# PROFILE OF SERUM LUTEINIZING HORMONE, FOLLICLE STIMULATING HORMONE, ESTRADIOL-17B AND PROGESTERONE IN NORMAL HUMAN MENSTRUAL CYCLE*

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Although several facets of the reproductive process vary widely among mammals, the interrelationship between hypothalamus, pituitary, ovaries and the reproductive tract that govern the repetitive events of the reproductive cycle is fairly similar. Availability of sensitive, specific and reproducible radioimmunoassay techniques for quantitation of glycoproteins and steroid hormones (Odell *et al* 1967; Midgley *et al* 1969; Abraham and Odell 1970; Niswender and Midgley 1970) have greatly facilitated assessment of the temporal relationship of these hormones in human menstrual cycle (Corker *et al* 1969; Johansson and Wide, 1969; Mishell *et al*, 1971; Abraham *et al* 1972; England *et al* 1974). Except for a recent study in Thai woman (Saxena *et al* 1974) not enough complete data on hormonal profile of human menstrual cycle is available in Asian population.

The present study describes the serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) estradiol-17B (E) and progesterone (p) in menstrual cycle of healthy North Indian women.

*Material and Methods*

Purified human LH (LER-960) for both standards and iodination, human FSH (LER-1366) for iodination and anti-human FSH rabbit serum (NPA-batch 3) were provided by the National Pituitary Agency, NIH, USA. The anti-human LH rabbit serum (MRC-70/355) and human FSH standard (MRC-68/39) were gifts from Division of Biological Standards, National Institute of Medical Research, London.

Crystalline progesterone and estradiol-17B were purchased from Sigma Chemicals, St. Louis, U.S.A. and were used as standards without further purification. 2,4,6,7 (n)-$^{3}$H-Estradiol in benzene/ethanol (95:5, v/v) with a stated specific activity of 313 $\mu$Ci/$\mu$g and 1,2,6,7 (n)-$^{3}$H-progesterone in benzene solution with a

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stated specific activity of 267 $\mu$Ci/$\mu$g were purchased from Radiochemical Centre, Amersham, England. Tyrosine methyl ester (TME) conjugates of estradiol-17B and progesterone were kindly provided by Prof. G. D. Niswender, Colorado State University, Fort Collins, USA; and anti-estradiol-11-BSA (Bovine serum albumin) and anti-progesterone-6-BSA rabbit serum were gifts from Prof. A. R. Midgely, Jr. of Reproductive Endocrinology Program, University of Michigan, Ann Arbor, U.S.A.

All solvents were of reagent grade, purchased from British Drug House, Laboratory Chemicals Division, Bombay and were used after redistillation. All glasswares were washed with methanol and oven cleaned for 12-16 hours, prior to use in radioimmunoassays.

The second antibody (2nd AB) anti-rabbit gamma globulin (anti RGG) was raised in goats in our laboratory.

Blood samples were collected between 8-10 a.m. on every third day from onset of menstruation to day 10, daily from day 11 to day 16 and again on every third day from day 18 till the next menstruation in 10 healthy volunteers. None of the subjects gave history of menstrual irregularity or any gynecological complaints and they had not been on contraceptive steroids for at least 6 months preceding the cycle under study. The sera were separated and kept frozen at $-20°C$ till assayed.

Radioimmunoassay of serum LH and FSH: The details of the procedure using second antibody precipitation technique have been described from this laboratory (Rastogi *et al*, 1973). For both LH and FSH determination 0.1 ml serum was used in duplicate and the mean value was taken to represent the serum concentration for that particular day. To avoid inter-assay variation, all samples from an individual were processed in the same batch. For the sake of uniformity in dat presentation and to avoid variations in cycle length, the day of highest serum level of LH during the cycle was taken to represent the mid cycle and was designated as day '0'. The days prior to LH peak in the follicular phase were prefixed with minus (—) and those following LH peak in the luteal phase were prefixed with plus (+).

Radioimmunoassay of estradiol 17B and progesterone: Radioiodination of TME-estradiol 17B, and TME-progesterone were carried out as described previously using chloramine-T as oxidising agent (Niswender, 1973; Bajpai *et al*, 1974). The iodination mixture was chromatographed on Sephadex G-25 (fine) column for the separation of radioiodinated hormone from free iodine and damaged hormone. For the radioimmunoassay, progesterone was extracted in petroleum ether and estradiol 17B, in benzene from aliquote of 500 ul of serum. The solvent extracts were dried under nitrogen, reconstituted in 3 ml of 0.01 M phosphate 0.15 M saline buffer containing 0.1% gelatin (0.1% gel PBS). The radioimmunoassay of estradiol-17B was carried out according to the method of England *et al* (1974) and that of progesterone according to Niswender (1973) with minor modifications. In brief, 500 $\mu$l of reconstituted sample or standard in buffer was incubated overnight (18 hours) with 100 ul of the appropriate dilution of the first antibody and 100 ul of tracer (approx. 10,000 CPM) at 4°C. Later, 100 $\mu$l of 1:4 anti RGG in 0.1 M EDTA were added and the incubation was allowed to proceed for 24 hours. The radioactivity precipitated in tubes with no cold hormone (buffer controls) minus that with

no first antibody (NRS) was assigned 100%. As increasing amounts of cold hormone (progesterone, 10 pg to 10 ng; estradiol 17B. 1 pg to 1 ng) were added to tubes, the radioactivity precipitated (bound) decreased progressively. Each sample's result was corrected for procedural losses according to the per cent of $^3H$-P or $^3H$-$E_2$ recovered during extraction and the amount expressed as ng/ml. or pg/ml.

*Results*

Fig. 1 illustrates the composite standard curves for LH, FSH estradiol-17B and

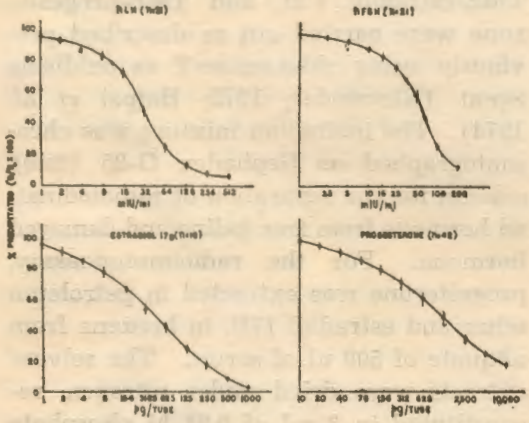

Fig. 1

Composite standard dose response curve for serum luteinizing hormone (LH), follicle stimulating hormone (FSH) estradiol 17B and progesterone.

progesterone. The minimum detectable amount of LH that could be differntiated from buffer control tubes (assay sensitivity) was 0.5 mIU/ml, 30 pg/ml and 0.3 ng/ml respectively. The inter- and intra-assay variations for all hormones were less than 15% and 10% respectively. Earlier reports have documented specificity of estradiol-17B and progesterone radioimmunossays and both these steriod assays have been used without chromatography of extracts with excellent correlations (England *et al* 1974; Niswender 1973).

The cycle length in our subjects varied between 26-30 days and all of them had normal luteal phase (13-15 days).

*Luteinizing hormone*

A single sharp major peak in serum LH was observed in each subject between 12 and 15 days (mean 14.3). It ranged between 14.3-98.3 mU/ml (mean 54.0 ± 9.1 SEM). Based on these data a composite curve was drawn showing serum LH (mean ± 1 SE) according to the days before and after the LH peak (Fig. 2). The

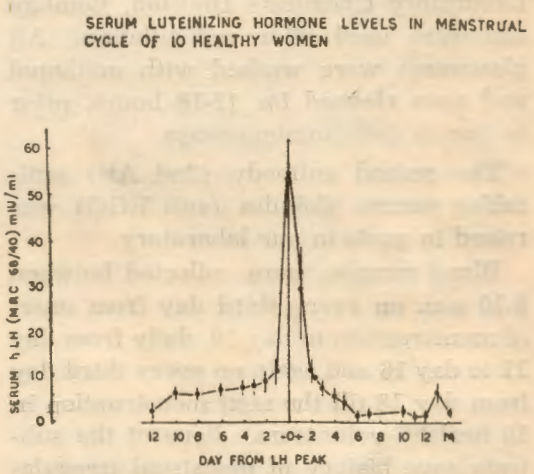


Fig 2

Composite data on serum LH mean ± SE) during normal menstrual cycle. Day '0' refers to mid cycle LH surge. Days before and after mid cycle surge are prefixed with (—) and (+) respectively.

midcycle peak of LH was more pronounced than that of FSH. The mean serum LH in the follicular phase was statistically not different ($p > 0.05$) from that in the luteal phase.

*Follicle Stimulating hormone*

A less pronounced but significant peak in serum FSH could be demonstrated in

each subject, it was coincidental to that of LH (Fig. 3). The levels in follicular

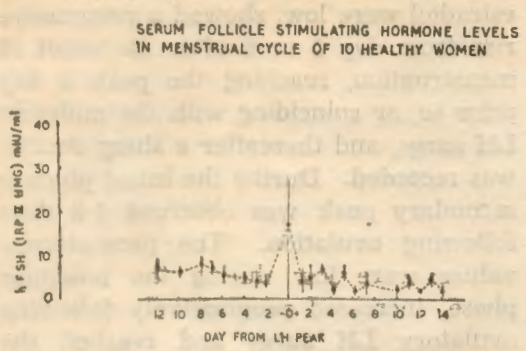


Fig 3

Composite data on serum FSH (mean ± SE) displayed around mid cycle LH surge.

and luteal phases were not statistically different ($p > 0.05$). The LH/FSH ratio during midcycle ranged between 1.8 to 7.8 ($3.4 \pm 1.5$).

*Estradiol-17B*

Serum levels of estradiol-17B were less than 100 pg/ml on 1st day of menses. Gradual elevation was recorded from fifth day of the cycle. In all but 3 subjects the peak levels coincided with LH peak and then decreased progressively till a significant secondary rise was observed 10 days after the LH peak (Fig 4).

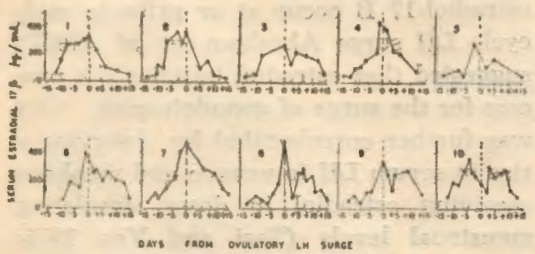


Fig 4

Serum estradiol-17B during normal menstrual cycle displayed in relation to the day of LH surge. Note peak levels correspond to the day of LH surge in 6 out 10 subjects.

*Progesterone*

Serum progesterone values throughout the follicular phase did not exceed 5 ng/ml. Rise was recorded 1-3 days after the midcycle LH surge, reaching peak values between 4-10 days, and then showing a sudden or gradual decline and remained below 5 ng/ml until the next menses (Fig. 5).

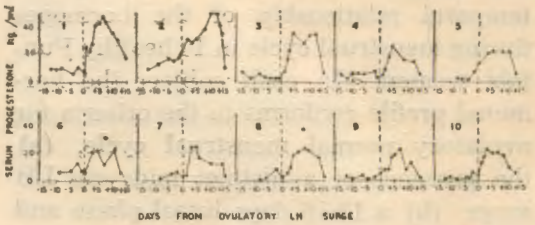


Fig. 5

Serum progesterone during normal menstrual cycle. Luteal phase progresterone was maximum during day 4-10 after the mid cycle LH surge (day 0).

The temporal relationship between gonadotropins, estradiol-17B and progesterone in relation to the LH peak is shown in Fig. 6. The peak elevation in FSH

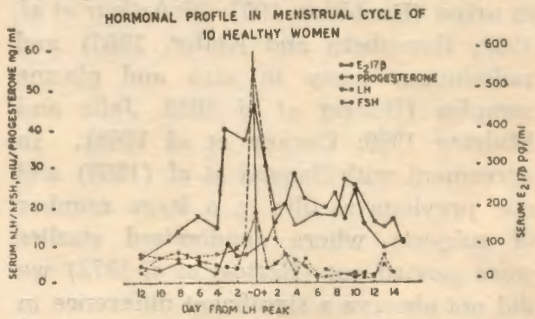


Fig 6

Profile of serum luteinizing hormone, follicle stimulating hormone, estradiol 17B and progesterone during normal menstrual cycle. Each point represents the mean of the hormone level in ten subjects in one full cycle. Note that peak levels of FSH and $E_2$ coincide with that of LH. A secondary peak of $E_2$ is seen in luteal phase.

and estradiol-17 B occurred coincidentally with the LH peak in the midcycle.

Significantly elevated levels in progesterone were observed in the luteal phase with peaks between 4-10 days. A smaller but significant secondary rise in estradiol-17 B was seen in the mid-luteal phase.

*Discussion*

Employing specific radioimmunoassays for the quantitation of LH, FSH, $E_2$17 B and progesterone we have defined the temporal relationship of the hormones during menstrual cycle in 10 healthy Punjabi women. In each subject the hormonal profile conforms to the criteria for ovulatory normal menstrual cycle, (a) the presence of a distinct midcycle LH surge, (b) a 12-16 days luteal phase and (c) plasma progesterone more than 5 ng/ml between days 5-8 after the ovulatory LH surge (Abraham *et al* 1972).

The patterns of serum LH and FSH seen in our subjects appear to be similar to those reported in literature. Peak levels of both serum LH and FSH in the midcycle are a consistent finding and confirm similar observations by bioassays in urine (Buchhloz, 1957; McArthur *et al*, 1958; Rosenberg and Keller, 1965) and radioimmunoassay in sera and plasma samples (Burger *et al* 1968; Jaffe and Midgley 1969; Corker *et al* 1969). In agreement with Saxena *et al* (1969) and our previous results in a large number of subjects where randomised studies were carried out (Rastogi *et al* 1973) we did not observe a significant difference in LH and FSH levels during follicular and luteal phases. The mean peak LH and FSH levels during midcycle compare favourably with reports from other centres where identical standards and reagents have been used.

The profile of $E_2$-17 B and progesterone in menstrual cycle in our subjects is similar to those published in the literature (Mishell *et al* 1971; Abraham *et al* 1972; Saxena *et al* 1974; England *et al* 1974). In early follicular phase the levels of estradiol were low, showed a progressive rise from day 5 or 6 after the onset of menstruation, reaching the peak a day prior to, or coinciding with the midcycle LH surge, and thereafter a sharp decline was recorded. During the luteal phase a secondary peak was observed 4-8 days following ovulation. The progesterone values were low during the follicular phase, increased progressively following ovulatory LH surge and reached the maximum between 3-7 days, after which they declined. While the serum estradiol levels compared favourably, the progesterone concentration was lower than those reported from Ann Arbor, (England *et al*, 1974). The reagents and assay conditions in the two studies were identical.

The interrelationship between pituitary gonadotropins and ovarian steroids in menstrual cycle has been well documented in different ethnic groups and they appear similar. While changes in serum levels of estradiol-17 B and progesterone are governed by follicular-luteal cell function, the mechanism(s) involved in the surge of gonadotropin secretion in midcycle still remains an enigma. Based on the observations that peak levels of estradiol-17 B occur at or prior to midcycle LH surge Abraham *et al* (1962) suggested that estradiol initiates the process for the surge of gonadotropins. This was further corroborated by observing a rise in serum LH in women and monkeys receiving estradiol in doses simulating menstrual levels (Tsai and Yen 1971; Monroe *et al* 1972; Karsch *et al* 1973). However, whether the midcycle surge of LH secretion in humans is brought about by an effect of estrogens on the release of gonadotropin releasing hormone (GnRH)

or by a direct sensitizing effect of estrogen on pituitary responsiveness to GnRH or by a combination of both, yet remains to be clarified. Based on the observation that the LH response to GnRH increased progressively as the time of ovulation approached, Yen *et al* (1972) have suggested an increase in pituitary sensitivity to GnRH dependent on increasing levels of circulating estradiol. Increase in pituitary sensitivity to GnRH has been shown in estradiol pretreated rats (Arimura and Schally 1971; Debeljuk *et al* 1972; Kulkarni *et al* 1974) and ewes (Reeves *et al* 1971; Arimura *et al* 1971). On the other hand, an actual increase in levels of circulating GnRH has also been documented concomitant with midcycle LH surge by a specific bioassy technique (Malacara *et al* 1972).

With these circumstantial evidences, it is speculated that rising concentrations of circulating estradiol-17 B perhaps stimulate release of GnRH from the hypothalamus as well as increase the senstivity of the pituitary gonadotrophs to GnRH, thus bringing about the midcycle surge of gonadotropins. However, a recent observation by Keye and Jaffe (1974) failed to document an enhanced response to GnRH in women in whom serum levels of estradiol comparable to midcycle period were achieved by exogenous administration. Therefore, it remains to be shown, whether 17-Hydroxyprogesterone whose serum concentration also rises prior to midcycle gonadotropin surge (Strott *et al* 1969; Abraham *et al* 1972) or any other related steroid, has a role in the initiation of midcycle surge of gonadotropins.

The importance of FSH surge in midcycle has largely remained unknown. I' is perhaps due to common releasing effects of GnRH on LH and FSH. Circulating FSH largely influences the growth and development of follicular cells in the maturing Graafian follicle. It has been suggested that when there is relative deficiency of FSH during the follicular phase and midcycle, as revealed by FSH/LH ratio, there is diminished follicular development as well as an inadequate luteal cell function (Strott *et al* 1970).

*Summary*

Serum levels of luteinizing hormone, follicle stimulating hormone, estradiol-17 B and progesterone were measured in sera samples from 10 healthy normally menstruating women by specific radioimmunoassays. The patterns were similar to those described in literature. The present literature on mechanism (s) concerning the midcycle surge of gonadotropins has been discussed.

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