

Fertility in adolescence

THE OVARY LEARNS TO OVULATE

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Regulation of the female reproductive system

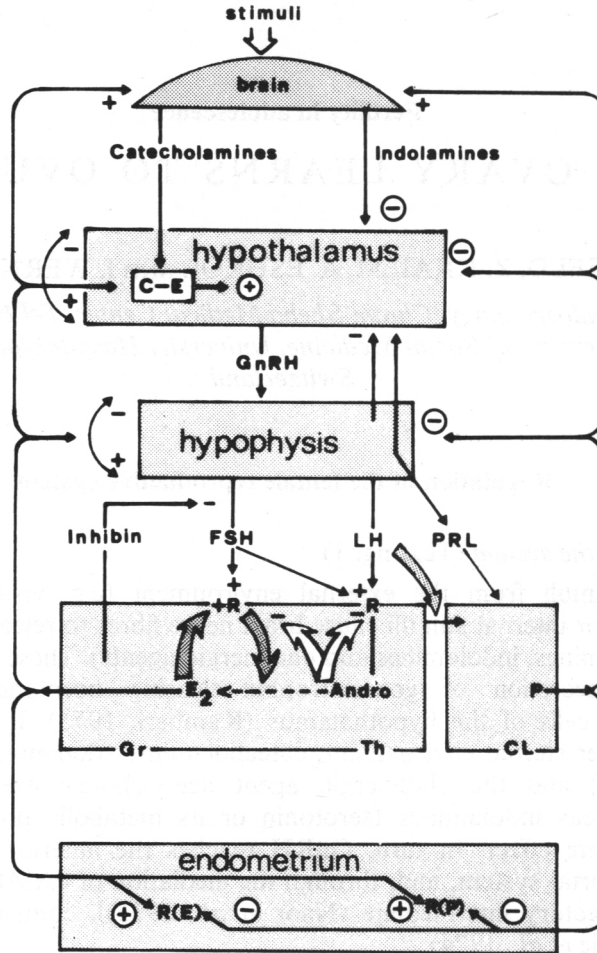
Components of the system (Text-fig. 1)

Sensory stimuli from the external environment (e.g. visual and olfactory stimuli, stress) or internal stimuli cause brain nerve fibres to release neurotransmitters (catecholamines, indolamines and cholinergic agents). These neurotransmitters regulate the secretion of gonadotrophin-releasing hormone (GnRH) from neurosecretory cells of the hypothalamus (Kamberi, 1975). It would seem that under the proper steroid environment, catecholamines (dopamine, norepinephrine or epinephrine) and the cholinergic agent acetylcholine exert a stimulatory influence, whereas indolamines (serotonin or its metabolic product, melatonin) have an opposite effect. In turn, GnRH reaches the anterior pituitary via the hypophyseal portal system, and, through the mediation of cAMP [though may be not as an obligatory intermediate (Naor *et al.*, 1975)], controls gonadotrophin secretion (Labrie *et al.*, 1974).

The gonadotrophins are synthesized in oval basophil cells of the adenohypophysis and evidently both gonadotrophins originate in the same type of cell. A simple mechanism in which a single releasing hormone regulates secretion of both gonadotrophic hormones would be insufficient to account for the fluctuations in the ratio of FSH to LH secretion observed during the menstrual cycle and in various pathological situations. This would be true irrespective of whether all gonadotrophin-secreting cells contained both FSH and LH (Leleux & Robyn, 1971; Phifer, Midgley & Spicer, 1973) or whether some cells contained only a single gonadotrophic hormone (Nakane, 1973). Cyclic fluctuations in the ratio of FSH/LH are probably achieved, as will be discussed later, by a differential sensitivity of the pituitary to GnRH under the influence of sex steroids.

The next message in this molecular relay is conveyed by means of FSH and LH which, besides controlling their own secretion through a short-loop feedback mechanism (Speroff & Van de Wiele, 1971), regulate ovarian function.

The first step in the action of gonadotrophins on the ovary is binding to specific receptor sites on the plasma membrane of ovarian cells (Channing & Kammerman,



Text-fig. 1. Regulatory mechanisms of ovarian function. The control of ovarian (Gr, granulosa cells; Th, theca cells; CL, corpus luteum) function is accomplished through actions of three different types: (1) stimulation along the brain–hypothalamus–hypophysis–ovarian axis by catecholamines, indolamines, catecholestrogens (C–E), gonadotrophin releasing hormones (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL); (2) intraovarian modulation of hormone receptors (R) by the positive influence of FSH and oestrogens (E₂) and the negative effect of androgens (Andro); (3) feedback signals, positive (+) and negative (–), by ovarian steroids (E₂ and progesterone, P) and inhibin. (Reprinted from Lunenfeld & Eshkol, 1977.)

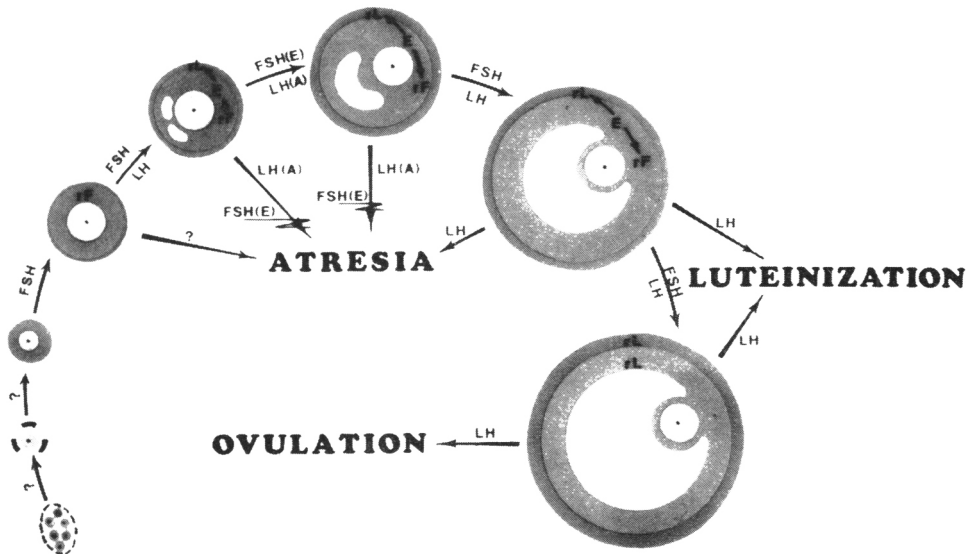
1974). Follicle stimulating hormone binds almost exclusively to granulosa cells whereas LH binds primarily to granulosa, thecal and corpus luteum tissue. The initial binding of gonadotrophins to receptor sites is followed by activation of a membrane-localized adenylate cyclase system, thereby resulting in an increase of intracellular levels of cAMP. In turn, cAMP—the second messenger—mediates

hormonal induction of ovarian RNA and protein synthesis necessary for ovum maturation and steroidogenesis (Jungmann, Hiestand & Schweppe, 1974; Tsafiriri *et al.*, 1973; Younglai, 1975). Regarding possible actions of cAMP on steroidogenesis, there is evidence (Marsh, 1976) for the following effects: (1) increase in amounts of cofactor, such as NADPH, required for many steroidogenic reactions; (2) increase in concentration of free cholesterol by activation of cholesterol esterase and inhibition of cholesterol ester synthetase; (3) increasing availability of this free cholesterol by promoting its transport into the mitochondria where the cholesterol side-chain cleavage enzyme system is located; (4) activation or increased synthesis of the enzyme cleavage system; (5) enhanced transport of pregnenolone out of the mitochondrion.

Steroid hormones produced by the ovary fulfil several functions: (1) they regulate follicular maturation by intraovarian mechanism(s); (2) they are necessary for providing the proper hormonal milieu in the reproductive organs for transport of gametes and sustaining nidation of the fertilized ovum; (3) they modulate FSH and LH secretion via feedback control at the hypothalamic and pituitary level.

Intraovarian regulation of follicular growth (Text-fig. 2)

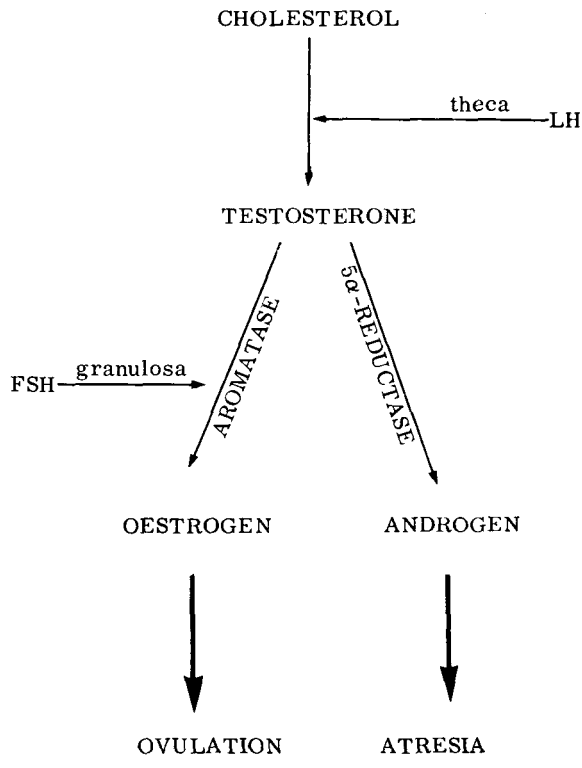
Ovarian production of oestrogens brings about follicular maturation by increasing follicular sensitivity to gonadotrophin stimulation through the following sequential mechanism; alone or together with FSH, oestrogen induces synthesis of ovarian FSH receptors which leads to increased FSH binding and a consequent



Text-fig. 2. Schematic representation of follicular development in relation to hormone action and receptor development. (Reprinted from Eshkol & Lunenfeld, 1976.)

stimulation of ovarian LH receptors (Channing & Tsafri, 1977a; Richards & Midgley, 1976).

Regarding intraovarian mechanism(s) involving progesterone, it has been suggested (Rondell, 1974) that the secretion of this steroid stimulates an 'ovulatory' enzyme (plasmin?—Beers, Strickland & Reich, 1975) which, by proteolytic action, causes a distortion of the collagen framework of the follicular wall, ultimately leading to follicular rupture and ovum expulsion. The LH-induced rise in prostaglandins (Le Maire, Leidner & Marsh, 1975; Bauminger & Lindner, 1975) has also been implicated in follicular rupture, possibly by inducing contraction of muscular elements (actin and myosin?) of the follicular wall (Lindner *et al.*, 1977).



Text-fig. 3. Follicular pathways leading to ovulation or atresia.

While some follicles grow to full maturity and ovulate, many more undergo atresia. Whereas atresia may be promoted by the local effect of ovarian androgens (Speroff & Van de Wiele, 1971; Louvet *et al.*, 1975), oestrogens seem to exert a restraining influence on this process. Moreover, since the 5α -reduced androgens, dihydrotestosterone and 5α -androstane-3,17-dione are effective inhibitors of aromatase (Schwarz, Kruggel & Brodie, 1973) the enzyme necessary for oestrogen production, and since ovaries contain abundant 5α -reductase activity (Armstrong, Kraemer & Hixon, 1975), as well as having been reported to secrete substantial amounts of 5α -reduced androgens (Eckstein & Ravid, 1974), it is

conceivable that one of these compounds might reach levels where it would cause atresia. Thus, the two products of testosterone metabolism—oestrogens and 5α -reduced androgens—may exert opposing actions upon the follicle: oestrogens leading to ovulation and androgens to atresia (Text-fig. 3).

It has been shown (Armstrong & Papkoff, 1976) that LH stimulates ovarian androgen production by theca interna cells whereas FSH enhances granulosa cell conversion of androgens to 17β -oestradiol by inducing aromatase activity. Thus, the fate of each follicle is essentially determined by a delicate interplay between the relative amounts of: (a) circulating FSH and LH available; (b) FSH and LH bound to follicular receptors; (c) oestrogens and androgens formed in response to gonadotrophic stimulation (Text-fig. 3). Hence, a follicle capable of binding FSH and LH in proper amount and sequence will produce enough intrafollicular oestrogens to mature and ovulate. On the other hand, a follicle incapable of binding enough FSH will synthesize mainly androgen and will be doomed to atresia.

In addition to the intraovarian control of follicular maturation exerted by steroids, non-steroidal follicular fluid constituents have been shown to inhibit several ovarian processes: oocyte maturation (Tsafri, Pomerantz & Channing, 1976); luteinization and cAMP accumulation (Ledwitz-Rigby *et al.*, 1977; Kraiem & Lunenfeld, 1976). Moreover, analogous to inhibin found in the male gonad, a non-steroidal factor derived from follicular fluid has been observed to modulate FSH secretion (Becker *et al.*, 1977). Finally, persistent high levels of prolactin may also interfere with LH binding and/or suppress steroid secretion before ovulation thus leading to inappropriate ovarian function (McNatty, McNeilly & Sawers, 1977).

Effects of steroids on uterine function

Ovarian steroids regulate endometrial growth and development. The frequency of endometrial mitosis during the phases of the primate cycle can be directly correlated with the sequential action of oestrogens, and oestrogens with progesterone (Bayard *et al.*, 1975). It seems that oestradiol enters the endometrial cells and binds non-covalently and specifically to a receptor protein present in the cytoplasm. This receptor–oestradiol complex is then translocated into the nucleus where it attaches itself to the chromatin (probably to the non-histone protein fraction). This will then elicit the biological response, i.e. increase in mitotic activity and synthesis of receptors of oestrogen and progesterone (Bayard *et al.*, 1975). As ovarian progesterone is released into the circulation, it also binds to the cytoplasmic receptors, is transported to the nucleus, and causes inhibition of mitotic activity, stimulation of secretory activity and inhibits synthesis of both its own as well as oestrogen receptors (Bayard *et al.*, 1975).

Role of steroids in feedback control of gonadotrophin secretion

The central role of ovarian steroids in feedback modulation of gonadotrophic secretion is well documented (Yen, 1977). Metaphorically speaking, as colourfully depicted by Short (1974): 'the hypothalamus and pituitary appear to dance to a tune played upon them by the ovarian steroids'. The transformation of oestrogens

to catecholestrogens (involving the replacement of hydrogen by a hydroxyl group at C-2 of oestradiol) by the hypothalamus establishes an oestrogen–catecholamine link which may mediate GnRH regulation of gonadotrophic secretion (Kamberi, 1975). Steroids exert a dual feedback action upon gonadotrophic secretion, having both negative-inhibitory and positive-stimulatory effects. The negative ('tonic') and positive ('cyclic') feedback centres have been localized in the rat: the ventromedial and arcuate nuclei in the posterior hypothalamus being responsible for the continuous 'tonic' secretion of gonadotrophins, whereas the hypothalamic preoptic nucleus controls the 'cyclic' discharge of gonadotrophins (Barraclough, 1973). It is this mid-cycle preovulatory surge of gonadotrophins triggered off and maintained by oestrogen, alone or—at least in some species—synergistically with progestagens (Speroff & Van de Wiele, 1971; Swerdloff, Jacobs & Odell, 1972), which leads to the final steps of follicular maturation, and by overcoming the inhibitory influence exerted by follicular fluid (Channing & Tsafriri, 1977b), leads to resumption of oocyte meiosis and ovulation. The shut-off mechanism of the LH surge may operate through a short-loop feedback mechanism: hypothalamic and pituitary cystine arylamidase, which inactivates GnRH, has been shown to be stimulated by LH acting synergistically with sex steroids (Kuhl & Taubert, 1975).

Ovarian steroids also appear to regulate gonadotrophic secretion by modulating, at the pituitary level, gonadotrophic response to the GnRH stimulus. This was studied in patients with primary hypothalamic amenorrhoea and apparently normal pituitary–ovarian axis under different steroidal environments (Lunenfeld *et al.*, 1974). In the presence of low endogenous oestrogen and progesterone levels (corresponding to the early follicular phase of a normal ovulatory cycle), the administration of GnRH resulted in a significant rise of both FSH and LH. In the presence of high oestrogen and low progesterone levels (corresponding to the periovulatory phase of the normal cycle), the administration of GnRH caused a diminished FSH response and an exaggerated LH secretion. On the other hand, the administration of GnRH in the presence of high oestrogen and elevated progesterone levels (compatible with the luteal phase of a normal cycle), did not evoke any appreciable release of either FSH or LH. It would thus seem that steroids, by selectively modifying pituitary responsiveness, can cause the preferential release of either LH or FSH, resulting in varying FSH/LH ratios secreted in response to the same stimulant.

The regulatory mechanisms described above culminate in the cyclic activity characteristic of the female reproductive system.

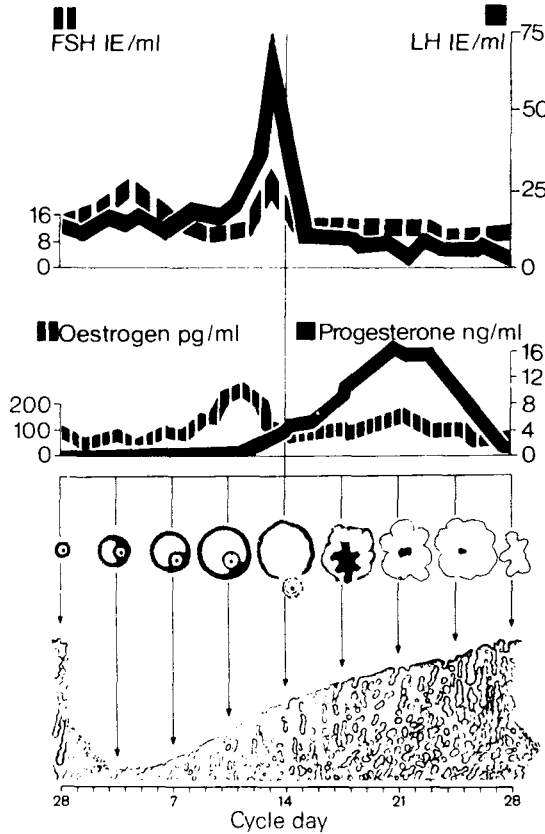
The human menstrual cycle

Marked changes in hormone levels accompany the menstrual cycle. The advent of radioligand techniques has allowed the development of assay methods sufficiently precise and sensitive to determine, simultaneously, multiple hormones in small amounts of plasma. Consequently, it has been possible to delineate in greater detail the shifting patterns of gonadotrophin and steroid levels during the human menstrual cycle and a number of investigations have been carried out seeking correlations between hormone levels during the cycle (Van de Wiele *et al.*, 1970; Ross *et al.*, 1970; Abraham *et al.*, 1972; Guerrero *et al.*, 1976; Aedo *et al.*, 1976).

More recently, in a multi-centre WHO-sponsored project, such correlations have been obtained in cases of certified ovulation. Partial results have been published from one group (Lunenfeld *et al.*, 1977).

The general profile of the cyclic hormonal secretion is shown in Text-fig. 4 and is briefly described here.

FSH. The levels of FSH are higher during the early follicular phase as compared to the late follicular phase. At mid-cycle, an FSH peak appears concomitant with that of LH but of lower magnitude. This is followed by a



Text-fig. 4. Schematic profile of the human menstrual cycle.

significant decrease in FSH concentrations which persists throughout the major part of the luteal phase. A few days before the onset of menstrual bleeding, FSH levels increase and continue to do so until the high levels of the early follicular phase are achieved.

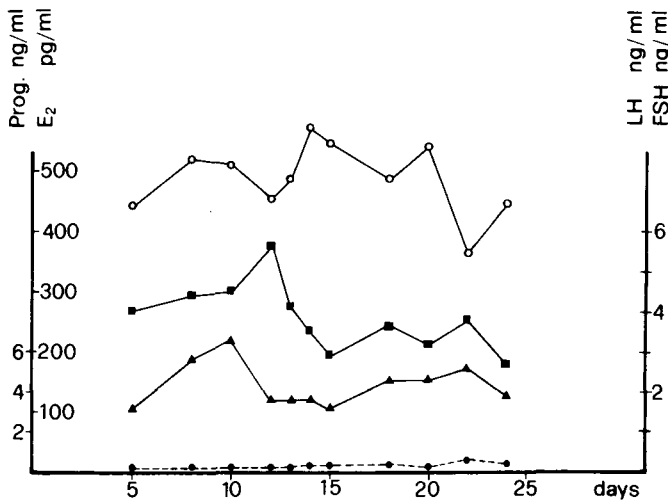
LH. LH levels are low, but detectable throughout both the follicular and luteal phases of the cycle. At mid-cycle, a sharp peak of short duration is observed. Actually, several successive LH peaks are superimposed upon one another, since—as for FSH—the hormone is released in pulses. The LH and FSH peaks precede ovulation by about 16–24 hr.

17 β -oestradiol. The first significant rise of this steroid starts 3–5 days, and reaches a maximum 1–3 days, before follicular rupture. Following ovulation, oestradiol levels decrease sharply then a steady rise of the hormone is observed reaching a maximum at mid-luteal phase. Three to four days before the beginning of menstruation the oestradiol levels begin to fall and remain low throughout menstrual bleeding and the early follicular phase.

Progesterone. The levels of this steroid are very low during the follicular phase of the cycle. A small but significant rise is detected at the start of the LH surge followed by a marked increase during the early luteal phase. Thereafter, the pattern of progesterone secretion roughly parallels that of 17 β -oestradiol.

Menstrual patterns during adolescence

The menstrual ovulatory cycle described above proceeds during adulthood and is not necessarily initiated with the onset of the first menstrual period. Analysing menstrual cycles from the start of menarche until cyclic ovulations occur, reveals a number of characteristic patterns.



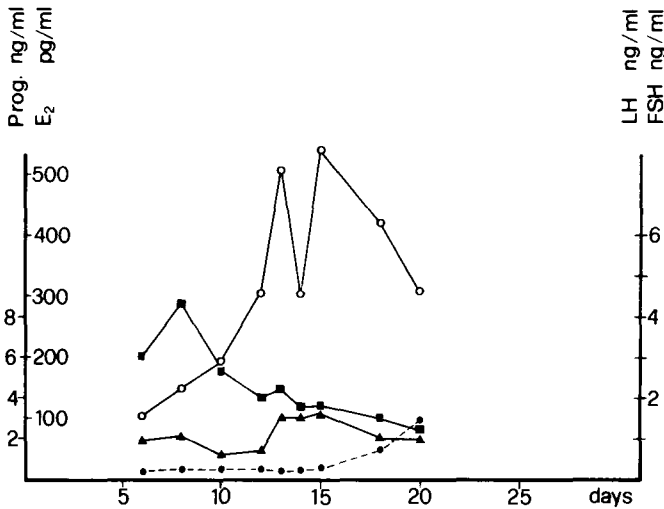
Text-fig. 5. Hormonal profile in a 13-year-old girl, 1 year post-menarche: ●—●, progesterone; ○—○, oestrogens; ▲—▲, LH; ■—■, FSH.

Text-figure 5 shows the hormonal profile in a 13-year-old girl, 1 year after menarche. Follicular development is evident from the high oestrogen levels. However, the high, fluctuating oestrogen concentrations are incapable of effectively suppressing the tonic secretion of FSH. Neither are they capable of inducing positive feedback, and hence no mid-cycle FSH and LH peaks occur. The result is anovulation as reflected by lack of progesterone production.

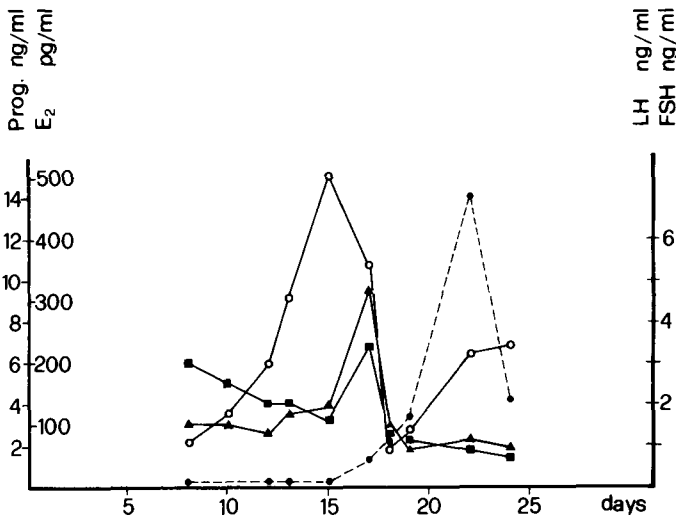
A different pattern is seen in a 15-year-old girl, 2 years post-menarche (Text-fig. 6). The characteristic elevated levels of FSH during the early phase of the cycle have appeared. The rising oestrogen concentrations are now capable of blunting

these FSH levels, i.e. inhibiting the tonic centre. However, when oestradiol reaches peak levels, they cannot elicit a positive feedback effect on the cyclic centre and the consequent absence of the mid-cycle gonadotrophic surge results in anovulation.

The third example is that of a girl aged 16 years, 2 years post-menarche (Text-fig. 7). As in the previous case, the elevated oestradiol levels inhibit the tonic centre and thus decrease FSH concentrations. As the oestrogens rise to a peak, they provide the positive signal for the mid-cycle FSH and LH surge. However, the

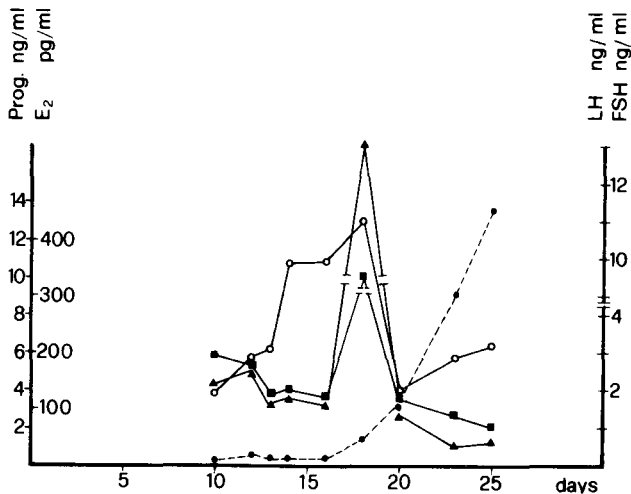


Text-fig. 6. Hormonal profile in a 15-year-old girl, 2 years post-menarche (symbols as in Text-fig. 5).



Text-fig. 7. Hormonal profile in a 16-year-old girl, 2 years post-menarche (symbols as in Text-fig. 5).

gonadotrophin peaks are of insufficient magnitude and hence they only promote follicular luteinization with no ovulation, as reflected by increased progesterone of only short duration.



Text-fig. 8. Hormonal profile of an 'ovulatory' cycle in a 15½-year-old girl, 1 year post-menarche (symbols as in Text-fig. 5).

The final example (Text-fig. 8) shows that this maturation process does not always take 2 years. A 15½-year-old girl, only 1 year post-menarche, displays a hormonal profile which has all the features of a normal ovulatory menstrual cycle, i.e. both the tonic and cyclic centres respond normally to the steroid environment, resulting initially in the FSH decrease followed by the mid-cycle gonadotrophin peaks which probably resulted in ovulation as indicated by increased progesterone levels.

A multi-centre project sponsored by WHO and the IPPF is now in progress in order to delineate menstrual patterns during adolescence in more detail. This project has the advantage that an attempt is made to follow-up—and hence be able to unmask—the changing profile in each of the girls under investigation.

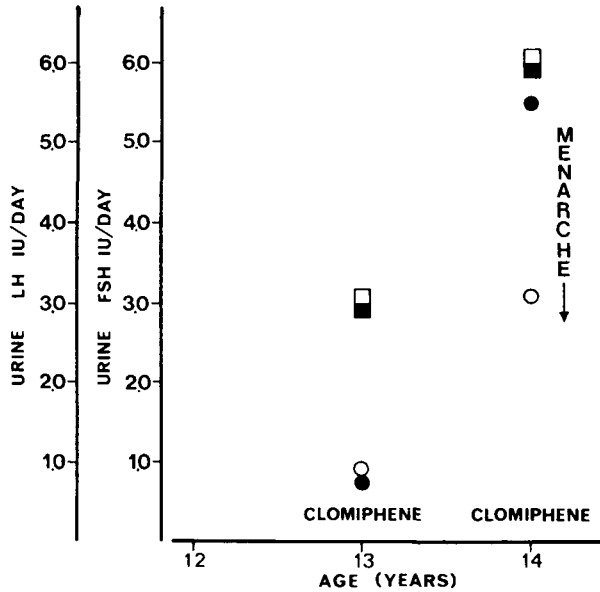
Maturation of the feedback mechanism

The menstrual patterns just described reveal a progressive maturation of the feedback regulatory centres in the hypothalamus. Initially, both tonic and cyclic centres remain unresponsive to physiological concentrations of oestradiol secreted by the ovary. At a later stage, the tonic centre alone responds adequately to oestrogens, and finally—as both centres become responsive—cyclic ovulations occur.

Studies by Kulin, Grumbach & Kaplan (1972) in children given clomiphene lend support to the gradual maturation of the hypothalamic feedback centres. The anti-oestrogen clomiphene, which in adults elicits an increase in FSH and LH secretion, had no effect on either FSH or LH before menarche (Text-fig. 9). One

year later, in the same girl, this drug provoked an increase in LH without altering the prepubertal elevated FSH levels; 17 days later, the girl had her first menstrual bleeding. These observations probably indicate that the LH tonic centre was capable of recognizing the clomiphene signal resulting in the LH increase which, in turn, promoted follicular maturation with an increase in ovarian androgens that served as substrate for the FSH-induced transformation into oestrogens. Steroid withdrawal following follicular regression then caused menstrual bleeding.

It would appear that maturation of the hypothalamic feedback centres can



Text-fig. 9. FSH and LH levels before and after clomiphene treatment given at different stages of sexual maturation. LH: ○, before and ●, after treatment; FSH: □, before and ■, after treatment. (Redrawn from Kulin *et al.* (1972).)

occur in the absence of ovaries and their secretions. This has become evident from studies in ovariectomized rats as well as the observed changes in gonadotrophin profile observed at puberty in patients with gonadal dysgenesis (Steele, 1977).

The central mechanism(s) which regulate the control of puberty remain obscure. A working hypothesis for the aminergic control of puberty has been proposed by Ruf (1973). The observations of Hyppä (1974) on the maturation of brain and hypothalamic neuroamines give support to this hypothesis. This investigator has shown that neuroamines are incapable of assuming control over neuroendocrine regulation before puberty. It is likely that this maturation process has its roots in the anatomical growth of neuroaminergic steroid-sensitive synapses in the hypothalamus. This growth can be inhibited by treatment with neurochemical drugs which lead to an increased activity of melatonin-synthesizing enzyme in the pineal gland. Consequently, melatonin is produced and an inhibition of hypothalamic GnRH follows. The hypothalamic noradrenergic pathway may be of key value in the process, becoming functionally competent during puberty, when

it starts to inhibit pineal metabolism through the multi-synaptic pathway to the gland. The hypothalamus then becomes able to secrete enough GnRH necessary for stimulating pituitary gonadotrophin secretion.

It would seem therefore that the critical factor in delaying cyclic ovulatory activity (i.e. sexual puberty) is the immaturity of the hypothalamic feedback centres. Investigations on the onset of puberty have shown that each of the separate components of the reproductive apparatus—pituitary, gonads and peripheral organs—is capable of responding to hormonal challenge. Job (1973) demonstrated that the pituitary can respond to GnRH as early as the age of 5 months. Peters (unpublished data) observed that ovaries of very young girls (who died of sudden accidents) showed active follicular growth and development. Furthermore, uterine bleeding in young girls with oestrogen-secreting granulosa cell tumours demonstrates that the uterus can respond to oestrogens even in childhood.

Although gonadotrophins can stimulate follicular growth in early infancy, they cannot induce ovulation until later on. This implies necessary changes in the ovarian machinery before it can be induced to ovulate. Indeed, Eckstein & Lerner (1978) have shown that the earliest event connected with the first ovulation in rodents is a sharp decrease in ovarian 5α -reductase activity. This change in steroid metabolism, and its consequent reduction in 5α -reduced androgens, would shift the balance, as described earlier (Text-fig. 3), away from atresia and towards ovulation.

The fragility of the female reproductive cycle during the adolescent years is evident from the many stress situations, such as college amenorrhoea and anorexia nervosa, which can perturb the system. Little is known about the effect of exogenous steroids during this critical period. Until more information becomes available, it is probably safest to follow recent WHO recommendations (1977) that for young females at risk of unwanted pregnancy during the early adolescent period, non-steroidal contraception should be preferred until studies have established the degree of risk they incur in using hormones.

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Discussion

Engstrom: I should like to address two questions to Dr Lunenfeld. First, is the occurrence of regular periods good evidence of the occurrence of ovulation? Second, what do we know about the interval between menarche and the start of regular ovulation in a girl with an early menarche as compared to that in a girl with a late menarche?

Lunenfeld: Menarche is certainly no proof that ovulatory cycles have commenced. I believe that the WHO/IPPF study on the onset of ovulation, which has recently started, hopefully should provide answers to this question. However, 2 or 3 years will pass before any answer will be available regarding the relationship between the time of menarche and ovulation.

Short: If I may anticipate, Professor Brown makes the point that there appear to be two different mechanisms that induce menstruation in the period before ovulation has started. There are those girls who are bleeding because they are having an oestrogen withdrawal bleed, presumably from follicular atresia. But there are other girls who are bleeding rather in the way that a woman with dysfunctional uterine bleeding might menstruate in spite of continuous, fairly elevated levels of oestrogens. The only comment he makes about it is interesting, that if the spinnbarkeit occurs immediately prior to menstruation, that is indicative of an oestrogen withdrawal bleed, whereas if the spinnbarkeit occurs about 14 days before the menstruation, perhaps this is a good indication that the girl has actually started to ovulate.

Edwards: Can Dr Lunenfeld tell us whether there is any information on changes in FSH and LH levels during the juvenile period before there is any evidence of ovulation?

Lunenfeld: I wish that I could answer that question in the way you and I would like to have it answered, namely, that there is more LH than FSH at that time, but we don't really know.

Short: The point about FSH and LH ratios is rather interesting. Dr Gerald Lincoln, in my Unit, has recently been experimenting in rams, giving them different pulse frequencies of LH–RH when they are in a state of sexual quiescence to see whether he can stimulate testicular development and spermatogenesis. If seven injections a day of 200 ng LH–RH are given at regular intervals, the FSH levels, instead of pulsing each time the LH rises, will gradually rise. If this treatment is continued for 10 days, at the end of that time the LH is still pulsing each time an LH–RH injection is given, but the FSH has now become constantly elevated.

So, puberty, in my view, is due to a change in the episodic pulse frequency of LH–RH, occurring initially during the early sleep period, which causes the dissociation between the FSH and LH levels, as we have seen. The dissociation is not due to two different releasing hormones, but to a differential response to different episodic firing rates of the one releasing hormone LH–RH.

Lunenfeld: These prepubertal levels of oestrogens provoke an increase in prolactin, which together with the pineal gland's melatonin might interfere with ovulation during the pre-menarche period. The pineal gland is another of the factors which we have to consider.

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