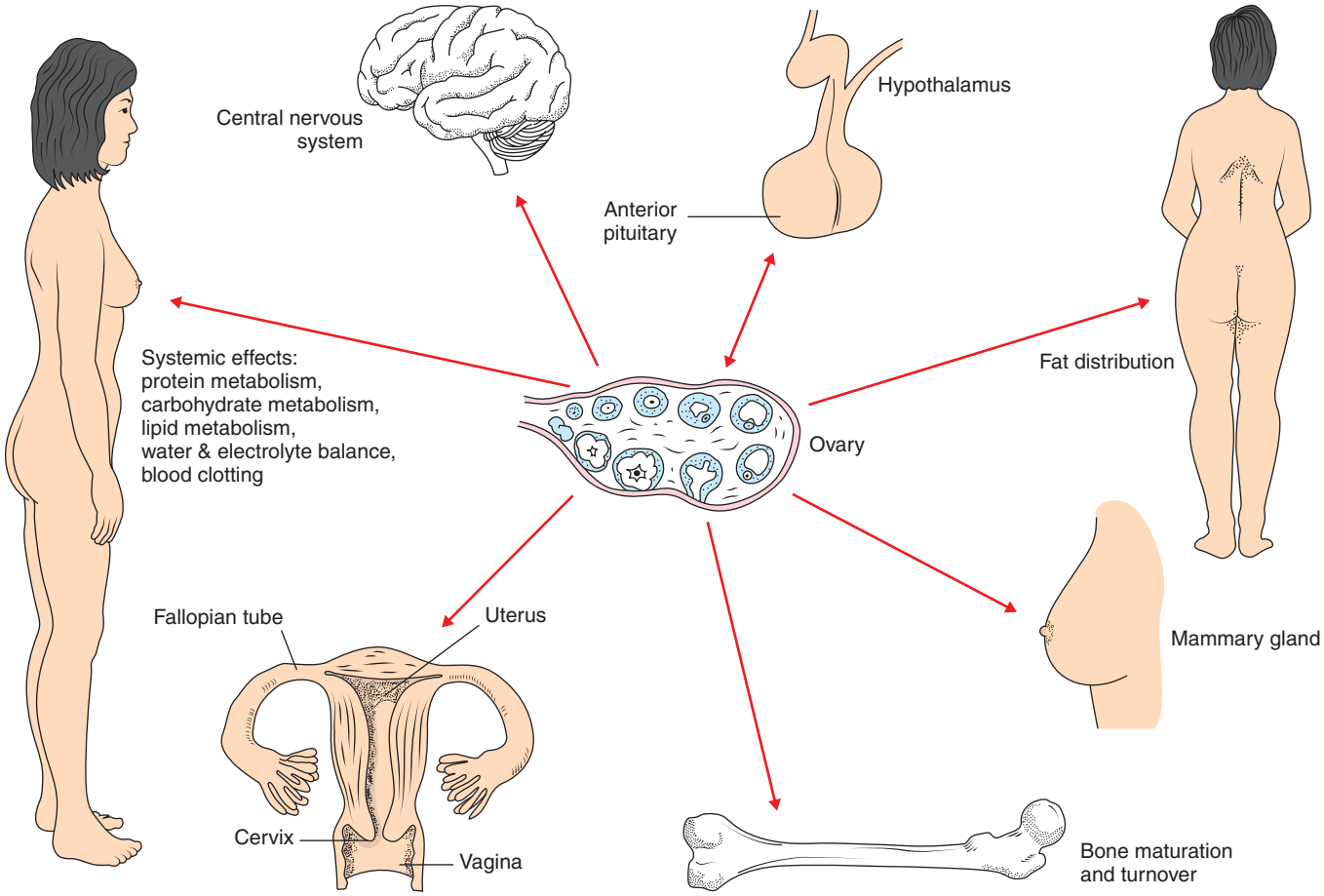

Figure 3.4 Physiological effects of oestrogen.

Ovary
No action.

3. **Breast**. Hypertrophy of the ductal and parenchymal tissue of the breast, increased vascularity, areolar pigmentation, but no galactogenic effect. Large doses suppress lactation.
4. **Action on other endocrine glands**. Oestrogen suppresses FSH and thyrotropic hormones. It can be used to inhibit ovulation as also production of milk in the puerperal patient. It is stimulant to the LH and thereby corpus luteum formation and, to a lesser extent, to ACTH.
5. **Skeletal system**. It increases calcification of bone and the closure of epiphyses in the adolescent and is antagonistic to somatotropin. In the postmenopausal women, decalcification of bone (osteoporosis leading to kyphosis) is, in fact, due to oestrogen deficiency.
6. **Water and sodium metabolism**. Oestrogen tends to cause water and sodium retention. An example is premenstrual tension, which is caused by congestion and water retention. It also causes calcium and nitrogen retention.
7. **Blood cholesterol**. Blood cholesterol levels are to a small extent controlled by oestrogen, hence the importance of ovarian conservation when performing hysterectomy in a young woman. HDL increases under oestrogen influence and is cardioprotective.
   - Oestrogen improves the skin by producing collagen.
   - By raising fibrinogen level, it can cause thromboembolism and is a major side effect of oestrogen.
   - It increases SHBG by the liver.

Progesterone

The corpus luteum is the main source of progesterone and a small amount is derived from adrenal gland (2–3 mg), seen in the proliferative phase. Although progesterone is an important intermediary product in the synthesis of adrenal corticosteroids, it has little, if any, biological action from this extra ovarian source. The plasma level of progesterone rises after ovulation and reaches a peak level of 15 ng/mL at mid-luteal phase. With the degeneration of the corpus luteum, its level falls and this brings about menstruation. In an anovulatory cycle, progesterone is absent or is in negligible amount (from extra ovarian sources). Menstruation is then brought about by a fall in the level of oestrogen. If pregnancy occurs, the corpus luteum persists, even enlarges and continues to secrete progesterone. This high level of hormone prevents menstruation and leads to amenorrhoea of pregnancy. It is excreted in the urine as sodium pregnanediol 3-glucuronide and recovered as such for assay in the secretory phase of the menstrual cycle. Progesterone is bound to albumin (80%) and corticosteroid-binding globulin (20%). Daily production in the luteal phase is 20–40 mg and daily urine excretion is 3–6 mg. Mid-luteal phase level of less than 15 ng/mL suggests corpus luteal phase defect (LPD) and ovulatory dysfunction.

Radioimmunoassay is currently used to estimate the plasma progesterone levels in mid-luteal phase in cases of infertility. However, with development of enzyme immunoassay, a home 'dip-stick' test can estimate urinary pregnanediol to determine occurrence of ovulation. Salivary progesterone level is estimated by direct use of solid-phase enzyme immunoassay (Dooley). Several synthetic progesterones (progestogens) are now available for commercial use (Figure 3.1A and B) .

Actions of Progesterones

Endometrium. Progesterones cause secretory hypertrophy and decidual formation if the endometrium has been previously primed with oestrogen. Glycogen and mucus collect in the tortuous glands.

Pregnancy. Progesterone initially from the corpus luteum and later from the placenta is essential for the continuation of pregnancy.

Uterus. Progestogens cause myohyperplasia of the uterus. They increase the strength but diminish the frequency of uterine contractions.

Fallopian tube. Progestogens cause hyperplasia of the muscular lining of the fallopian tube and make peristaltic contractions more powerful as well as increase the secretion by the tubal mucous membrane.

Cervix. Progestogen causes hypertrophy of the cervix and makes the cervical mucus more tenacious. It renders the internal os competent and holds the pregnancy to term.

Vagina. During early pregnancy the vagina becomes violet coloured due to venous congestion. The epithelial cells fail to mature and cornify. They are classically basophilic with fairly large nuclei and folded edges. Karyopyknotic index falls to below 10%.

Breasts. Progestogens, with oestrogen, cause breast hypertrophy. They increase acinar epithelial growth.

Pituitary. The exact action of progestogens on the pituitary is not known. Progestogens may inhibit the production of FSH and suppress ovulation. A certain percentage of progestogens is metabolized to oestrogen, and it may well be that the oestrogen so produced is responsible for inhibiting pituitary activity.

Fluid retention. Progestogens cause water and sodium retention and is a contributory factor in premenstrual tension and weight gain.

Smooth muscle. Progestogens relax smooth muscles. The uterine muscles therefore relax in pregnancy. Ureter dilates under its effect.

Thermogenic. Progestogens raise the body temperature by 0.5°C. Basal body temperature (BBT) chart is based on its thermogenic effect during the menstrual cycle.

Anabolic effect. Progestogens exert anabolic effect and this partly accounts for some of the weight gain which may follow their administration.

Libido. Diminution of libido infrequently occurs.

Virilization. While part of the administered progestogen is metabolized to oestrogen, it is also partly metabolized to testosterone. If administered to a patient during pregnancy, some progestogens have virilizing effect upon a female fetus.

- Lipid metabolism decreases HDL but increases low-density lipoprotein. Thus, it is harmful to the heart.
- It improves the immune response.

Side Effects

If given in large doses, progestogen can cause gastrointestinal symptoms, nausea and vomiting. Headache and mild elevation of temperature are also seen. In fact, all symptoms of pseudopregnancy state may be observed—water retention, breast enlargement and tenderness, and moderate uterine enlargement. Virilism has been reported with some synthetic progestogens, especially 19-nortestosterones. Some exhibit adverse effects on lipid metabolism and increases the risk of breast cancer. Thrombosis of deep veins, pulmonary embolism and arterial thrombosis are rare but are reported with third generation of synthetic progestogens (gestodene, desogestrel) (Table 3.1).

Relaxin

This hormone relaxes the connective tissue and is probably secreted by the ovary. Relaxin is a water-soluble protein and nonsteroid. It may have a role in pregnancy and may be responsible for relaxation of pelvic joints and pelvic floor muscles.

Inhibin

Inhibin is a nonsteroidal water-soluble protein (peptide) secreted by the Graafian follicle. McCullagh identified this protein and named it inhibin, because it is known to suppress pituitary FSH. Inhibin consists of two peptides, namely inhibin A (α-fraction) and inhibin B (β-fraction). In normal ovarian folliculogenesis, FSH and LH initiate secretion of oestrogen by the Graafian follicle. Oestrogen is responsible for secretion of inhibin in the Graafian follicle, which in turn suppresses FSH but stimulates LH secretion. Administration of inhibin in the early follicular phase can delay folliculogenesis and inhibit ovulation and luteinization. Inhibin may have an important role in the control of fertility both in the males and the females. It causes agglutination of sperms, prevents cervical mucus penetration and interferes with egg interaction. In polycystic ovarian disease (PCOD), there is an increased secretion of inhibin. This causes a low FSH but a high LH secretion by the anterior pituitary gland and is responsible for anovulation. Although the extraction of purified inhibin is not yet successful, there is a possible hope of its availability in the near future. Normal level of 50 pg/mL (>45 pg) drops to less than 15 pg/mL after menopause due to oestrogen deficiency. It is studied by ELISA test.

TABLE 3.1 Effects of oestrogen and progesterone on the female genital tract

Organ	Oestrogen	Progesterone
Breasts	Ductal/stromal growth	Alveolar growth
Vagina	Superficial cells with glycogen	Intermediate cells
Cervix	Abundant mucus thin, viscous, penetrable to sperms	Thick tenacious mucus, impenetrable to sperms
Uterus	Myohyperplasia	Myohyperplasia
Endometrium	Proliferative endometrium	Secretory endometrium
Fallopian tube	Secretion	Increased peristaltic movements
Ovary	No action	No action

Activin

Activin is secreted by the anterior pituitary gland and the granulosa cells, and stimulates FSH release, and enhances action in the ovary.

Follistatin suppresses FSH activity by acting against activin.

Anti-Müllerian Hormone (AMH)

AMH is a peptide secreted by the Sertoli cells in the testis and granulosa cells in the ovary. In the male, AMH starts to be secreted by the seventh week of intrauterine life and it continues until puberty. It inhibits the development of **Müllerian** system. Absence of AMH results in hermaphrodite.

In the female, AMH is secreted by the granulosa cells after puberty. It helps in the follicular development and oocyte maturation.

Normal value is 2–6.8 ng/mL; level <1 ng/mL shows poor ovarian reserve, >10 ng/mL is seen in PCOD and hyperstimulation syndrome. Its level is related to precocious and delayed puberty, infertility and premature menopause. Its level is related and reflects the number of growing follicles.

Estimation of serum AMH is used in the study of ovarian reserve in an infertile woman and a woman with secondary amenorrhoea. In in vitro fertilization programme, it carries a prognostic value and helps to decide on donor egg.

Sex Hormone-Binding Proteins

Most of oestrogens and androgens are bound to sex hormone-binding protein (SHBP) secreted by the liver and remain inactive. Only free hormones are biologically active and influence their target organs (1–2%). Oestrogen and thyroid hormones increase the secretion of these proteins, but androgens lower their levels.

Testosterone

Fifty per cent testosterone comes from the ovaries and the rest from adrenal gland. The ovarian stromal tissue secretes androgenic products, namely testosterone, dehydroepiandrosterone (DHEA) and androstenedione. Androstenedione gets converted in the peripheral fat to oestrone. The normal increase in stromal tissue at ovulation causes a slight increase in the secretion of these hormones. After the menopause, the increased ovarian stroma is responsible for the rise in these hormones and development of hirsutism in some postmenopausal women. Total daily

production of testosterone is 0.2–0.3 mg and plasma level is 0.2–0.8 ng/mL. The daily production of androstenedione is 3 mg and plasma level is 1.3–1.5 ng/mL. Normal 17-ketosteroid level is 5–15 mg in 24 h. More than 25 mg indicates adrenal hyperplasia. Plasma level of DHEA sulphate over 5 mcg/mL is seen in adrenal hyperplasia.

Eighty to eighty five per cent androgens are bound to SHBP and 10–15% to albumin. One to two per cent free testosterone remains biologically active and acts at the peripheral targets, i.e. hair growth and acne by conversion to dihydrotestosterone by hydroxylase enzyme. Clinically, administration of androgen causes follicular atresia and anovulation.

Physiology of Menstruation

The proliferative phase of the endometrium represents the oestrogenic part of the menstrual cycle. It is initiated and controlled by oestrogen. The secretory phase of the endometrium is controlled by progesterone, although the effect of progesterone is obtained only after the endometrium has been sensitized with oestrogen. This is because oestrogen produces progesterone receptors to which progesterone acts.

Although the activity of the endometrium is directly controlled by the ovarian function and by the two hormones secreted by the ovary, the ovary itself is activated by the pituitary gland, the secretion of which is under the nerve control of the hypothalamus.

At birth, the ovaries are populated with lifetime complement of eggs located in the primordial follicles, but most of these follicles undergo atresia throughout childhood and only about 400 of these primordial follicles are present during reproductive age. At puberty, the hypothalamus starts a pulsatile secretion of GnRH, resulting in the activation of H–P–O uterine axis and in the establishment of menstrual cycles.

Pulsatile GnRH initiates secretion of FSH and LH. FSH released by the anterior pituitary gland stimulates the growth of a few primordial follicles into Graafian follicles. Multiple follicles start growing in both the ovaries, but only one dominant Graafian follicle is selected which ripens to full maturity and ovulates, whereas other follicles become atretic. The Graafian follicles under the influence of FSH together with only a minimal amount of the LH secrete 17-β-oestradiol (Figure 3.5A and B). 17-β-Oestradiol has several functions: in the first place, it produces proliferative changes in the endometrium, it

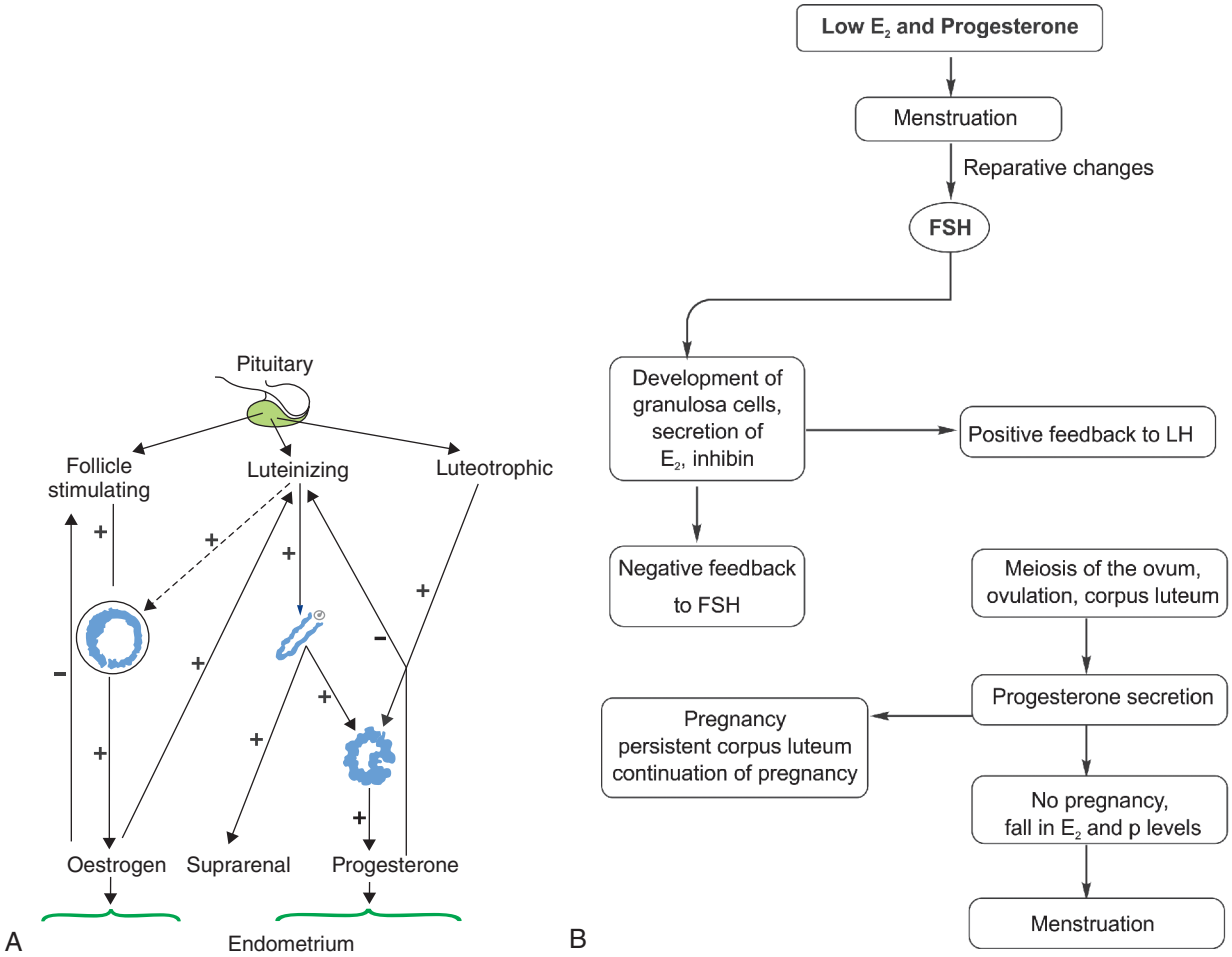

Figure 3.5 **(A)** A scheme illustrating interrelation of pituitary gonadotropic hormones. '+' indicates stimulation and '–' indicates inhibition. **(B)** Flowchart of menstruation.

secretes inhibin and inhibits further secretion of FSH by the anterior pituitary and it stimulates LH receptors in the theca cells and stimulates anterior pituitary to secrete LH. Inhibin produced by the Graafian follicle under oestrogenic effect is also responsible for a fall in the FSH level and stimulation of LH secretion. The maximum peak of oestrogen secretion is seen about 48 h before ovulation, whereas the LH peak occurs about 24–36 h before ovulation. LH has following functions. In the first place, it stimulates a Graafian follicle to secrete 17-β-oestradiol, and secondly, it causes the follicle to rupture at ovulation and to form a corpus luteum (Figure 3.6). It also stimulates the secretion of testosterone and androstenedione by theca cells.

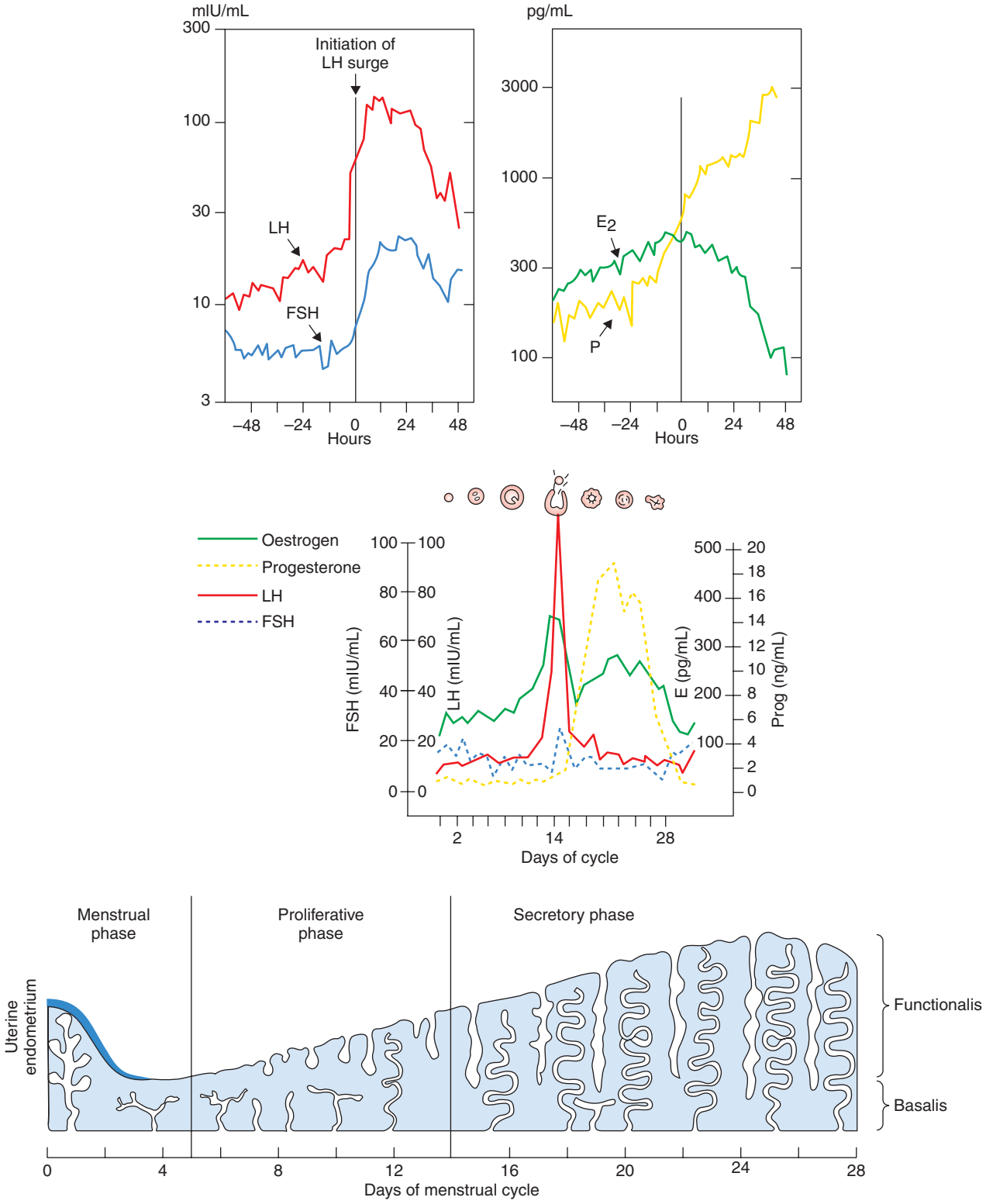

Figure 3.6 Plasma hormone levels in the normal menstrual cycles.

The corpus luteum secretes progesterone, the level of which starts rising. The hormone progesterone has two functions. In the first place, it stimulates the endometrium to undergo secretory hypertrophy, and secondly, it inhibits further production of LH by the anterior pituitary. *The gonadotropins seem to have no direct effect upon the endometrium of the uterus* (Figure 3.6).

In the absence of pregnancy, both oestrogen and progesterone levels decline gradually and the fall in the level of these hormones brings about menstruation. A fall in the level of these hormones also starts off a fresh positive feedback mechanism and triggers the hypothalamus to release gonadotropin. This is how a menstrual cycle is regulated. The luteal phase, i.e. time between ovulation and menstruation, is fairly constant at 14 days in a menstrual cycle. The growth of the ovarian follicles and endometrial thickness can be studied by serial ultrasound. Oestrogen, LH and mid-luteal progesterone levels can be conveniently and speedily measured by radioimmunoassays (Figure 3.7; Table 3.2).

As mentioned earlier, thyroid hormones and adrenal hormones react with sex hormones and alter the H–P–O pathway by inhibiting GnRH secretion. Oral combined pills, by virtue of inhibiting GnRH and preventing ovulation,

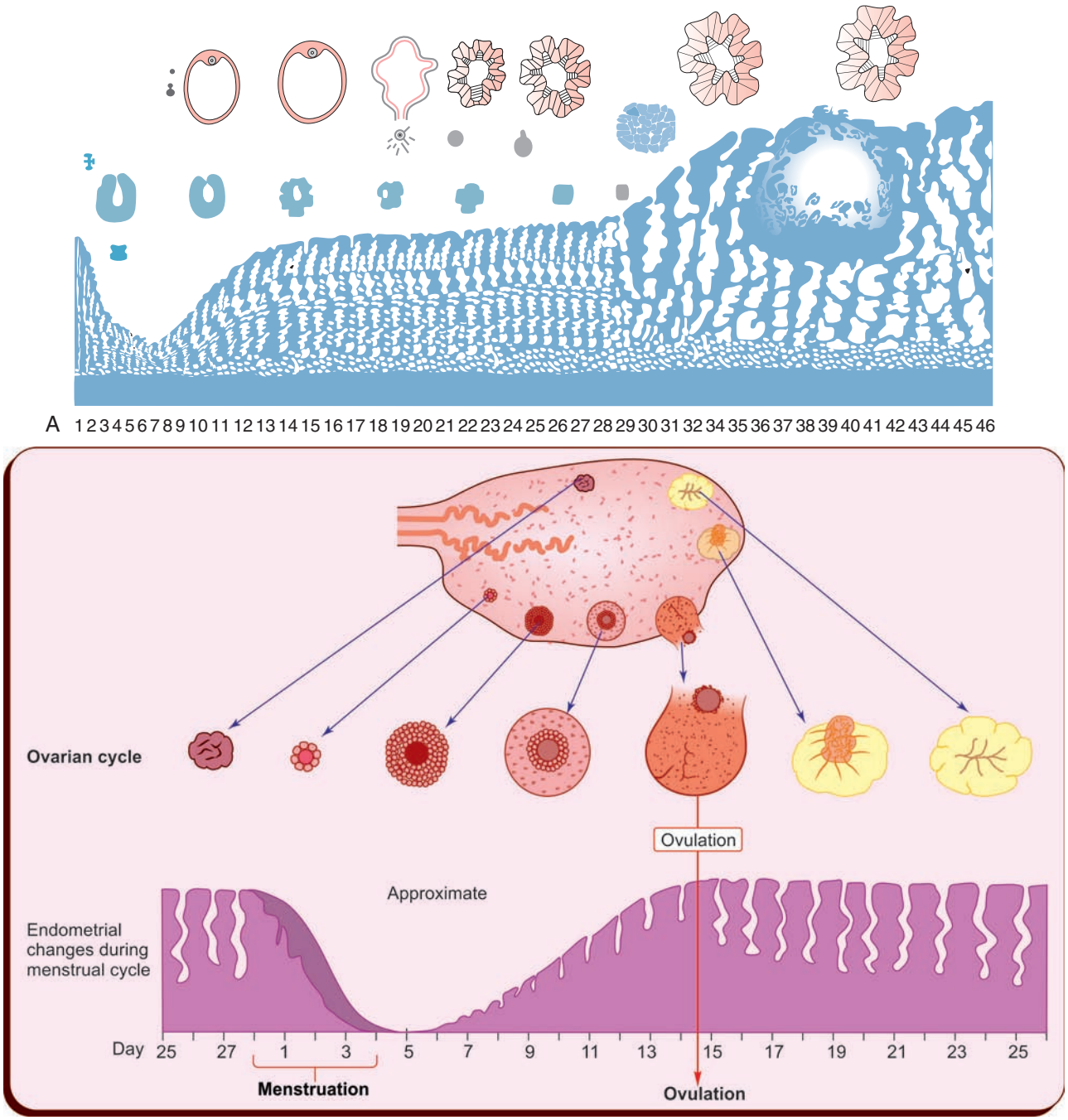

Figure 3.7 **(A)** Schroder's illustration of the relation between ovarian function and the changes in the endometrium during early pregnancy. **(B)** Ovarian cycle with corresponding endometrial thickness.

TABLE 3.2 Hormonal levels in different phases of menstrual cycle

	NORMAL			
Hormone	**Follicular Phase**	**Ovulation**	**Luteal Phase**	**Menstrual Phase**
FSH	5–15 mIU/mL	12–30	2–9	3–15 mIU/mL
LH	6–14 mIU/mL	25–100	2–13	3–12 mIU/mL
E_2	100/200 pg/mL	300–500 pg/mL	100–200	–
P	1 ng/mL	–	15 ng/mL	–
17 ketosteroid	Normal	5–10 mg/daily	–	>25 mg in adrenal hyperplasia
Testosterone	Normal	0.2–0.8 ng/mL	–	>2 ng/mL in ovarian tumours
Androstenedione	Normal	1.3–1.5 ng/mL	–	–
DHEA	Normal	<5 mcg/mL	–	>5 mcg in adrenal hyperplasia
Cortisol	–	<5 mcg/dL	–	–
DHEAS	800 ng/mL	–	–	Adrenal hyperplasia, tumour

cause atropic endometrium. Continuous oestrogen stimulation leads to endometrial hyperplasia (Figure 3.8).

Feedback Mechanism in the H–P–O axis

As mentioned in the beginning, the various hormones liberated by the hypothalamus, anterior pituitary gland and the ovaries are dependent upon each other, each reaching in positive as well as negative feedback at different levels.

The following are the feedbacks:

1. Long feedback mechanism from the ovaries to pituitary and hypothalamus.
2. Short feedback mechanism between the anterior pituitary gland and hypothalamus.
3. Ultrashort feedback mechanism.

Autoregulation of release of GnRH by hypothalamus. Increased secretion of GnRH suppresses its own synthesis and vice versa.

Leptin

Since its discovery in 1994, leptin (adipocyte protein hormone) is linked to nutrition and may bear an important role in the control of hypothalamic–pituitary–ovarian axis. A diet restriction has a negative impact on hypothalamus and decreases LH secretion causing amenorrhoea as seen in anorexia nervosa. Leptin is found in the follicular fluid in the ovaries and presumably stimulates pulsatile secretion of GnRH around puberty. Hence, an obese adolescent reaches menarche earlier than a lean girl. Lean girls have a delayed puberty. More research is required in this field.

Menstruation

Menstruation is the end point in the cascade of events starting at hypothalamus and ending in the uterus. The menstrual cycle is usually of 28 days, measured by the time between the first day of one period and the first day of the next. The duration of bleeding is about 3–5 days and estimated blood loss is between 50 and 200 mL. The regular cycle of 28 days is seen only in a small proportion of women. A deviation of 2 or 3 days from the 28-day rhythm is quite common. The menstrual rhythm depends on the H–P–O function, whereas the amount of blood loss depends upon the uterine condition.

A study of the coiled arteries of the endometrium shows that there is a slight regression of endometrium shortly after ovulation and that a rapid decrease in thickness can be demonstrated even before menstruation starts. In the regression that starts a few days prior to the onset of menstruation, there is a decreased blood flow which may cause shrinkage of the endometrium from dehydration. During menstruation itself, the reduction in the thickness of the endometrium is determined by both desquamation and resorption. The coiled arteries become buckled with subsequent stasis of blood flow. The necrosis of the superficial layers of the endometrium is produced either by local stasis or by the clearly demonstrated vasoconstriction of the coiled arteries. Menstrual bleeding occurs when the open arteries damaged by necrosis relax and discharge blood in the uterine cavity. Some degree of venous haemorrhage also occurs. Fragments become detached from the superficial layer of the endometrium by the end of the first day (Figures 3.7–3.9).

The important feature of the menstrual changes is the contraction and constriction of the coiled arteries. The ischaemia causes necrosis and disintegration of the superficial zone. The regeneration of the vascular system is probably brought about by the development of anastomosing arteries. The re-epithelialization is brought about by the cells growing from the mouth of the base of the glands that remain in the unshed basal layer of the endometrium.

In anovulatory menstruation, there is the same shedding of a thin necrotic superficial layer of the endometrium, and it is to be presumed that exactly the same factor is at work to cause the vascular changes with resultant ischaemia.

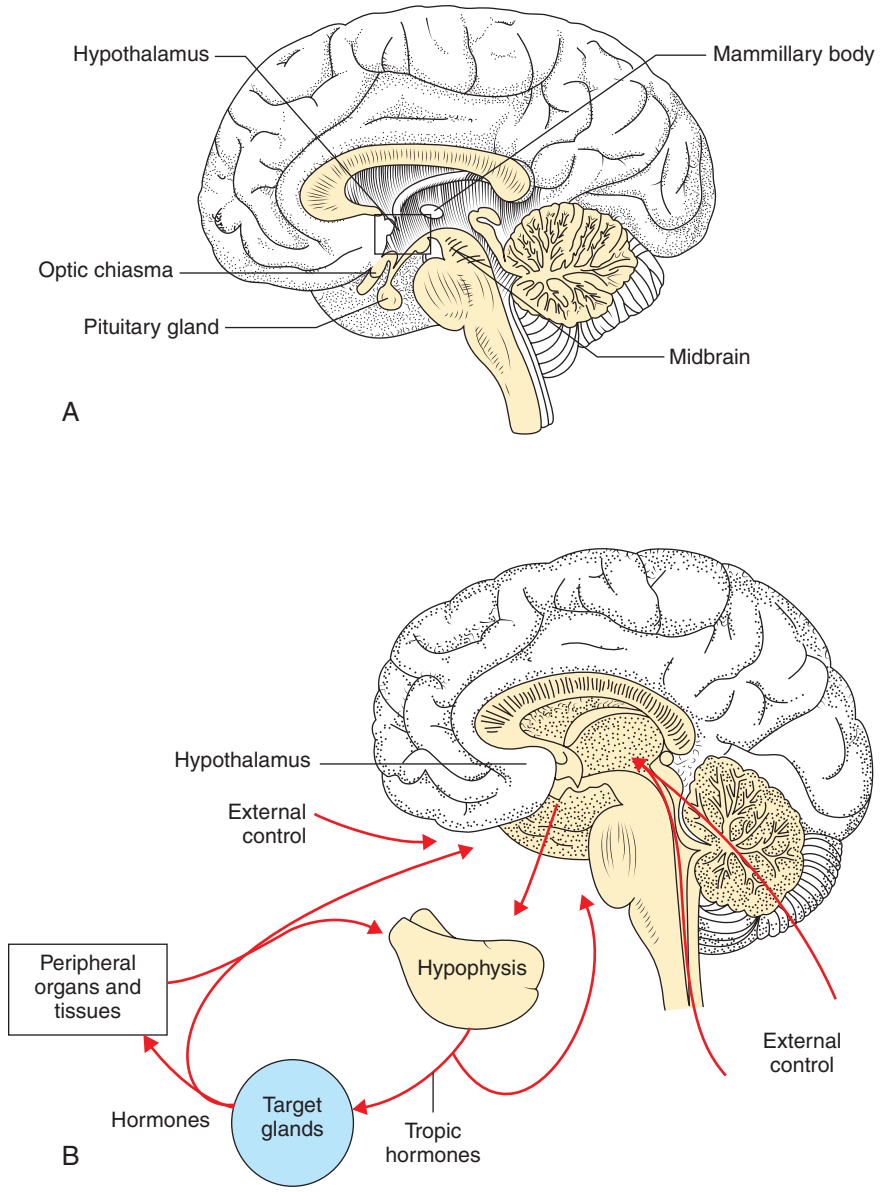

Figure 3.8 Neuroendocrine control of menstruation.

The vascular changes in the endometrium and the amount and duration of the menstrual bleeding are controlled by the interaction of different prostaglandins secreted by the endometrium.

Prostaglandin E_2 (PGE_2) causes myometrial contractions but vasodilatation of the vessels. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes vasoconstriction as well as myocontraction. Prostacyclin (PGI_2) is responsible for muscle relaxation and vasodilatation. According to this, PGE_2 and $PGF_{2\alpha}$ are responsible for dysmenorrhoea, and PGI_2 can cause menorrhagia.

Improved ultrasonic imaging and colour Doppler study of the endometrium have improved our knowledge related to menstrual disorders.

Menstrual Fluid in 'Stem Cell' Therapy

The stem cells are the basic building blocks of every other cell in the body. Whereas organ cells have specific functions, the stem cells are 'blank' but have the potential to take up any function. Under suitable environment and surrounded by specific organ cells, the stem cells divide into either stem cells or another type of cells with their attached functions. Thus, the stem cells have a vital role in 'regenerative medicine' in degenerative and life-threatening diseases such as Alzheimer disease, atherosclerosis, diabetes, heart disease, bowel disease, Parkinsonian disease and rheumatoid arthritis.

The sources of stem cells were until recently seen in bone marrow, embryo, amniotic fluid and umbilical cord blood but now in menstrual fluid as well. The menstrual fluid contains mesenchymal cells such as mononuclear cells and fibroblasts. These cells, however, deteriorate with advancing age. Therefore, cells from young women are suitable for donation, and self-use at a later age if needed. The kit contains antibiotics to prevent infection, and the menstrual fluid is cryopreserved and harvested. The procedure is simple, noninvasive and painless as well as possible.

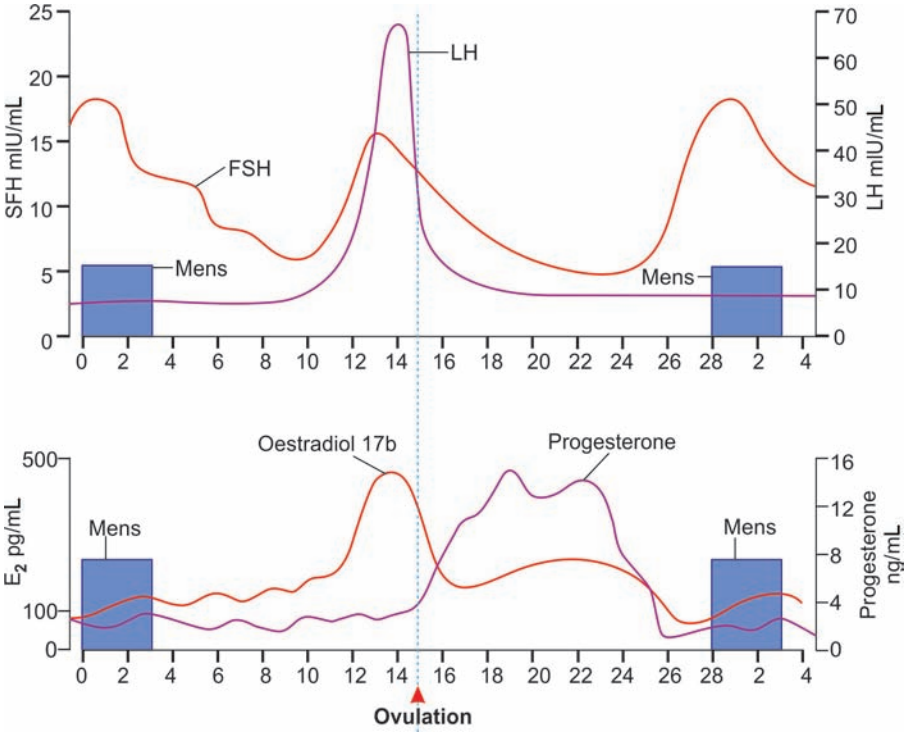

Figure 3.9 Hormonal level during menstrual cycle.

Key Points

- Neuroendocrinology with its vast hormonal network is key to normal menstrual cycles and reproductive function in a woman.
- Hypothalamus, with its secretion of GnRH (decapeptide), is the main neuroendocrine gland and regulatory factor in the chain of hypothalamic–pituitary–ovarian axis. The higher cortical centres can modify or influence hypothalamic secretion.
- Proliferative phase of endometrium represents oestrogenic action of the ovary.
- Progesterone causes secretory endometrium only if the latter is primed with oestrogen.
- Therapeutic management in infertility, family planning and gynaecological disorders is based on the knowledge of neuroendocrinology and the interaction of various hormones.
- Synthetic analogues of GnRH, FSH and LH are used in infertility and amenorrhoea.
- Oestrogen and progesterone have specific roles in the menstrual cycle and in the development of genital organs.
- Other hormones participate in the maintenance of normal menstruation.
- LH surge is the key marker of imminent ovulation.
- LH causes maturation of Graafian follicle, meiosis of ovum before ovulation, ovulation and development of corpus luteum.
- Leptin appears to have a role in the development and onset of puberty.
- Menstrual fluid is recently discovered to contain the stem cells and may prove useful in stem cell therapy. Only young women are suitable for donation.
- Fifty per cent of total testosterone and a small amount of androstenedione produced in the stroma by LH are needed for conversion to oestrogen by the granulosa cells. Excess of production causes acne and hirsutism.

Self-Assessment

1. Describe the neuroendocrine control of the menstrual cycle.
2. Describe the formation and processes that lead to the formation of the Graafian follicle.
3. Describe the mechanism of ovulation.
4. Describe the microscopic appearance of the endometrium during the various phases of the menstrual cycle.
5. Describe the rheological properties of cervical mucus during different phases of the normal menstrual cycle.

Suggested Reading

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