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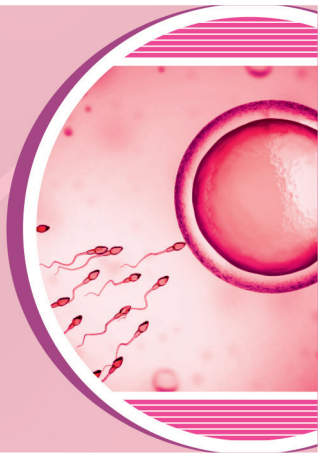
Puberty



BN Chakravarty

INTRODUCTION

Events Preceding Advent of Puberty

Puberty is a transitional period between immaturity of childhood to semimaturity of adolescence. Apart from genetic influence, endocrinological, nutritional and environmental factors primarily initiate and regulate the advent and onset of puberty. Endocrinological factors primarily initiate—nutritional and environmental factors subsequently control and regulate advent and onset of sequential events of puberty. Endocrinological modulation through hypothalamic-pituitary-ovarian (HPO) axis is the primary trigger which initiates and coordinates different physiological and physical changes of puberty from intrauterine (foetal) to pubertal development of human life. The initiative starts from hypothalamic pulse generator, located in hypothalamus.

What is Hypothalamic Pulse-Generator?

The term "hypothalamic pulse-generator" was coined by Ernst Knobil. The hypothalamic pulse generators are clusters of neurohormonal secretory cells (specialized) located in the arcuate nucleus in the medial basal hypothalamus. The number is approximately 1500 to 2000. These are known as GnRH neurons. They have 'autorhythmicity'. They perform their function as oscillator for the pulsatile release of GnRH. Under the influence of 'signal', they start secreting GnRH in a pulsatile fashion which stimulate gonadotroph cells in pituitary. These cells, in turn start secreting follicle stimulating hormone (FSH) and luteinizing hormone (LH), also in a pulsatile rhythm. Pituitary gonadotropin stimulates maturation of ovarian follicles. Granulosa and theca cells of the ovary, under influence of FSH and LH, also start secreting in a similar pulsatile manner. To stimulate ovarian steroids (estrogen and progesterone) in a similar pulsatile manner.

Consequently, the pattern of pituitary gonadotropin and gonadal steroid secretion during the entire period of life, foetal, infancy, childhood, adolescence and adulthood will reflect changes in the activity of the hypothalamic pulse-generator.

Pattern of gonadotropin and gonadal steroid secretion from intrauterine life till onset of puberty:

Hypothalamic neurons which synthesize GnRH originate primarily in the olfactory placode and migrate to the hypothalamus during 6–9 weeks of gestation.[1] By 10 weeks, hypothalamus contains significant number of GnRH neurons. Meanwhile, hypothalamic-portal venous system develops from 9–10 weeks and the development is completed by 19–20 weeks. GnRH secreted by hypothalamic neurons can now be transported through hypothalamic-pituitary portal venous system to stimulate pituitary gonadotropin release. Foetal serum gonadotropin rises progressively

reaching a peak between 20 and 24 weeks.[2] Thereafter, the level starts declining progressively during the last 10 weeks of pregnancy possibly because of negative feedback effect of placental oestrogen and progesterone.[3–5]

After birth, following a small gap of persisting negative feedback of placental steroid effect, FSH and LH in the newborn start rising again (Fig. 1.1). This is because auto-rhythmicity of neonatal GnRH pulse-generator starts functioning again following removal of negative feedback effect of placental sex steroid hormones. The characteristic pulsatile pattern of GnRH function now emerges.[5,6] The serum gonadotropin level (both FSH and LH) rise again. Type of gonadotropin rise has a specific sex difference. FSH rises to a greater extent in females and LH in males.[7,8] The maximum rise reaching to a peak is observed about 3–6 months in boys and 12–18 months in girls. The gonadotropin starts to decline thereafter presumably because normal feedback mechanism becomes fully functional. By approximately 9–12 months of age in boys and 24–36 months in girls, gonadotropin concentration reaches *to a* ***typical pre-pubertal level remaining at a very low concentration till the advent of puberty***.[8] The suppressing effect is more intense in boys than in girls which probably reflects the influence of testosterone on hypothalamic programming.[9]

Advent of Puberty

Biological expression of interlinked hormones (neurohormone-GnRH), protein hormones (FSH and LH) and sex steroid hormone (estrogen and progesterone) remain suppressed during childhood and early puberty. With advent of puberty, the expression of these hormones become apparent both biochemically and clinically. The controlling mechanism of this dramatic change is not very clear.

Previous theory which tried to explain 'Juvenile pause' which precedes puberty, speculated, a hypothalamic '**gonadostat**' was probably controlling the level of 'sensitivity'

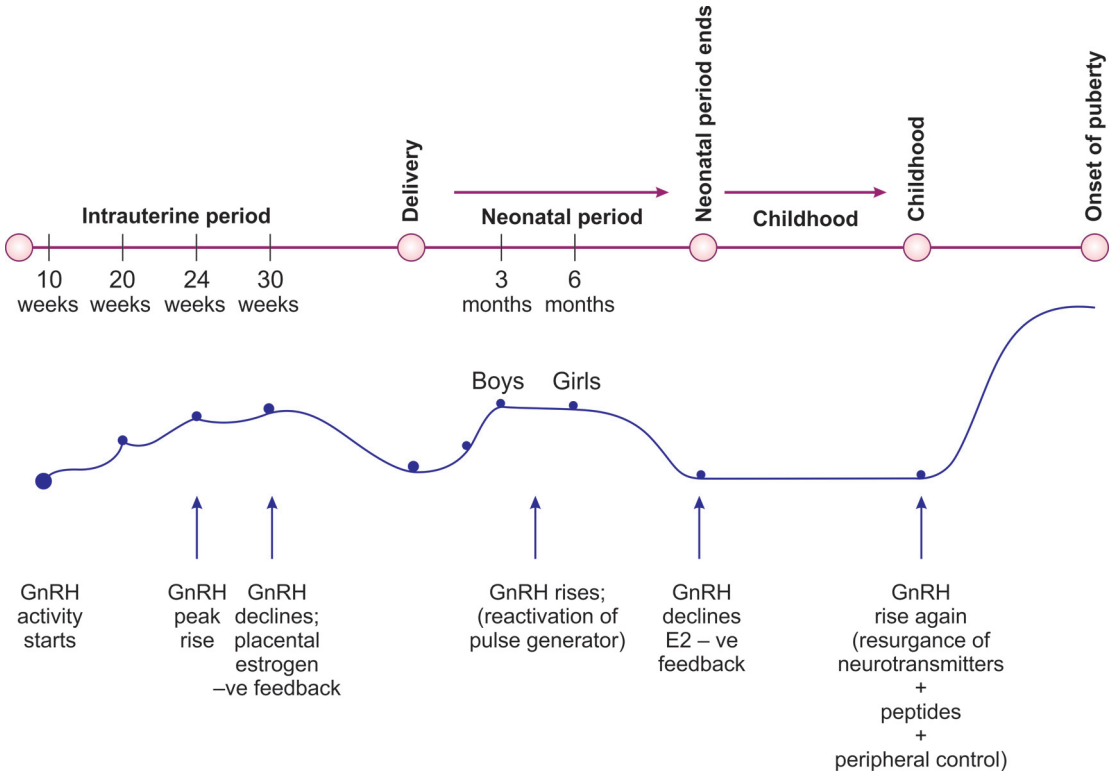


Fig. 1.1: Pattern of GnRH release (GnRH pulse generator); functioning from intrauterine life till onset of puberty

of the central negative feedback action of gonadal steroid. The view was based on the fact that changing pattern of gonadotropin secretion depends on changes in the gonadostat setting, i.e. primarily on the gonadal steroid (estrogen) feedback mechanism. But it is becoming more clear that the typical **'biphasic pattern'** of gonadotropin secretion from infancy to puberty is primarily because of changing level of central inhibition of pulsatile GnRH secretion and to an insignificant extent from a high sensitivity to low level of gonadal steroid feedback. In other words, the control of 'pulsatile release' or 'inhibition' is more on central factors and not on simple 'gonadal steroid' feedback mechanism as was explained by 'gonadostat' theory. The central factor has a neuroendocrine switch and this switch controls the GnRH pulse-generator. The switch when 'on' will release GnRH, pituitary gonadotropin and ovarian steroids in a pulsatile fashion and when the controlling switch is 'off'—the entire chain becomes non-functioning.

Some of the factors governing the 'neuroendocrine switch' for the GnRH pulse-generator have now been identified.

The list of factors which modulate the activity of hypothalamic-pituitary gonadal axis include both 'inhibitory' and 'excitatory' neurotransmitters and peptides.

In summary, it appears that primary controlling factors for 'initiation' and 'inhibition' of HPO axis for pulsatile release of GnRH are centrally located 'neurotransmitters' and 'peptides'. 'Negative ovarian steroid feedback mechanism' has minor role to play.

Neuroendocrine Factors Controlling Neurotransmitters and Peptides

Some of these factors (neurotransmitters and peptides) (Fig. 1.2) governing the functioning

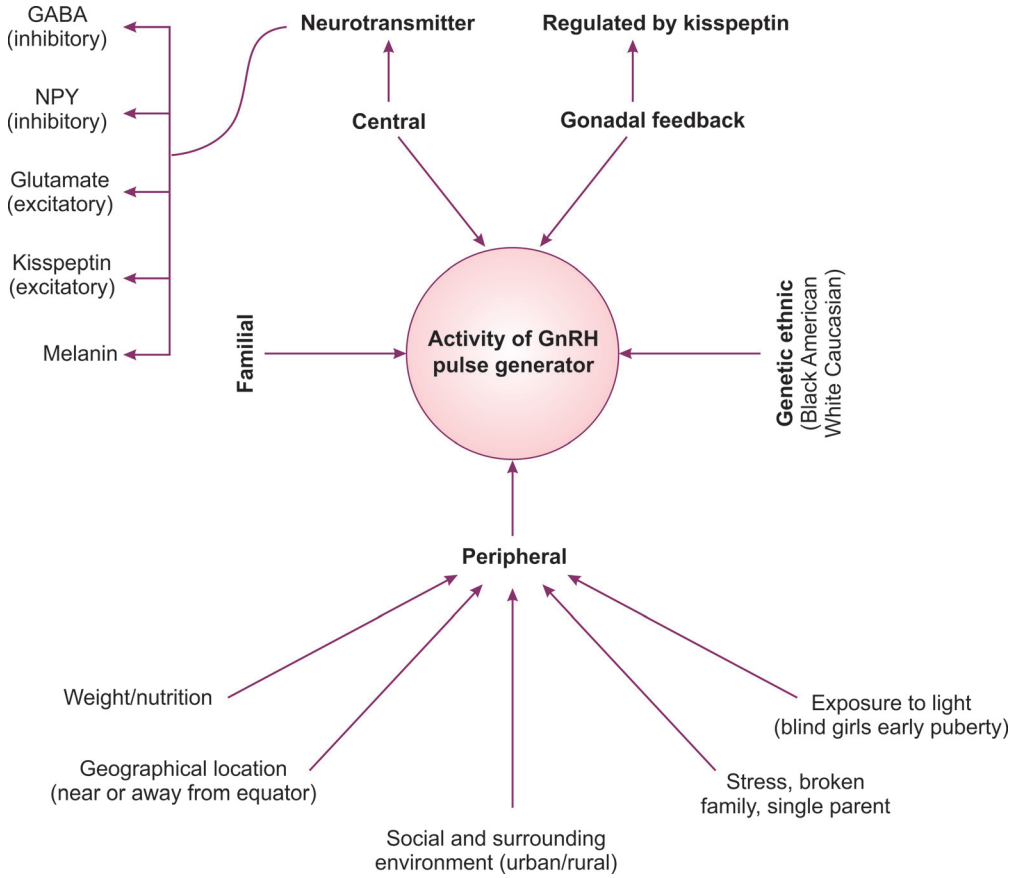


Fig. 1.2: Control of GnRH pulse-generator activity; central-peripheral-genetic-familial

of HPO axis have been identified and they are briefly outlined as follows:

- **Gamma amino butyric acid (GABA):** It is an ***inhibitory*** neurotransmitter. Experimental evidences have suggested that declining concentration of GABA at the onset of puberty helps to accelerate increasing release of pulsatile GnRH. In other words, central GABA signaling is one of the factors which inhibits GnRH neuronal activity during childhood.
- **Neuropeptide (NPY):** This is also a hypothalamic peptide involved in the control of food intake behaviour and reproductive function in adults. Majority of experimental evidences have suggested that NPY, like GABA is an important component of 'neurobiologic break' that inhibits activity of GnRH in the pre-pubertal period.[10] However, the observation was not substantiated by other observers who hold an opposite view.[11] Additional work will be required to verify the role of NPY in regulation of hypothalamic pulse-generator and the onset of puberty.
- **Glutamate:** This is an ***excitatory*** neurotransmitter and stimulates release of pulsatile GnRH both *in vivo* and *in vitro*.[12] Experimental evidences[13,14] suggest that glutamate stimulates resurgence of pulsatile GnRH release at the onset of puberty.
- **Kisspeptin:** Kisspeptin is a neuropeptide (encoded by kiss-1 gene) and like GnRH neurons, are resident in hypothalamus. Evidences suggest that Kisspeptin signaling might play a major role in resurgence of pulsatile GnRH secretion at puberty.[15] Melatonin produced by the pineal gland also regulates the production of kisspeptin, and synthesized mostly in the hypothalamic tissue. Also, decreased melatonin blood levels lead to increased kisspeptin. These are two different views of controlling the level of kisspeptin for the developmental of puberty. The results of subsequent studies in non-human primates and also in human, strongly support this view.

Neurons expressing kiss-1 gene are located exclusively in arcuate nucleus[16,17] where GnRH neurons also express their neurohormones.[18] Hypothalamic kisspeptin secretion is distinctly pulsatile and highly correlated with GnRH.[19] The biological expression of kisspeptin regulates pulsatile GnRH secretion.[20] It has also been observed that kisspeptin might only amplify and not stimulate GnRH pulse-generator directly.[15]

In summary, it is more or less accepted that kisspeptin signaling is the key component of the neurobiological mechanism that triggers the onset of puberty. Kisspeptin neurons provide the ingredients for functioning of the hypothalamic GnRH pulse-generator.

These are all about central activity of kisspeptin in initiating pubertal resurgence of hypothalamic pulsatile GnRH release at puberty with activation of HPO axis.

There is another mechanism through which kisspeptin controls advent of puberty. It has been suggested that kisspeptin mediates the negative feedback action of both testicular and ovarian hormones. There are evidences which suggest that kisspeptin neuros play an important role in the negative feedback loop that regulates hypothalamic GnRH secretion in the male which also involves the opioid and GABA neuronal input.[21] Similar activities of kisspeptin have also been observed in the ovarian steroidal feedback mechanism.

Hence, it is obvious that kisspeptin neurons are critical components of neurohormonal mechanism which activate and regulate hypothalamic pulse-generator at puberty.

Impact of Peripheral Signaling

In addition to 'central' and 'loop feedback' mechanism, GnRH pulse generator is also controlled by peripheral signaling.

Since last few decades, the age of onset of puberty has been declining steadily. This may be also due to increasing prevalence of obesity. It is possible that critical body weight[22] or body composition[23] may be important factors in determining the timing and progression of puberty. The negative impact of fasting (anorexia nervosa) is well known. Possibly the impact of

malnutrition and lean body weight negatively affects pulsatile release of hypothalamic GnRH secretion.[24]

Impact of Leptin

Adipocyte (fat) cells produce leptin. Leptin may provide metabolic signals and indirectly, have peripheral control on the higher center, regulating the activity of the hypothalamic pulse generator at the onset of puberty. The clinical observations suggests that leptin plays an important, but only permissive role in the onset of puberty. However, available evidences suggest that leptin might have the ability to influence or modulate the activity of the hypothalamic GnRH pulse generator.[9]

Other Metabolic Signals

In addition to leptin, various other metabolic signals have been implicated in the process of nutritional regulation for the normal function of hypothalamic GnRH pulse generator. Some of the metabolites which provide these signals are—insulin, ghrelin (endogenous ligand of growth hormone secretagogue with a small role of energy balance),[25] gallamine like peptide (potential neuronal target of leptin and free fatty acid).[26] However, the exact mechanism in which these peripheral impulses regulate release of pulsatile GnRH and onset of puberty is not known.

Clinical Landmarks of Puberty

Puberty is a period of immense physical, physiological, endocrinological and psychological changes. The physical and physiological changes are regulated primarily by endocrine maturation and partly by environmental and ethnic variation. Physiological changes are totally dependent on a combination of physical, endocrinological and psychological changes. Although the timing of appearance may vary, the sequential events of puberty generally follow a predictable pattern. The sequence of events are briefly summarized in following paragraphs:

Adrenarche: The first physical indicator of puberty is adrenarche. This means activation and stimulation of adrenal androgen. Adrenal androgen originates from zona reticulosa of adrenal cortex. It is significant to realize that adrenarche is independent of maturation of hypothalamic-pituitary gonadal axis, but the two (GnRH and ACTH) often are temporarily interrelated.[27]

The steady increase in adrenal androgen stimulates growth of pubic hair (pubarche) and activates pilosebaceous unit consisting of hair follicles and sebaceous gland.[27] Androgen increases bone density, suggesting that androgen also contributes growth of cortical bone.[28] Adrenarche precedes maturation of HPO axis by 2 to 3 years. This observation initially suggested the idea that 'adrenarche' stimulates 'gonadarche' or in other words, adrenarche has the fundamental role for the 'onset of puberty'. But other clinical evidences do not support this view. The contradictory evidences are:

- Premature 'adrenarche' is not generally associated with earlier onset of thelarche or menarche.
- Adrenarche occurs in those girls who have hypergonadotropic hypogonadism (gonadal dysgenesis), hypogonadotropic hypogonadism (Kallmann syndrome).
- Even without adrenarche like Addison's disease (hypoadrenalism) gonadarche occurs.
- In precocious puberty under the age of six gonadarche precedes adrenarche.

If adrenarche occurs earlier than gonadarche, then pubic hair should appear before 'growth spurt' and 'thelarche'. But during normal pubertal development, pubic hair develops after growth spurt and thelarche. Possibly in adrenarche, weak androgen DHEA is secreted from zona reticulosa for which clinical impact (pubic and axillary hair) appears after full development of gonadarche, i.e. after the onset of growth spurt and thelarche.

Growth Spurt

'Growth spurt' is an important landmark at puberty. Growth includes both in height and body composition. 17–18% of adult height is gained at puberty.[29] Pubertal growth occurs usually two years earlier in girls than boys and in girls the peak velocity reaches approximately 6 months before menarche.[30]

Accumulation of bone mass is critical during puberty. This is a major determinant of occurrence of osteoporosis in later life. Bone mass accumulates from circulating calcium. About one half of total body calcium is utilized during puberty in girls and one half to two third in boys.[31, 32] In girls the maximum accumulation of bone mass occurs 9–12 months after peak height velocity has been reached.

Apart from height velocity, pubertal weight changes, reflect deposition of lean body mass and fat. Adolescent girls have more body fat than boys—the maximum deposition occurs in upper arms, thighs and back. The difference in increase in girls and boys continues throughout puberty. The increase in BMI before the age of 16 is primarily due to increase in lean body mass and, thereafter, due to fat mass.[33]

Apart from genetic factor, pubertal growth spurt is primarily dependent on growth hormone, including IGF-1, nutritional factors and also on sex steroid. These factors are being briefly described below.

Growth Hormone

Growth hormone (GH) is synthesized and released by 'somatotrophs' (like gonadotroph) in the pituitary. It is also released in a pulsatile fashion. Release and rhythmicity are controlled by central (growth hormone releasing hormone) and peripheral negative feedback effect of IGF-1 (insulin-like growth factor 1) which inhibit GH release. The peripheral action of growth hormone on target cell is mediated through IGF-1. Other peripheral factors like nutritional or hormonal (oestrogen, glucocorticoids) also contribute to release and functioning of growth hormone. Fasting[34] and 'high' protein meal[35] stimulate GH release, whereas hyperglycemia and leptin inhibit GH secretion.[35] Similarly oestrogen stimulates and excess glucocorticoids inhibit GH release. The peak rise of growth hormone is observed during puberty and thereafter declines with aging. Approximately every 7 years GH acts through stimulation of hepatic synthesis and secretion of IGF-1 which accelerate bone growth and differentiation. GH is also involved in a number of metabolic factors, namely increase of lipolysis, stimulation of protein synthesis, insulin antagonism, water and sodium retention.

Insulin Like Growth Factor 1 (IGF-1)

IGF-1 is synthesized and released by liver in response to GH stimulation. IGF-1 circulates in serum in bound form—IGF binding protein (IGFBP). The family of IGFBP includes six proteins having greater affinity for IGF-1. The IGFBP-1 concentration is regulated by insulin, increases during fasting when insulin levels are low and decreases after fasting or administration of insulin.[36]

IGF-1 augments the effect of FSH and LH in the ovary—the effect of ACTH on adrenal steroidogenesis and also thyroid response to thyroid stimulating hormone (TSH). IGF-1 levels are low at birth; peak values are observed during puberty, thereafter, fall rapidly approximately by 50% by the age of 20 and then decline gradually as age advances.[37]

Impact of Sex Steroid Hormone

Undoubtedly, pubertal growth is primarily dependent on growth hormone and IGF-1. But clinical evidences suggest that sex steroid hormones also play important role. This is evident from the fact that precocious puberty can induce a substantial growth spurt even in the absence of a normal pubertal increase in circulating hormone or IGF-1. Normally, pubertal 'growth spurt' requires combined action of sex steroid and growth hormone. It is already well known that sex steroids limit adult height by stimulating epiphyseal fusion.

The Age of Onset of Puberty

The triggering mechanism of onset of puberty has still remained unknown. Apart from genetic factor, the age of onset of puberty and the mechanisms are influenced by overall health, social environment and environmental exposure. Family history is also closely linked with early or delayed onset of puberty or menarche. Age of onset of puberty and menarche are often well correlated between mother and daughter and between sisters.[38] Apart from

family linkage, environmental factors play a great role. Girls living close to equator, at lower altitude in urban areas, and those who are mildly obese, generally begin puberty earlier than those who live away from equatorial region, at higher altitude, in rural areas and those who are of normal weight. Environmental pollution, which probably acts as endocrine 'disrupters' may also be involved in the timing of sexual development.[38] The age of onset of puberty is gradually declining globally over the past few decades irrespective of location and ethnicity. The trend to an earlier onset of puberty and sexual development has been attributed to improved nutrition and stressful living conditions (Fig. 1.2).[38] Early onset of puberty is directly associated with increased weight and higher body fat mass.[39,40]

Early pubertal development has two adverse impacts during adult life: (1) increase of obesity; and (2) decrease in adult height.[41,42] The age of pubertal development, thelarche and menarche in India are 10.2 and 12.6 years respectively (1988 to 1991).

Landmarks of Onset of Puberty—Sequence of Development

There are basically four landmarks of onset of puberty. These are: (1) Growth spurt, (2) thelarche, (3) pubarche, and (4) menarche—onset of menses.

Growth spurt in preadolescent girl is due to combined effect of "adrenarche" followed by 'gonadarche'. It is due to combined effect of adrenal and gonadal hyperactivity, which includes increase both in height and bulk. Details have already been discussed in earlier part of this chapter.

A staging system to describe physiological changes of puberty was first described by Marshall & Tanner in 1969 for girls[43] and for boys in 1970.[44] The staging system describes secondary sexual characteristics, including breast development in girls, pubic hair growth in both sexes and genital development in boys. There are five Tanner stages of breast and pubic hair development, in girls. This has been discussed in further details in Chapter 2: "Precocious Puberty". Stage 1 represents the prepubertal state and stage 5 maintaining the adult development.

Menarche occurs about 2.6 years after the onset of puberty and after completion of attaining the peak growth.[45] The total period from onset to completion of pubertal growth (accelerated growth, thelarche, pubarche and menarche) covers a period of 4.5 years (range 1 to 6 years). After menarche, the growth rate declines and generally does not increase more than 6 cm (2.4 inches). Initially menses are infrequent and anovulatory (Fig. 1.3). Anovulation and infrequent menses continue to persist on

Early puberty	Mid puberty	Late puberty
Age 6–8 years	Age 8–10 years	Age 10–12 years
Adrenarche	Gonadarche	
• Release of weak androgen DHEA-induces pubarche slowly; development of pubic hair after growth spurt thelarche • Induces bone mass	• Growth spurt • Thelarche • Pubarche	• Menarche • Co-ordinated function of HPO axis • Initially anovulatory irregular, finally ovulatory and regular

Fig. 1.3: Sequence of chronological development of clinical landmarks of puberty-regulated by combined endocrinological, environmental, genetic and ethnic control

an average about 12 to 18 months to as long as up to 4 years after menarche.[45,46] The criteria for completion of pubertal development and landmarks for maturity of hypothalamic–pituitary ovarian axis is the development of oestrogen function which stimulates the mid-cycle LH surge and ovulation.

Take Home Message

- Onset of puberty depends on step-wise development, maturation and regulation of HPO axis.
- HPO axis develops and differentiates during early intrauterine life (6–10 weeks) and becomes fully functional before birth.
- Activity of HPO axis reaches a peak around 20 week of intrauterine life and thereafter, declines and remains at a low level through later part of intrauterine and earlier part of neonatal life after birth.
- The decline is due to negative feedback effect of placental oestrogen which continues up to earlier period of neonatal life.
- HPO axis becomes active again in the later neonatal period because of its autorhythmicity and LH and FSH remain elevated for 3–6 months in boys and 12–18 months in girls.
- Again there is decline in HPO axis and FSH, LH levels remain very low during the entire period of childhood till onset of puberty due to higher sensitivity of ovarian steroid negative feedback by kisspeptin.
- Somatotrophic axis (GHRH) through growth hormone (GH) and insulin-like growth factor-1 (IGF-1) provide partial support (adrenarche) to GnRH-HPO axis.
- This support also helps in 'growth spurt' of puberty.
- The 'prime mover' however is HPO axis.
- The 'specialized' neurohormonal secretory cells within the hypothalamus (n = 1500–2000) aggregate to form a functional organ within the hypothalamus known as 'hypothalamic pulse generator'.
- The cells of pulse generator originate from olfactory placode, and migrate to arcuate nucleus and are known as GnRH neurons.
- The pulse generator has an 'oscillatory' pattern of function and also has 'autorhythmicity'.
- Hypothalamic pulse generator produces neuro-hormone 'GnRH' in a pulsatile fashion—which stimulate pituitary gonadotroph cells to synthesize and release pituitary hormones—FSH and LH also in a pulsatile fashion.
- Gonadotropins (FSH and LH) induce release of episodic ovarian steroids (oestrogen and progesterone) from granulosa and theca cells of ovary.
- This chain of events starts functioning when hypothalamic pulse generator becomes fully active (around 18–20 weeks of intrauterine life) and again become suppressed in later part of pregnancy because of negative impact of placental oestrogen.
- The activity of hypothalamic pulse generator and consequently HPO axis is controlled primarily by 'central', 'excitatory' and 'inhibitory' factors and secondarily by oestrogen 'feedback' mechanism.
- The central control, which is more significant, is a composite combination of several neuro-transmitters, which are the products of neuro secretory cells and peptides.
- All of them are residents of hypothalamus. Some of them act as inhibitors while others work as excitatory stimulators.
- Kisspeptin has been accepted as the most significant 'excitatory' neurotransmitter for GnRH pulse generator.
- In addition to its excitatory function, kisspeptin also provides a 'high' hypothalamic sensitivity to respond to the regulatory mechanism of negative feedback loop of ovarian steroid hormones.
- These functions become fully functional before birth.
- After birth, beginning in late infancy and continuing through childhood, the HPO axis remains inactive because pulsatile release of hypothalamic GnRH is suppressed to a very low level.
- The suppression is due to low level of activity due to central inhibitory mechanism, and to a lesser extent, due to high sensitivity to low ovarian steroidal feedback.
- The first endocrine change in prepuberty is 'adrenarche'.
- The stimulus is unknown. ACTH does not increase. Adrenal androgen is liberated from 'zona reticulosa' of adrenal cortex which differentiates around 3 years of age.
- Weak adrenal androgen DHEA is elevated. It increases slowly and stimulates 'pubarche' (growth of axillary and pubic hair) after establishment of two of the three landmarks of subsequent gonadarche (growth spurt and thelarche).
- Previously it was presumed that 'adrenarche' stimulates the onset of 'gonadarche'.

- Currently this hypothesis is not accepted.
- After a long gap (from late infancy), pulsatile release of GnRH starts functioning again and hypothalamic-pituitary gonadal axis is reactivated (gonadarche).
- Probably the resurgence of HPO axis is in response to metabolic signals from the periphery (nutrition, weight gain, etc.). This is apart from genetic and familial influence.
- FSH and LH start rising from around 8 years of age followed by gradual increase of estrogen resulting in development of breast and also participating in growth spurt.
- Before 'thelarche' and 'pubarche' there is rapid increase in growth (growth spurt).
- This is primarily due to steroid induced increase of GH and IGF-1 secretion and to a lesser extent sex steroid concentration.
- Finally, rising steroid (estrogen) concentration will restrict adult height by stimulating epiphyseal fusion.
- Following growth spurt, thelarche, pubarche, and menarche (onset of menses) start. This is the final landmark of onset of puberty.
- Initially menses are irregular, infrequent and anovulatory. With maturation of estrogen feedback mechanism, ovulatory cycles increase in frequency and by late puberty become well established.

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