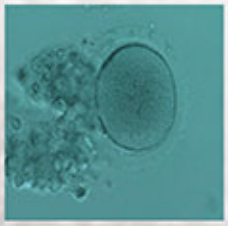
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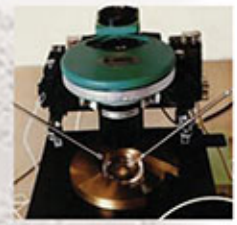
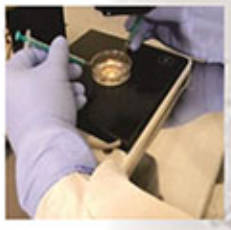




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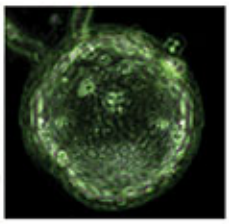
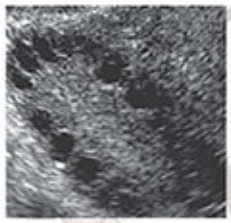
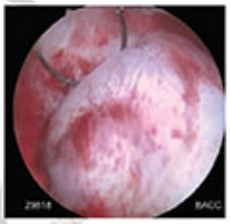
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Principles and Practice of ASSISTED REPRODUCTIVE TECHNOLOGY

VOLUME 1
INFERTILITY

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CHAPTER 2

Regulation and Physiology of Menstrual Cycle

Arveen Vohra

JAYPEE BROTHERS

INTRODUCTION

The normal menstrual cycle is one of the most complex, elegant, and yet one of the simplest physiologic processes. It is mandatory for a clinician and infertility consultant to completely understand the menstrual cycle. The menstrual cycle, under the control of the endocrine system, is necessary for reproduction. It is the cycle of changes that occurs in the ovary and uterus for the purpose of sexual reproduction, being essential for the production of eggs and for the preparation of the uterus for pregnancy. The normal menstrual cycle is the orderly cyclic hormone production and parallel proliferation of the uterine lining in preparation for implantation of the embryo failing, which it prepares for the next cycle.

Menstruation is defined as the cyclic, orderly sloughing of the uterine lining in response to the interactions of hormones produced by the hypothalamus, pituitary, and ovaries. Menarche is typically followed by 5–7 years of relatively long cycles that gradually decrease in length and become regular. Variations in cycle length reflect differences in the length of the follicular phase, as the luteal phase is fairly consistent.

In humans, the menstrual cycle is a monthly cycle that averages 28 days per cycle. It varies in different women and even in the same woman during various cycles. Menstrual cycle is counted from the first day of menstrual bleeding. Each menstrual cycle can be divided into three phases based on the following ovarian and endometrial events:

1. *Ovarian cycle (events in the ovary):* It consists of the follicular phase, ovulation, and luteal phase along with the luteal–follicular transition phase.
2. *Uterine cycle (events in the uterus):* It consists of menstruation, proliferative phase, and secretory phase.

This chapter encompasses in detail the neuroendocrinology and physiology of menstrual cycle, which in synchrony are responsible for the regulation of the menstrual cycle in women. The genomic profiling, which has now highlighted the various hormonal and the hormone receptor polymorphisms, is also discussed along with newer concepts in primordial follicle activation.

REPRODUCTIVE NEUROENDOCRINOLOGY

Introduction

The female and male reproductive systems function as an orchestra in harmony with a set of reproductive hormones under the influence of regulatory hormones secreted by the hypothalamus and pituitary gland. Initially, the pituitary gland was considered as the master of the endocrine orchestra, but recently, the hypothalamus has equitably replaced this status. The hypothalamus exerts its influence via neurotransmitters transported to the pituitary gland by means of a portal vessel network in response to both peripheral and central nervous system signals.

The hypothalamus and the pituitary are essential for the operation of the menstrual cycle, in synchrony with the endocrine feedback by the ovary on the anterior pituitary that leads to ovulation. The physiology of the menstrual cycle is dependent on signaling whereby the "ovarian clock" modulates hypothalamic–pituitary function as well as endometrial proliferation and secretion.

The predominant hormones involved in the menstrual cycle are gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone. GnRH is secreted by the hypothalamus, the gonadotropins FSH and LH are secreted by the anterior pituitary gland, and estrogen and progestins are secreted at the level of the ovary. GnRH stimulates the release of FSH and LH from the anterior pituitary, which in turn stimulate the release of estrogen and progestin at the level of the ovary.

Hypothalamo-hypophyseal Portal Circulation

Hypothalamus

The hypothalamus is a small, cone-shaped neural structure situated at the base of the brain above the optic chiasma

TABLE 1: Pituitary releasing factors from the hypothalamus.

Hypothalamic hormone	*Action*
Gonadotropin-releasing hormone (GnRH)	Controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH)
Corticotropin-releasing hormone (CRH)	Controls the release of adrenocorticotropic hormone (ACTH)
Growth hormone-releasing hormone (GHRH)	Regulates the release of growth hormone (GH)
Thyrotropin-releasing hormone (TRH)	Regulates the secretion of thyroid-stimulating hormone (TSH)

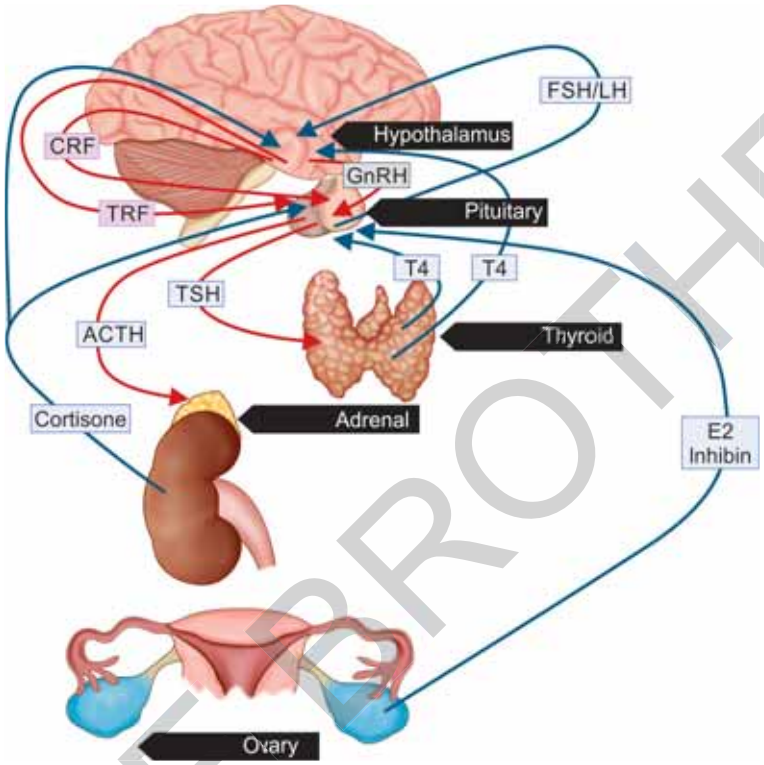


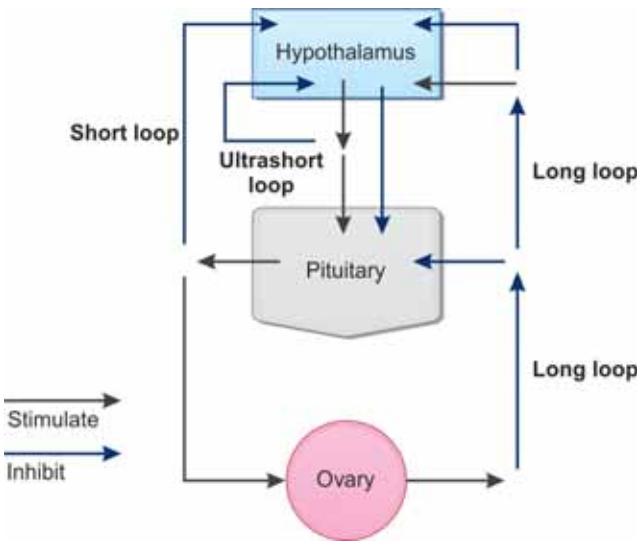
Fig. 1: Pituitary-releasing factors that control the endocrine function of the ovaries, thyroid, and adrenal glands. Note the feedback from the target hormones (marked in blue lines).
(ACTH: adrenocorticotropic hormone; CRF: corticotropin-releasing factor; E2: estradiol; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing factor; LH: luteinizing hormone; TRF: thyrotropin-releasing factor; TSH: thyroid-stimulating hormone; T4: thyroxine)

and below the third ventricle forming the lower part of diencephalon. It is connected directly to the pituitary gland and is the source of many pituitary secretions.

The hypothalamus has peptidergic neural cells that secrete releasing and inhibiting hormones. These cells resemble both neurons and endocrine gland cells and respond to signals in the bloodstream as well as to neurotransmitters within the brain in a process known as *neurosecretion*. These neurotransmitters are secreted at the nerve terminal. Neuroendocrine agents originating in the hypothalamus have positive stimulatory effects on growth hormone (GH), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and the gonadotropins **(Table 1 and Fig. 1)**.

Several levels of feedback to the hypothalamus exist and are known as the long, short, and ultrashort feedback loops **(Flowchart 1)**. The long feedback loop is composed of endocrine input from circulating hormones, just as feedback

Flowchart 1: Hypothalamic–pituitary–ovary feedback loop mechanisms.



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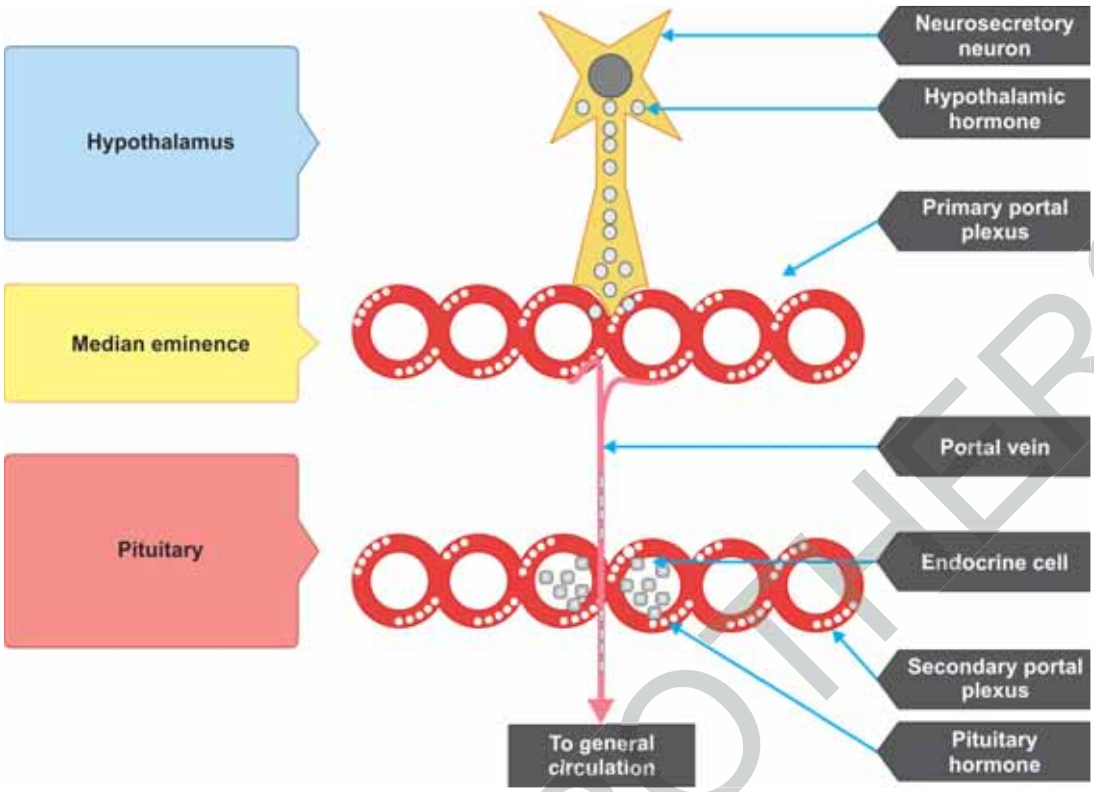


Fig. 2: Hypothalamic–pituitary axis with the portal system.

of androgens and estrogens on to steroid receptors is present in the hypothalamus. In short-loop feedback, pituitary hormones send feedback to the hypothalamus and serve important regulatory functions. Hypothalamic secretions may directly feedback to the hypothalamus itself in an ultrashort feedback loop.[1,2]

Pituitary

The pituitary consists of two lobes—anterior (adenohypophysis) and posterior (neurohypophysis), which are structurally different. The unique fenestrated capillaries in this region eliminate the blood–brain barrier in the median eminence and permit bidirectional feedback control. The anterior pituitary is under the influence of the hypothalamus by means of neurohormones released into this portal circulation. There also exists retrograde flow so that pituitary hormones can be delivered directly to the hypothalamus, creating the opportunity for pituitary feedback on the hypothalamus. Short vessels that originate in the posterior pituitary that in turn receive their arterial supply from the inferior hypophyseal arteries provide an additional blood supply **(Fig. 2)**.

HORMONES IN FEMALE REPRODUCTION

Gonadotropin-releasing Hormone

Gonadotropin-releasing hormone (also called luteinizing hormone-releasing hormone or LHRH) is the controlling factor for gonadotropin secretion. There is a single neurohormone (GnRH) for both FSH and LH. It is a decapeptide with the gene located on chromosome 8p and works through its own receptor, encoded on chromosome 4q. It is produced by neurons with cell bodies primarily in the arcuate nucleus of the hypothalamus and in many extrapituitary tissues, such as the ovarian follicle and the placenta. GnRH has autocrine–paracrine functions throughout the body **(Fig. 3)**.

Pulsatile secretion of GnRH: The half-life of GnRH is only 2–4 minutes. A prominent feature of the reproductive system is the absolute requirement for pulsatile secretion of GnRH from the arcuate nucleus of the hypothalamus into the pituitary portal system for normal gonadotropin secretion. The increase in the GnRH pulses marks the onset of puberty. GnRH is unique as it regulates the secretion of two hormones—FSH and LH—and must be secreted in a pulsatile fashion within a critical range in frequency and amplitude. Knobil et al. showed that in hypothalamic-lesioned monkeys receiving GnRH, intermittent stimulation of the pituitary results in secretion of LH and FSH, whereas constant GnRH stimulation leads to suppression of gonadotropin levels.[3]

The frequency of GnRH secretion is once per 90 minutes in the early follicular phase, which increases to once per 60–70 minutes in the late-follicular phase. Lower GnRH pulse frequencies favor FSH secretion and higher frequencies favor LH secretion. Pulsatile GnRH secretion is more frequent but lower in amplitude during the follicular phase.

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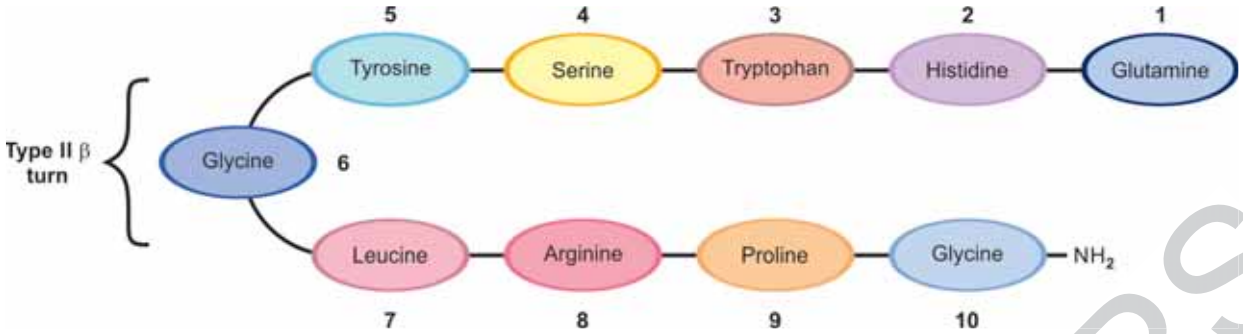


Fig. 3: Structure of gonadotropin-releasing hormone molecule.

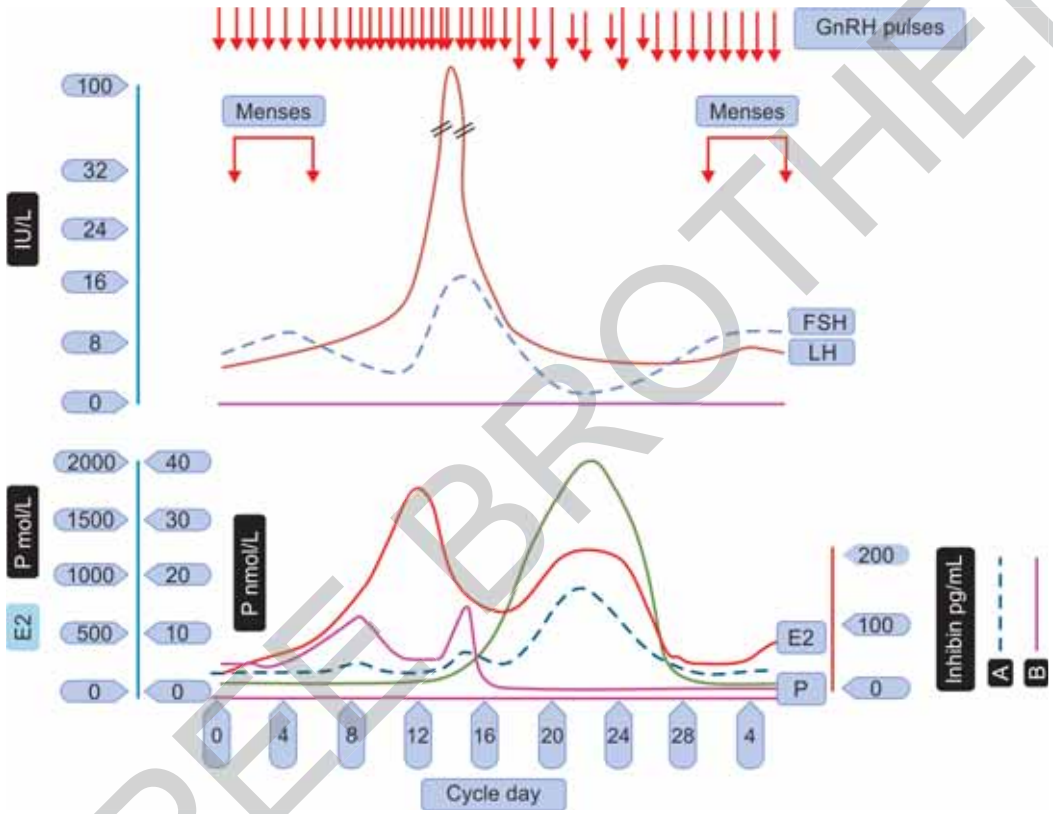


Fig. 4: Gonadotropin-releasing hormone (GnRH) pulsatility affecting gonadotropin and steroid release during menstrual cycle. (E2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; P: progesterone)

An increase in frequency and amplitude during mid-cycle favors LH surge necessary for ovulation. Slowing of pulse frequency in the late-luteal phase favors FSH synthesis and allows the rise in FSH essential for the next cycle **(Fig. 4)**.

Control of GnRH pulses: Catecholamines such as norepinephrine, dopamine, and serotonin, and transmitters, such as opiates and prostaglandins, also appear to influence the secretion of GnRH from the hypothalamus. Norepinephrine seems to stimulate GnRH release, whereas dopamine, serotonin, endogenous opioids, and corticotropin-releasing hormone (CRH) appear capable of inhibiting or suppressing GnRH, as witnessed by the association of stress with hypothalamic amenorrhea in some women. Biogenic catecholamines modulate GnRH pulsatile release by influencing the frequency (and perhaps the amplitude) of GnRH discharge **(Flowchart 2)**.

The *dopamine* tuberoinfundibular tract arising within the medial basal hypothalamus and terminating in the median eminence provides the dopaminergic effect on the pituitary. Dopamine is directly secreted into the portal blood, thus behaving like a neurohormone. It directly suppresses GnRH activity and is transported via the portal system to directly and specifically suppress pituitary prolactin secretion.

Neuropeptide Y (NPY), a polypeptide, stimulates appetite, decreases heat production, and increases insulin and cortisol secretion. The secretion and gene expression of NPY in hypothalamus are regulated by gonadal steroids.

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It stimulates pulsatile release of GnRH and potentiates gonadotropin response to GnRH.[4]

Kisspeptin is a hypothalamic neuropeptide encoded by the gene *Kiss1*, which has recently emerged as a key central regulator of gonadotropin secretion. The kisspeptin neurons have estrogen and progesterone receptors and are essential for the normal development of puberty.[5]

Kisspeptins and their receptors are expressed by neurons in the arcuate and anteroventral periventricular nuclei of the hypothalamus. **Figure 5** shows structure of kisspeptin.

Puberty is regulated by the maturation of kisspeptin neurons as well as by interactions between kisspeptins and leptin.[6]

The obligatory kisspeptin signaling for normal puberty is evidenced by a failure to progress through puberty in individuals with mutations in genes encoding kisspeptin and its receptor, G-protein coupled receptor 54 (GPR54).[7]

Neurokinin B (NKB) and dynorphin (Dyn) are cotransmitters of kisspeptin signaling **(Figs. 6A to D)**.[6]

Sex steroids can regulate *Kiss1* gene expression both positively and negatively, depending on the physiological environment. Some studies have demonstrated that administration of kisspeptin, as the longer kisspeptin fragment kisspeptin-54 (Kp54), stimulates gonadotropin secretion in healthy women and in women with hypothalamic amenorrhea. However, another study suggested that kisspeptin-10 does not stimulate gonadotropin secretion in women.[8]

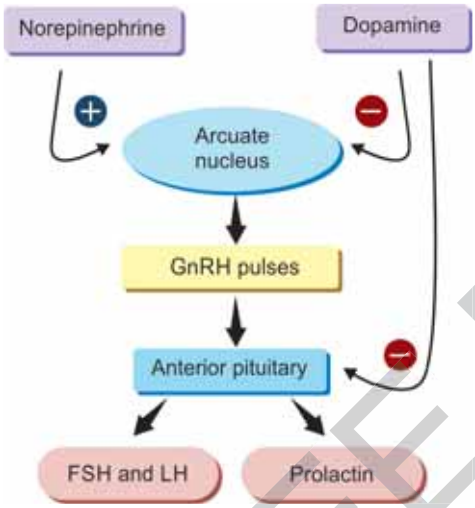
Various studies have shown that intravenous infusion of kisspeptin-10 can stimulate LH pulse frequency in men. This is, however, still a controversial area where more research is needed.[9] Assessment of gonadotropin response to the administration of exogenous kisspeptin provides a novel investigative tool to assess GnRH neuronal function in vivo in reproductive health medicine **(Figs. 7A to C)**.

Kisspeptin agonists may be used to localize lesions causing hypothalamic–pituitary–gonadal axis dysfunction and evaluate the gonadotrophic potential of subfertile individuals. Kisspeptin antagonists may be used as contraceptives in women through the prevention of premature luteinization during in vitro fertilization and also in the treatment of sex steroid-dependent diseases and metastatic cancers.

The role of kisspeptins as ovulation triggers is also being studied extensively.

KISS1R/Kiss1r is desensitized with continuous kisspeptin exposure. In animals, intermittent kisspeptin administration can raise LH levels. However, LH gets suppressed after continuous administration of kisspeptin, as it induced long-lasting depolarization in GnRH neurons, followed by

Flowchart 2: Control of catecholamines and dopamine on gonadotropins.



(FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone)

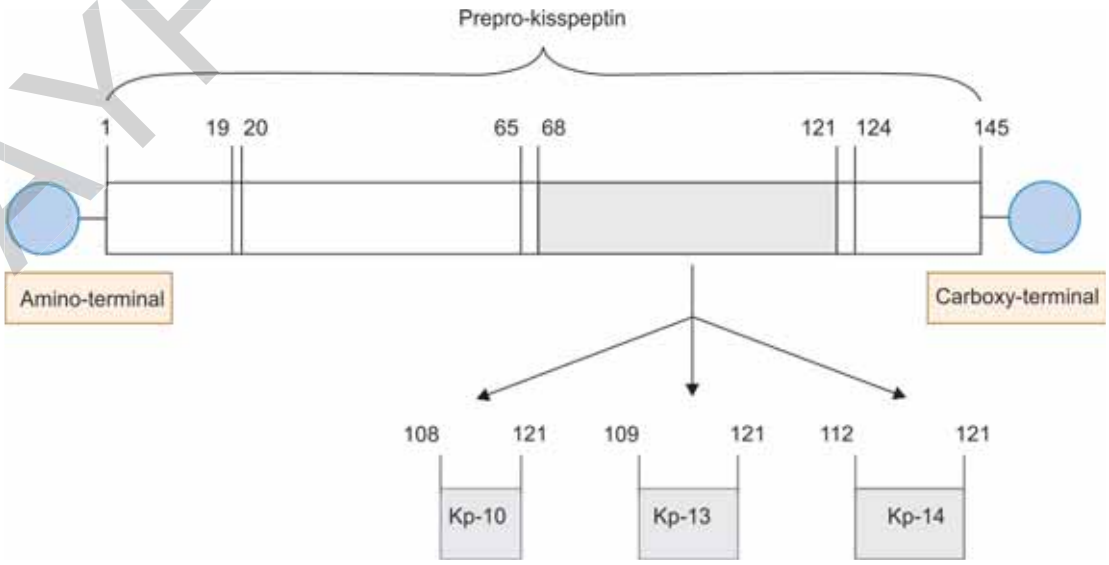
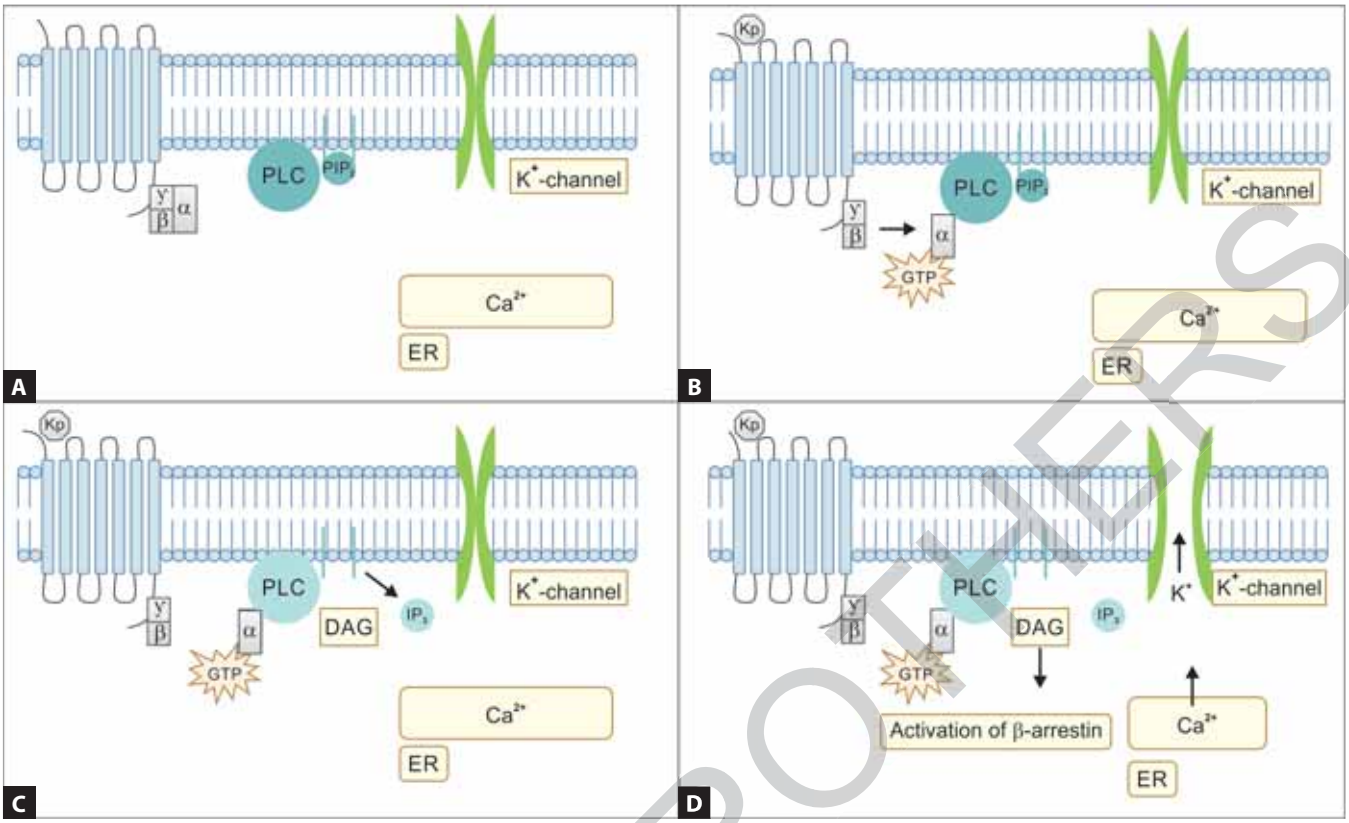


Fig. 5: Structure of kisspeptin (Kp) protein.

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Figs. 6A to D: (A) KISS1R in the dormant state; (B) kisspeptin ligand (Kp) binding activating the G protein subunit, Gq/11α, and subsequently phospholipase C (PLC); (C) PLC activation leading to the formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG); (D) IP_3 causing the release of intracellular calcium (Ca^{2+}) from the endoplasmic reticulum (ER). PLC-independent mechanisms open the potassium (K^+) channels. These events depolarize the kisspeptin neuron. (GTP: guanosine triphosphate; PIP_2: phosphatidyl inositol bisphosphate)[6]

desensitization. This desensitization is not due to GnRH depletion but due to clathrin-mediated internalization of arrestin and KISS1R in internalized vesicles after kisspeptin activation.[6]

Activins, Inhibins, and Follistatins

Intrapituitary cytokines and growth factors provide an autocrine–paracrine system for regulating pituitary cell development and replication as well as pituitary hormone synthesis and secretion. Activins and inhibins are peptide members of the transforming growth factor-beta (TGF-β) superfamily.

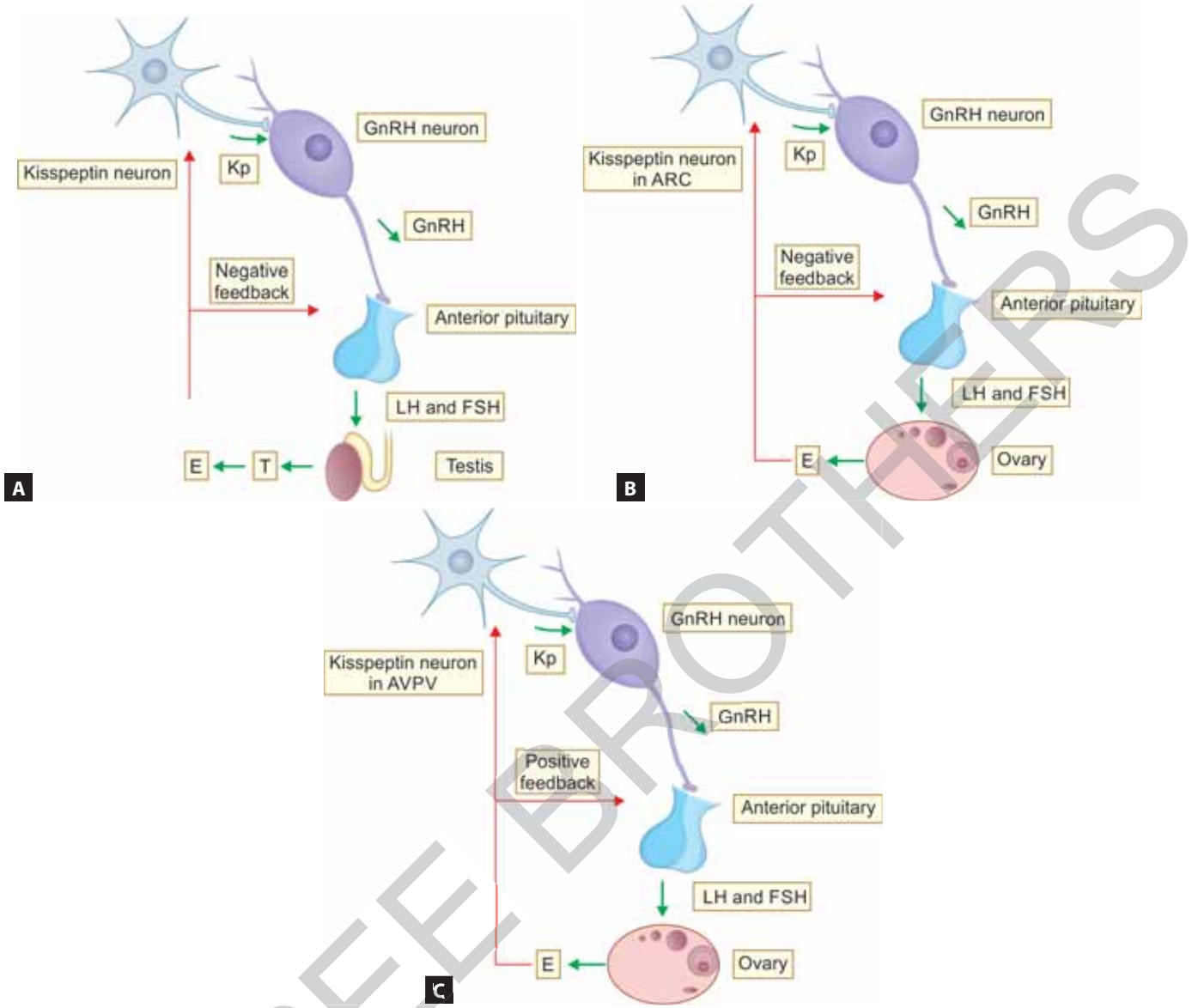
Inhibins are secreted by granulosa cells. FSH stimulates secretion of inhibin, and inhibin suppresses FSH secretion. Inhibin blocks synthesis and secretion of FSH, prevents upregulation of GnRH receptors, and at high levels, may also promote degradation of gonadotropins.

The inhibins, named for their inhibitory control of FSH, are composed of two dissimilar peptides linked by disulfide bonds. There are two forms of inhibin that are composed of one of the two inhibin β subunits and a closely related α subunit to form inhibin A or inhibin B. Inhibin B, peaking in mid-follicular phase, amplifies the withdrawal of FSH from other follicles allowing emergence of the dominant follicle. After appearance of LH receptors and formation of corpus luteum (CL), it comes under control of LH and inhibin A dominates. Inhibin has little or no effect on GH, ACTH, and prolactin production.[10,11] Inhibin blocks the synthesis and secretion of FSH, prevents the upregulation of GnRH receptors, and at high levels, may also promote degradation of gonadotropins.

Activins are derived from granulosa cells and pituitary gonadotrophs. They are composed of two subunits identical to the β subunits of inhibin A and B. They augment FSH secretion but inhibit prolactin, ACTH, and GH responses.[11-13] Marked increase in activin tone helps in FSH-dependent follicle selection. Activin increases pituitary response to GnRH by enhancing GnRH receptor formation. The effects of activin are blocked by inhibin and follistatin.[14,15]

Follistatin, also called the FSH-suppressing protein, is a glycopeptide secreted by a variety of pituitary cells, including the gonadotrophs. Its main action is inhibiting FSH synthesis, secretion, and the FSH response to GnRH, probably by binding to and decreasing activity of activin.[16,17] Activin stimulates follistatin production and inhibin prevents this response.

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Figs. 7A to C: The diagram shows that (A) kisspeptin (Kp) stimulates gonadotropin-releasing hormone (GnRH) secretion and subsequent gonadotropin release. Testosterone (T) is aromatized to estrogen (E), which exerts negative feedback on the anterior pituitary gland and hypothalamus. (B) E exerts negative feedback on GnRH via Kp neurons in the arcuate nucleus (ARC). (C) E exerts positive feedback on GnRH via Kp neurons in the anteroventral periventricular nucleus (AVPV).
(FSH: follicle-stimulating hormone; LH: luteinizing hormone)

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Endogenous Opioids and Effects on Gonadotropin-releasing Hormone

The endogenous opioids are three related families of naturally occurring substances produced in the CNS that represent the natural ligands for the opioid receptors.[18-20] They exert their effects by binding to the G protein-coupled receptors, delta opioid receptor (DOR), kappa opioid receptor (KOR), and mu opioid receptor (MOR). These include:

- *Endorphins* (α, β, γ) are produced in the hypothalamus from the precursor proopiomelanocortin (POMC) with morphine-like activity and help in regulation of temperature, appetite, mood, and behavior.
- *Enkephalins* are most widely distributed opioid peptides in the brain and function primarily in regulation of the autonomic nervous system. Proenkephalin A is the precursor for the two enkephalins of primary importance: Methionine–enkephalin and leucine–enkephalin.
- *Dynorphins* are produced from the precursor prodynorphin that serves a function similar to that of the endorphins, producing behavioral effects and exhibiting high analgesic potency.

Endorphins appear to inhibit GnRH release within the hypothalamus, resulting in inhibition of gonadotropin secretion but have no effect on the pituitary response to GnRH.[21] The principal endogenous opiates affecting GnRH

release are β-endorphin and Dyn, probably by modulation of the catecholamine pathway, principally norepinephrine. β-endorphins are 5–10 times more potent than morphine. Ovarian sex steroids can increase the secretion of central endorphins, further depressing gonadotropin levels.[22] Endorphin levels vary significantly throughout the menstrual cycle, with peak levels in the luteal phase and a nadir during menses.[23] The dysphoria experienced by some women in the premenstrual phase of the cycle may be related to a withdrawal of endogenous opiates.

The emerging evidence indicates the participation of opioid peptides in the regulation of reproductive function through a direct local action within reproductive tissues, mainly the endometrium. Various studies have reported the role of these peptides in different phases of endometrial development and implantation; however, more research is needed.[24]

Prolactin

Prolactin is a 198 amino acid polypeptide, with the gene expression occurring in the lactotrophs of the anterior pituitary gland, decidualized endometrium, and the myometrium. The prolactin secreted in these various sites is identical, but differences in messenger ribonucleic acid (mRNA) indicate differences in gene regulation. Three types of prolactin have been identified based on size and structural modifications that are the result of glycosylation, phosphorylation, additions and deletions—little, big, and big–big prolactin.

Prolactin secretion is under tonic inhibitory control by the hypothalamic secretion of dopamine. The dopamine action in the pituitary is mediated by receptors coupled to inhibition of adenylate cyclase. Among the five forms of the dopamine receptor divided into two functional groups, D1 and D2, the D2 type is the predominant receptor in the anterior pituitary gland. Prolactin secretion follows a circadian rhythm, with the maximum secretion 5–8 hours after the onset of sleep. The release is pulsatile with the frequency of pulses reaching maximum in the mid-cycle and then declining in the luteal phase.

Prolactin homeostasis is regulated mainly by prolactin itself, feeding back on the dopamine-releasing neurons. Prolactin gene transcription is principally stimulated by estrogen, thyroid-releasing hormone (TRH), breast manipulation, drugs, stress, exercise, hormones including TRH (explaining the hyperprolactinemia seen with TRH elevation in hypothyroidism), vasopressin, γ-aminobutyric acid (GABA), β-endorphin, vasoactive intestinal peptide, epidermal growth factor (EGF), angiotensin II, and possibly GnRH.[25,26]

It has a direct action on the ovaries, which is responsible for the menstrual irregularities associated with hyperprolactinemia **(Box 1)**.

BOX 1: Effects of hyperprolactinemia on female reproductive function.

- Disrupts normal follicular development
- Inhibits aromatase enzyme
- Premature destruction of the corpus luteum
- Atresia of the dominant follicle
- Inhibits progesterone synthesis by the corpus luteum
- Induces uterine adenomyosis

Other Factors

Prostaglandins and catechol estrogens possibly act as neuromodulators by altering the function of catecholamines through inhibition of tyrosine hydroxylase and competing for the enzyme catechol-O-methyltransferase.

Leptin induces weight loss due to decreased appetite and food consumption and an increase in heat production and activity, associated with a decrease in the NPY expression. The *Lep* gene in humans is located on chromosome 7q31 and leptin receptor gene is located on chromosome 1. Fasting and exercise decrease leptin secretion and increase NPY in the arcuate nucleus. The NPY neurons stimulate feeding and inhibit heat production by inhibiting sympathetic nervous activity.[27]

Ghrelin is a complex hormone that stimulates the release of GH and is secreted mainly in the stomach, intestine, pituitary, hypothalamus, kidney, ovary, testes, and placenta. It is the only hormone known that stimulates food intake. The regulation of food intake occurs via the NPY pathway, with ghrelin and leptin having opposing actions. It is reduced with food intake and increased with fasting.

Differential Control of Follicle-stimulating Hormone and Luteinizing Hormone Secretion

The anterior pituitary secretes the major hormone releasing factors—FSH, LH, TSH, and ACTH along with GH and prolactin. FSH and LH are both glycoproteins that share identical α subunits along with TSH and placental human chorionic gonadotropin (hCG) and differ only in the structure of their β subunits, which confers receptor specificity.

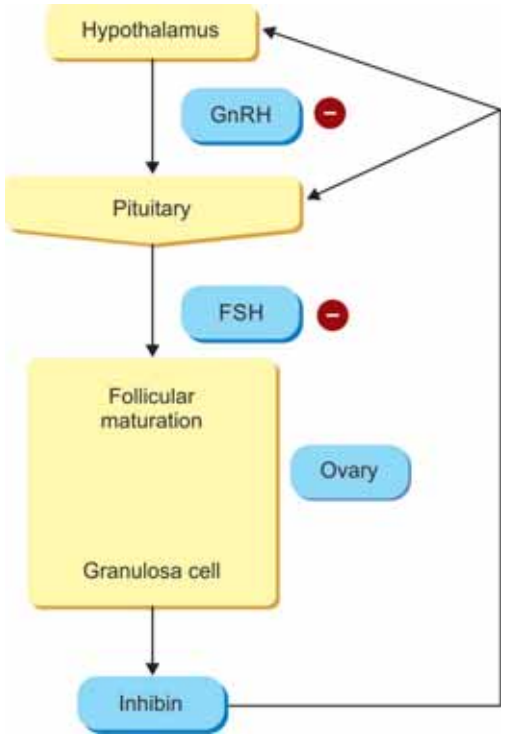
Although secreted from common gonadotrophs, FSH and LH have markedly different functions in the control of ovarian physiology. The divergent control of FSH and LH is achieved through a combination of differential control of their synthesis and secretion by GnRH. The preferential control of FSH synthesis is by the activin/follistatin system, and differential feedback by ovarian steroids and inhibins **(Flowchart 3)**.

Follicle-stimulating Hormone

Follicle-stimulating hormone is essential for follicular growth. It induces estrogen and progesterone secretion at the level of the ovary by activating aromatase and p450 enzymes and exerts negative feedback on GnRH secretion.

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Flowchart 3: Role of follicle-stimulating hormone (FSH) and inhibin in controlling ovarian function.



(GnRH: gonadotropin-releasing hormone)

BOX 2: Functions of follicle-stimulating hormone (FSH) in menstrual cycle.

- Selection and maturation of dominant follicle
- Proliferation of granulosa cells
- Aromatization within granulosa cells
- Induction of FSH and LH receptors on granulosa cells
- Production of autocrine–paracrine factors—activin and inhibin
- FSH surge helps in ovulation
- Prevents germ cells from atresia in luteal–follicular transition

(LH: luteinizing hormone)

FSH further induces the proliferation of granulosa cells and expression of FSH and LH receptors on granulosa cells. The rise in FSH during the LH surge helps in ovulation by stimulating plasminogen activator activity **(Box 2)**.

Luteinizing Hormone

Luteinizing hormone is secreted by the anterior pituitary gland and is required for both growth of preovulatory follicles, and ovulation and luteinization of the dominant follicle. During the follicular phase of the menstrual cycle, LH induces androgen synthesis by theca cells that is essential for robust steroidogenesis; stimulates proliferation, differentiation, and secretion of follicular thecal cells; and increases LH receptors on granulosa cells. The preovulatory LH surge drives the oocyte into the first meiotic division and initiates luteinization of thecal and granulosa cells. The resulting CL produces high levels of progesterone and some estrogen.

TABLE 2: Impact of different concentrations of luteinizing hormone (LH) on the menstrual cycle.

Effects below LH threshold	*Effects within LH window*	*Effects above LH ceiling*
Absent paracrine signaling between granulosa and theca	Normal follicular growth and development	Suppression of granulosa proliferation
Inadequate androgen and estrogen synthesis	Adequate granulosa proliferation and functional maturation	Follicular premature luteinization
Compromised oocyte maturation	Normal estrogen and androgen biosynthesis	Compromised oocyte development
	Optimum oocyte maturation	

The LH-dependent phase of preovulatory follicular development proceeds normally only if LH is present at concentrations within the *LH window*, which is over the threshold level and beneath the ceiling value **(Table 2)**.

Pharmacogenomics—Follicle-stimulating Hormone and Luteinizing Hormone Polymorphisms

Response to hormones depends on variations in the gene sequence of the receptor or the ligand. Association studies assess whether a gene variant is present more often than expected in a population and evaluate whether a medical condition or a phenotype is associated with a gene variant. Gene variants may consist of insertions or deletions of one or more bases, or may be single-base changes, known as single-nucleotide polymorphisms (SNPs). A genetic variant is considered a polymorphism when it reaches a frequency higher than 1% in a sample population; otherwise, it is considered a gene mutation.

With the advent of pharmacogenomics, various polymorphisms have been identified that may lead to impaired responses to various hormones (FSH and LH) and steroids (estrogen and progesterone). The effects of variations in different biochemical pathways involved in estrogen production and action (aromatase and estrogen receptor genes), folliculogenesis [bone morphogenetic protein 15 (BMP15), growth differentiation factor-9 (GDF-9), and anti-Müllerian hormone (AMH)], and other areas have been observed. These variations may alter the response to controlled ovarian stimulation (COS). In this chapter, we will discuss about the common variations in FSH and LH hormones and their receptors.

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Follicle-stimulating Hormone Polymorphism

With advancements in the pharmacogenomics, various polymorphisms have been identified that attribute to altered function and efficacy of FSH.

Follicle-stimulating hormone works by binding to its specific receptor, the follicle-stimulating hormone receptor (*FSHR*, gene ID: 2492, location: 2p21–p16, OMIM: 136435). Research now highlights the role of common genetic variants of *FSHR* and FSH beta subunit (*FSHB*, gene ID: 14308, location: 11p13, OMIM: 136530) in the determination of individual serum hormone levels and target organ response. However, controversies exist concerning the impact of genetic polymorphisms of these genes on gonadotropin treatment and some contradictory findings have been published, necessitating further research.[28]

The current evidence suggests that the combination of *FSHR* and *FSHB* genotypes is predicted to have a much stronger impact than either one alone, on both male and female gonadal function. This is because about 20% of people are carriers of the allele combinations associated with lower serum FSH levels and lower *FSHR* expression or activity. The advent of powerful, sensitive, and inexpensive techniques assessing several SNPs simultaneously will be very helpful in the identification of the patients at infertility risk.[29]

The *FSHR* gene is located at chromosome region 2p21 and consists of 10 exons. A large number of SNPs in the gene have been identified (1,488 SNPs). The most common and well-studied SNPs in *FSHR* are Thr307Ala (rs6165) and Asn680Ser (rs6166). Both polymorphisms are located within exon 10, where Thr307Ala is located in the extracellular domain of the protein (the hormone-binding area) and Asn680Ser is located in the intracellular domain. These two polymorphisms are in near complete linkage disequilibrium.

The *FSHR* 680Ser variant is usually associated with elevated basal levels of FSH and elevated gonadotropin requirements during COS. A29 polymorphism reduces expression of *FSHR*. S680 is a "resistance factor" to FSH stimulation determining a higher ovarian threshold of the gonadotropin and reflecting a different pattern of ovarian secretion during the cycle transition phase. Another *FSHR* polymorphism, G/A, located in the promoter region at position 29, could modulate ovarian response to FSH and may require higher gonadotropin doses for COS.

The Asn680 allele has been found to be a risk factor and predictor of severity of symptoms for ovarian hyperstimulation syndrome (OHSS).[29]

Luteinizing Hormone Polymorphism

Luteinizing hormone beta (LHB) polypeptide encodes the beta subunit of the hormone and determines the specificity of binding to luteinizing hormone/choriogonadotropin receptor (LHCGR). LH has a key role in steroidogenesis and folliculogenesis (with FSH), ovulation, and luteinization.

The *LHB* gene is located at chromosome region 11p13 and has three exons. A number of polymorphisms in the gene have been identified (179 SNPs). Three polymorphisms in the coding area have been found to lead to decreased LH activity: Polymorphisms Trp8Arg and Ile15Thr have been associated with slightly suppressed fertility, menstrual irregularities causing infertility and recurrent pregnancy loss, and the Gly102Ser SNP has been associated with infertility and menstrual disorders.[29]

Luteinizing hormone receptor: LH exerts its actions by binding to its cell surface receptor, *LHR* (also known as LHCGR, as LH and hCG bind to the same receptor). The *LHR* gene is located at chromosome region 2p21, consisting of 11 exons, and several common polymorphisms have been identified (over 520 SNPs). Polymorphisms 18insLeuGln, Asn291Ser, and Ser312Asn in the *LHR* gene are associated with increased receptor activity, and their possible effects in steroid hormone-related diseases have been suggested, though still not established.[29]

Hypothalamic–Pituitary–Ovarian Axis

In the presence of a GnRH pulse, the pituitary and ovarian hormones exert mutual control over the circulating levels of one another. The complex interactions between pituitary and ovarian hormones involve forward control, positive feedback, and negative feedback mechanisms and serve to sustain a self-perpetuating monthly endocrine cycle **(Fig. 8)**.

Ovarian Feedback on the Hypothalamus and Pituitary

Estrogen

Estrogen produced at the level of the ovary is crucial for the development of the antrum and maturation of the

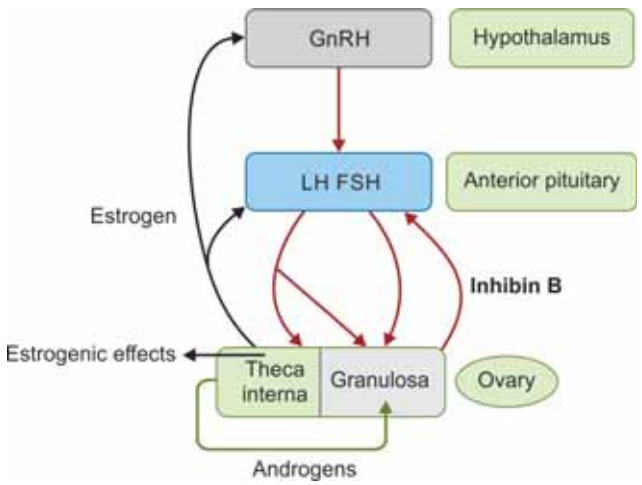
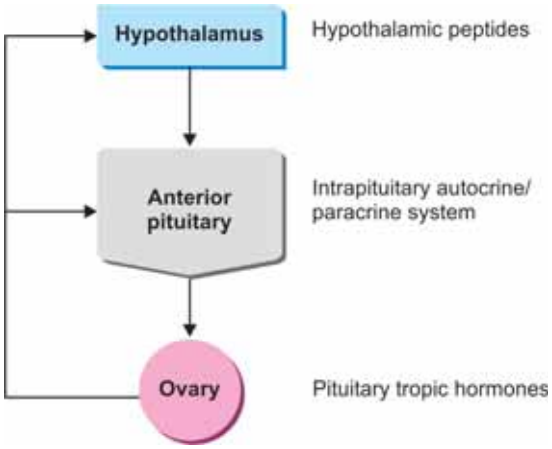


Fig. 8: Hypothalamic–pituitary–ovarian axis.
(FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone)

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Flowchart 4: Hypothalamic and ovarian control of pituitary secretions.



Graafian follicle. Estrogen is predominant at the end of the follicular phase, directly preceding ovulation. Estradiol, the most potent and abundant estrogen, is primarily derived from androgens produced by thecal cells. The androgens migrate from the theca cells to the granulosa cells, where they are converted into estradiol by aromatase enzyme. The actions of estradiol include induction of FSH receptors on granulosa cells, proliferation and secretion of follicular and thecal cells, induction of LH receptors on granulosa cells, and proliferation of endometrial stromal and epithelial cells. At low circulating levels, estrogens exert negative feedback on FSH and LH secretion, and at very high levels, estrogens exert positive feedback on LH secretion.[30] Estrogen further induces proliferation of estrogen-converting granulosa cells and synthesis of estrogen receptors, establishing a positive feedback loop on itself. In the endometrial cycle, estrogen induces proliferation of the endometrial glands **(Flowchart 4)**.

Progesterone

Progesterone is secreted at the level of the ovary, primarily by luteinized follicles. The levels increase just prior to ovulation and peak 5–7 days postovulation. It stimulates the release of proteolytic enzymes from thecal cells that ultimately prepare for ovulation. Progesterone further induces migration of blood vessels into the follicle wall and stimulates prostaglandin secretion in follicular tissues. During the luteal phase, progesterone induces swelling and increased secretion of the endometrium **(Flowchart 4)**.

Gonadotropin-releasing Hormone Dynamics and Pituitary Responsiveness

The interplay between pituitary and ovarian hormones gives rise to a stereotyped pattern of hormone levels during the menstrual cycle. The graph shows relative hormone trends in an average 28-day cycle **(Fig. 9)**.

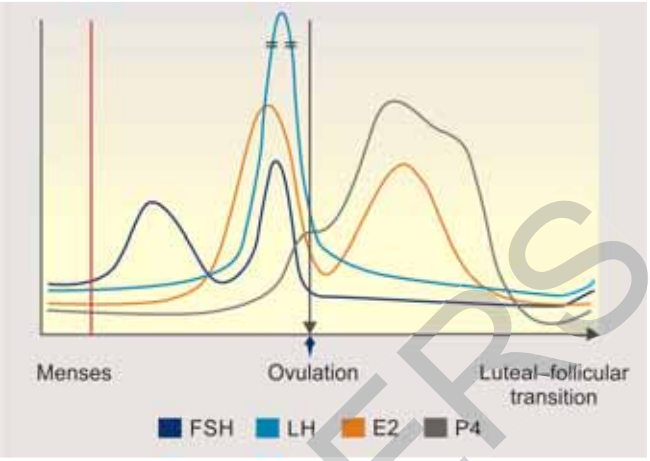


Fig. 9: Pituitary and ovarian hormonal interrelationships during the menstrual cycle.
(E2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; P4: progesterone)

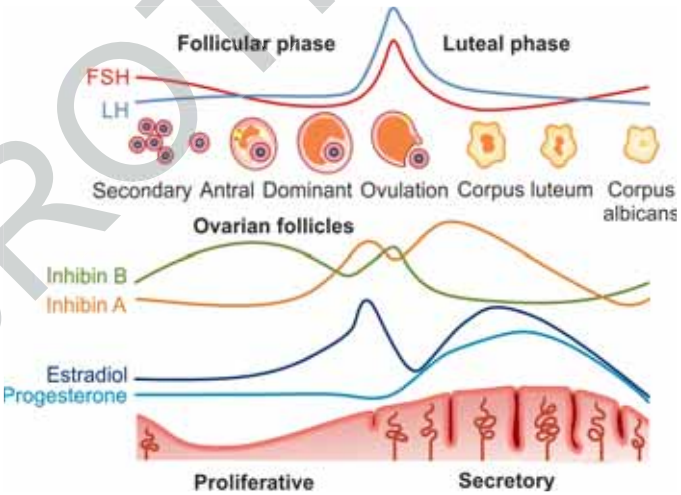


Fig. 10: Overview of hormonal and peptide variations in the menstrual cycle.
(FSH: follicle-stimulating hormone; LH: luteinizing hormone)

Physiology of Menstrual Cycle

Following the exhaustive discussion about the neuro-endocrinological control and the hypothalamic–pituitary–ovarian (HPO) axis, the understanding of the physiology of menstrual cycle becomes comprehensive. The menstrual cycle is divided into four phases based on the ovarian cycle as described below **(Fig. 10)**.

1. Follicular phase (further classified into early- and late-follicular phases)
2. Ovulatory phase
3. Luteal phase
4. Luteal–follicular transition phase.

Based on the uterine cycle, it may be divided into:

- Proliferative phase
- Secretory phase
- Menstruation

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Ovarian Cycle

The menstrual cycle is divided into four phases based on the ovarian cycle.

Follicular Phase

The follicular phase of the menstrual cycle spans from the first day of menstruation until ovulation. The primary goal is to develop a viable follicle capable of undergoing ovulation. The early events of the follicular phase are initiated by a rise in FSH levels at the luteal–follicular transition attributed to a decrease in progesterone, estrogen, and inhibin levels at the end of the previous cycle and the subsequent removal of inhibition of FSH. Rise of FSH rescues a cohort of preantral follicles from apoptosis—a phenomenon called recruitment, which requires a certain minimum concentration of FSH called *FSH threshold*. The time frame for which this threshold surpasses the level of FSH needs to be maintained for follicular recruitment and growth. This is called *FSH window* **(Fig. 11)**.

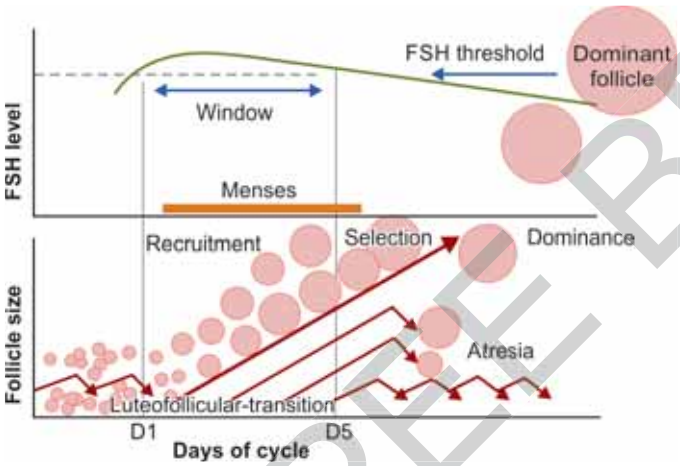


Fig. 11: Concept of follicle-stimulating hormone (FSH) window and threshold.

This requires an orderly sequence of events and a series of sequential actions of hormones and autocrine-paracrine peptides on the follicle, through a period of initial growth from a primordial follicle through the stages of the preantral, antral, and preovulatory follicle.[31,32] As folliculogenesis has already been described in another chapter, this chapter only discusses the physiological attributes of the ovarian events **(Fig. 12)**.

Primordial Follicle

The primordial germ cells originate in the endoderm of the yolk sac, allantois, and hindgut of the embryo and by 5–6 weeks of gestation, they have migrated to the genital ridge. A rapid mitotic multiplication of germ cells begins at 6–8 weeks of pregnancy, and by 16–20 weeks, the maximum number of oocytes reaches to a total of 6–7 millions in both ovaries.[33] The primordial follicle is nongrowing and consists of an oocyte, arrested in the diplotene stage of meiotic prophase, surrounded by a single layer of spindle- shaped granulosa cells.

Until the follicle numbers are exhausted, some grow while some undergo atresia, uninterrupted by even pregnancy, ovulation, or periods of anovulation, continuing through infancy and up to menopause. The rate of decrease in follicle number is proportional to the total number present, implying that the most rapid decrease occurs before birth resulting in 2 million oocytes at birth and 300,000 at puberty. Out of these, about 400 follicles will ovulate during a woman's reproductive years[33] **(Fig. 13)**.

Intrafollicle signaling regulating primordial follicle activation: The phosphatidylinositol 3-kinase (PI3K) pathway, the mechanistic target of rapamycin complex 1 (mTORC1) pathway, the p27Kip1 (p27) cyclin-dependent kinase (CDK) system, and several oocyte-specific transcription factors have been shown to play roles in regulating the activation of dormant oocytes in the mammalian ovary.

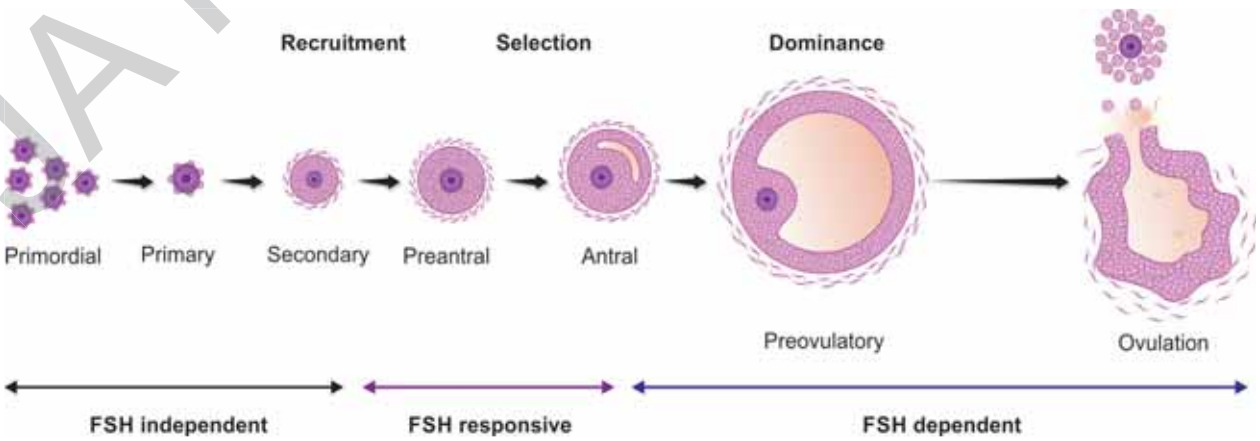


Fig. 12: Stages of follicular growth. (FSH: follicle-stimulation hormone)

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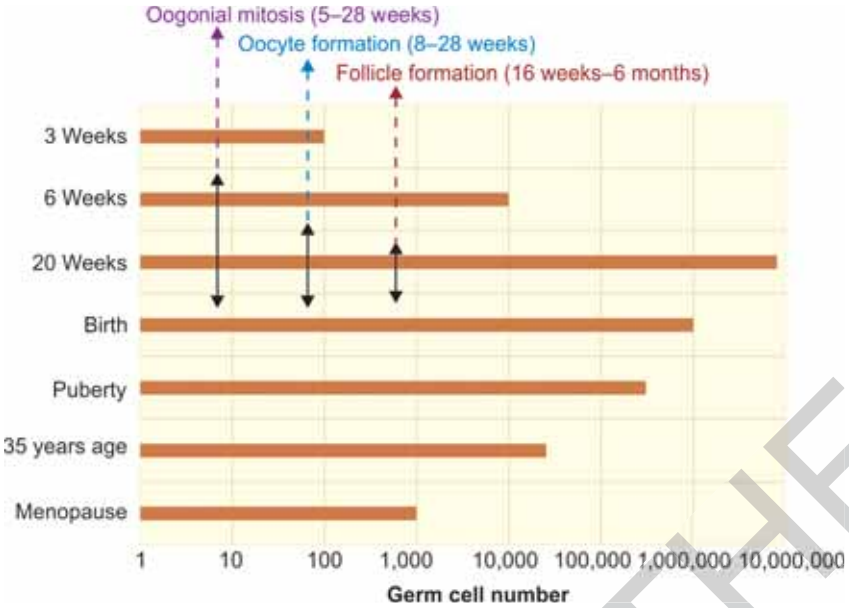


Fig. 13: Changes in germ cell numbers in relation to age.

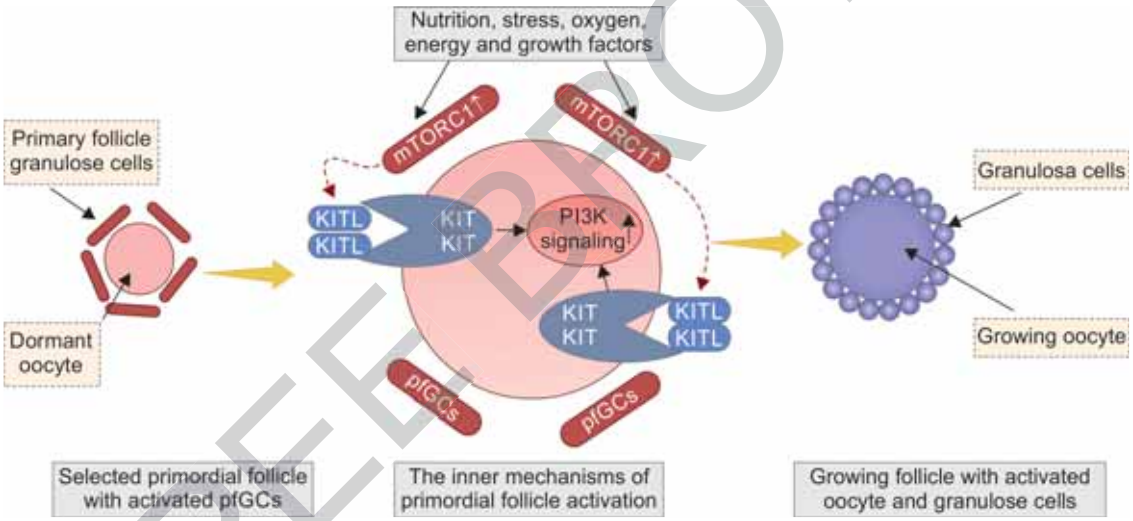


Fig. 14: Primordial follicle activation model for the mammalian ovary.
(KITL: KIT ligand; mTORC1: mechanistic target of rapamycin complex 1; pfGCs: primordial follicle granulosa cells; PI3K: phosphatidylinositol 3-kinase)

Recent evidence suggests that the somatic primordial follicle granulosa cells (pfGCs) play an important role in initiating the activation of primordial follicles in the adult ovary. It is primarily achieved through mTORC1 activation in pfGCs, which mediates the differentiation and proliferation of the flattened pfGCs into cuboidal granulosa cells and simultaneously promotes KIT ligand (KITL) expression. KITL secreted from the pfGCs subsequently binds to the KIT receptor on the oocyte surface, resulting in the phosphorylation of KIT Y719, which then activates intraoocyte PI3K signaling. The PI3K signaling in the dormant oocytes is then responsible for triggering the events that awaken the oocyte from its quiescence into a robust developmental state.[34]

In the adult mammalian ovary, only a small proportion of dormant primordial follicles are awakened to move on to further developmental stages. In response to stimulation by nutrition and other factors, the mTORC1 signaling is activated in the pfGCs of selected primordial follicles, leading to their differentiation and proliferation. The activated mTORC1 signaling in pfGCs also stimulates an upregulation of KITL secretion. KITL binds to KIT on the surface of the dormant oocyte, which leads to activation of PI3K signaling. Activated PI3K signaling in oocytes awakens the dormant oocytes and stimulates their growth **(Fig. 14)**.

The application of this understanding of primordial follicle activation could be beneficial for women with low ovarian reserve and for cancer survivors.

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Primary Follicle

With multiplication of the cuboidal granulosa cells, the primordial follicle becomes a primary follicle. The granulosa cell layer gets separated from the stromal cells by a basement membrane called the basal lamina. The stromal cells are further subdivided into theca interna and theca externa.

Rescue from atresia: The early growth of follicles occurs over the time span of several menstrual cycles, approximately 85 days. The follicle destined to ovulate is one of a cohort that is recruited at the luteal–follicular transition.[35] Until a late stage, the growth is independent of hormonal regulation.[36] Eventually, this cohort of follicles reaches a stage where unless recruited or rescued by FSH, it is doomed toward apoptosis, a programmed physiologic cell death.[37]

Recruitment is the process by which the cohort of follicles responding to FSH at the beginning of the cycle gets rescued from apoptosis and competes for the selection of the dominant follicle.

Early signs of follicular development are an increase in the size of the oocyte, cuboidal pattern of granulosa cells—a change from their original squamous shape and the appearance of gap junctions between the granulosa cells and the oocyte, essential for nutritional, metabolite, and signal interchange **(Fig. 15)**.

Follicular atresia is regulated by endocrine factors, such as FSH and LH, and by paracrine factors, including insulin-like growth factor 1 (IGF-1), EGF, basic fibroblast growth factor (bFGF), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and others.

Preantral Follicle

With accelerated growth promoted by the gonadotropins, the granulosa cells undergo a multicellular proliferation leading to an increased production of not only estrogen but also other steroids, such as androgens and progestins. The oocyte also enlarges and is surrounded by zona pellucida, an acellular glycoprotein membrane. FSH receptors are acquired through which FSH aromatizes androgens to estrogens, generating an *estrogenic microenvironment*.[38] Together, FSH and estrogen synergistically promote a rapid accumulation of FSH receptors, allowing the follicle to respond to relatively low concentrations of FSH **(Fig. 15)**.

Androgens have a complex role in early follicular development. At low concentrations, androgens serve as a substrate for FSH-induced aromatization to the extent of even enhancing the enzyme. But, in androgen-rich environment, the limited capacity of aromatization is overwhelmed and the preantral granulosa cells instead favor the conversion of androgens to more potent 5α-reduced androgens.[39] These androgens inhibit aromatase activity and FSH-induced LH receptor formation as they cannot convert to estrogen leading the follicle toward atresia.[40,41] Hence, the fate of the preantral follicle is in delicate balance depending on the ability to convert an androgen-dominant microenvironment to an estrogen-dominated microenvironment for survival **(Box 3)**.

Formation of gap junctions: Oocytes are unable to use glucose, as an energy source to support meiotic maturation, cannot transport certain amino acids, and lack both the enzymes

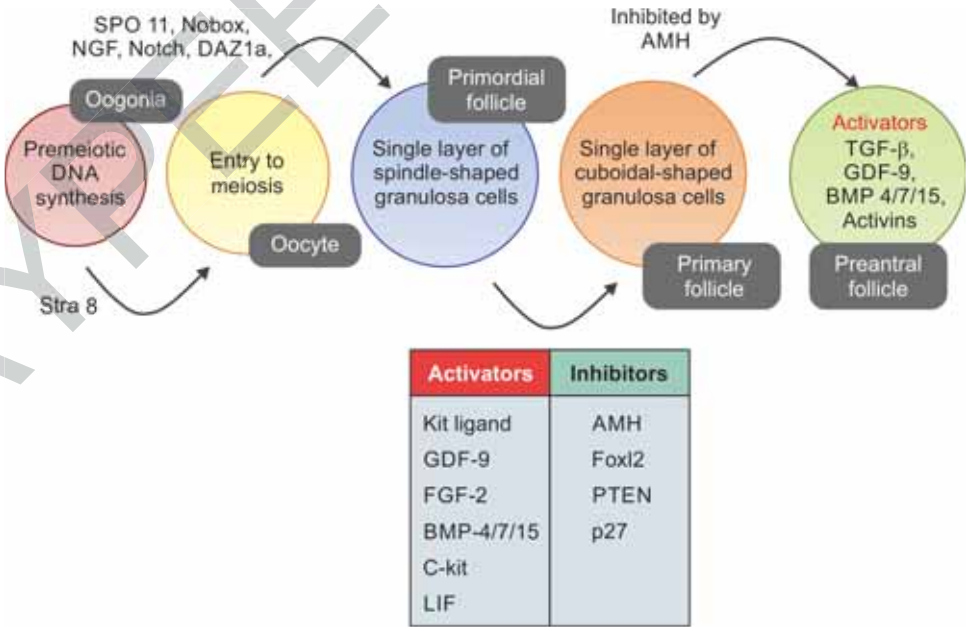


Fig. 15: Series of events in follicular development. Note the genes involved in early stages of folliculogenesis.
[AMH: anti-Müllerian hormone; BMP: bone morphogenetic protein; DNA: deoxyribonucleic acid; FGF-2: basic fibroblast growth factor; GDF-9: growth differentiation factor-9; LIF: leukemia inhibitory factor; NGF: nerve growth factor; Nobox (expressed in germ cells): newborn ovary homeobox; PTEN: tumor suppressor gene (phosphatase and tensin homolog deleted on chromosome 10); TGF-β, transforming growth factor-β]

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A recipient of the Padma Shri, **Kamini A Rao** MBBS DGO MCh FRCOG FNAMS FICOG PGDMLE is a pioneer of the assisted reproduction fraternity in India. She is the Founder Director of Medline Academics an Online Learning Platform and Founder and Chief Mentor for the Milann Chain of Fertility Centers. She has served as the President of the Federation of Obstetric and Gynaecological Societies of India (FOGSI) and of the Indian Society for Assisted Reproduction (ISAR). She is a recipient of the Rajyotsava Award and Lifetime Achievement Awards from FOGSI, ISAR, BSOG and KSOGA and has been bestowed with an Honorary Fellowship of the International Academy of Assisted Reproduction (IAHR) for her contributions to the field of reproductive medicine. She is an educationist of repute and has authored and published over 50 medical books and over 40 research publications in national and international journals. She is a popular teacher and an eminent Academician with the credit of teaching and training over 10,000 students both in India and abroad. She has received the distinguished NBE Teacher Award by the Association of National Board Accredited Institutions and was conferred with a Doctorate of Science (DSc) by Bangalore University recently. She is the Founder Editor of the prestigious Journal of Human Reproductive Sciences (JHRS), the official organ of the Indian Society of Assisted Reproduction (ISAR) and Founder, Editor-in-Chief of the International Journal of Infertility and Fetal Medicine (IJIFM) published by Jaypee Brothers Medical Publishers, both of which are peer-reviewed and indexed journals. She is one of the stalwarts of the medical profession in India and her contributions to the medical field and in particular to assisted reproduction are immeasurable.



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