

Progesterone Modulation of Pulsatile Luteinizing Hormone Secretion in Normal Women*

MICHAEL R. SOULES, ROBERT A. STEINER, DONALD K. CLIFTON,
NANCY L. COHEN, SEZER AKSEL, AND WILLIAM J. BREMNER

Division of Reproductive Endocrinology, Departments of Obstetrics-Gynecology (M.R.S., R.A.S., D.K.C., N.L.C.), Physiology, Biophysics and Zoology (R.A.S.), and Medicine (W.J.B.), University of Washington, and Veterans Administration Medical Center, Seattle, Washington 98195; and the Department of Obstetrics-Gynecology, University of South Alabama (S.A.), Mobile, Alabama 36688

ABSTRACT. Recent studies show that the frequency and amplitude of pulsatile LH secretion change during the normal human menstrual cycle; however, the neuroendocrine mechanisms underlying these changes are poorly understood. To assess the role of progesterone (P) in regulating LH secretion patterns, we treated normal women ($n = 5$) with im P in oil during the follicular phase of their cycle and compared LH pulse frequency, amplitude, and mean plasma level during treatment to those in normal cycling women. Normal women were studied five times in five menstrual cycles. Each study lasted 24 h, with a sampling interval of 20 min. The cycle phases studied were early follicular

(twice), late follicular (LF), midluteal, and LF with P therapy to simulate luteal phase plasma P levels. LH pulse frequency was slower ($P \leq 0.001$) in the midluteal phase than in either the early follicular phase or LF, and furthermore, P, administered in the normal follicular phase, slowed LH pulse frequency, augmented pulse amplitude, and reduced mean plasma LH levels compared to those in untreated women studied at the same cycle phase ($P \leq 0.02$). We infer that P secretion by the ovary mediates the change in the LH secretory pattern during the luteal phase of the normal menstrual cycle, and that at least part of this effect is mediated by the central nervous system. (*J Clin Endocrinol Metab* 58: 378, 1984)

EVIDENCE from human and other animal studies indicates that LH and FSH are secreted by the pituitary in an intermittent (pulsatile) fashion (1-7). Studies performed in normal women demonstrate that LH pulse frequency and amplitude vary during the course of the menstrual cycle (5-7). Although this phenomenon is widely acknowledged to occur, the supporting evidence is incomplete. The few studies addressing this issue reported only small numbers of subjects (often a single individual in a given cycle stage), used short sampling intervals (usually 4-8 h), and used various analytical methods to assess episodic hormonal profiles (5-9). Moreover, the mechanisms underlying the changes in the gonadotropin secretory patterns during the menstrual cycle have not been elucidated. In this study, we first characterized the gonadotropin secretory pattern in various phases of the menstrual cycle in normal women. Second, to test the hypothesis that progesterone (P) causes the apparent slowing of LH pulse frequency in

the normal luteal phase, we studied the effects of P administration on LH and FSH secretion profiles in these women during the follicular phase of their cycle.

Materials and Methods

The subjects were five normal women (aged 24-34 yr), of normal height and weight. All had a history of normal menstrual cycles and a biphasic basal body temperature chart before entering the study. All subjects had normal plasma levels of LH, FSH, estradiol (E), and P when daily venous samples were obtained in the first study cycle.

The pulsatile patterns of LH and FSH were determined during five admissions (24 h each) to the Clinical Research Center (University of Washington) during five menstrual cycles in each volunteer. The cycle phases studied were: early follicular (EF; days 1-4; studied twice, admissions in first and fifth cycles), late follicular (LF; days 9-12; third cycle), midluteal (ML; days 21-24; admission in first cycle), and LF with P therapy (admission in fifth cycle). The second and fourth menstrual cycles were rest cycles (basal body temperature chart only). The cycle phases were confirmed by analyzing plasma E and P levels at the beginning and end of each admission and in relation to the LH surge.

During each study each volunteer was at bedrest, with caffeine and smoking prohibited. Blood samples were obtained through an indwelling iv line every 20 min. In the fifth study cycle, each woman had her second EF admission followed by

Received April 19, 1983.

Address requests for reprints to: Michael R. Soules, M.D., Department of Obstetrics-Gynecology, University of Washington, Seattle, Washington 98195.

* This work was supported by: Parent NIH Grant BSRG 2-507-RR05432, NIH Grants P50 HD-12629-04 and HD-12625-04, CRC Grant RR-37, and the V.A.

an 8-day regimen of P treatment, with her final admission on the last treatment day. P in oil (P injection USP, Eli Lilly Co., Indianapolis, IN) was administered every 12 h (im) in increasing doses (Table 1). P levels were determined in blood samples obtained immediately before each injection.

Blood samples were analyzed for LH and FSH by double antibody RIAs (10, 11). Standard NIH reagents were used, and results are expressed as nanograms per ml LER-907 reference preparation. The sensitivity of the FSH assay was 25 ng/ml; intra- and interassay coefficients of variation were 7.3% and 9.7%, respectively.

Serum samples for plasma E and P determinations were assayed in duplicate by RIA (12). The sensitivity of the E assay was 23 pg/ml; intra- and interassay coefficients of variation were 6% and 7.8%, respectively. The sensitivity of the P assay was 140 pg/ml; intra- and interassay coefficients of variation were 6% and 8%, respectively.

Bioactive plasma LH levels were measured using a modification (2) of procedures described by Van Damme *et al.* (13) and Dufau *et al.* (14). This assay is based on the measurement of testosterone production by dispersed immature mouse Leydig cells. Serial dilutions of serum samples containing high LH levels yielded results parallel to the standard, LER-907 (biological potency, 1 mg = 6000 IU LH). The minimally detectable amount of LH was 0.54 ng/tube (0.027 μ g/ml at 20- μ l sample volume). This represents the LH value read from the cell blank plus 5% of the maximum testosterone produced with excess standard. Samples were assayed in triplicate in volumes ranging from 5–20 μ l. The mean intra- and interassay coefficients of variation were 14.4% and 24.3%, respectively.

Pulsatile LH patterns were analyzed using a modification of the Santen and Bardin method (8). For each sample set, measurement error was assessed based on replicate variability. A pulse was defined as an increase from nadir to peak that was 2 SD greater than the assay variability. Based on computer simulations, we found that this procedure worked well when there were at least 10 real pulses (in data sets consisting of 73 samples). When less than 10 real pulses were present, false positives were a problem. Therefore, when the initial analysis indicated less than 10 pulses, we set a more stringent criterion, requiring an increase of greater than 5 SD in the assay variability. Overlapping 10- and 20-min sampling intervals were employed for 16 h in the EF phase (4-h segments in 4 volunteers) and for 16 h in the LF phase (8-h segments in 2 volun-

TABLE 1. P treatment (n = 5)

Day	Dose (mg P in oil)		Mean serum level (ng/ml)
	am ^a	pm	
1	2.5	1.25	0.9
2	2.5	1.25	0.7
3	5.0	2.5	1.5
4	15.0	7.5	4.1
5	15.0	7.5	6.3
6	20.0	10.0	9.4
7	20.0	10.0	9.9
8	20.0	10.0	7.9

^a Doses were administered 12 h apart; the exact hours varied among individuals.

teers). When these data were subjected to pulse analysis, there were no differences in LH pulse frequency between the 10- and 20-min data.

Statistical comparisons among groups were assessed by analysis of variance and paired *t* tests. A correlation coefficient was calculated between LH and FSH at each sampling point and over each sampling interval. A similar correlation was calculated between immunoreactive LH and bioactive LH over selected sampling intervals.

Results

Details of the menstrual cycle phases studied in the five women are presented in Table 2. A comparison between the first and second EF studies (three menstrual cycles apart) in each individual revealed no significant differences in the LH secretory parameters (data not shown). The data from the two EF admissions in each volunteer were averaged. The LH secretory pattern in the EF phase was characterized by pulses that occurred

TABLE 2. Characterization of menstrual cycle phases

Cycle phase	Day to LH surge	E (pg/ml)	P (ng/ml)	Large LH pulses, change \geq 20 ng/ml over 24 h (% large pulses) ^a	LH/FSH correlation (r)
EF	-11.6 \pm 1.1	57.5 \pm 6.7	0.3 \pm 0.03	3.3 (23)	0.50 \pm 0.04
LF	-5.4 \pm 1.0	121.1 \pm 17.8	0.3 \pm 0.03	3.4 (20)	0.35 \pm 0.14
ML	+8.2 \pm 0.4	156.6 \pm 29.0	7.5 \pm 1.7	2.4 (55)	0.51 \pm 0.05
LF + P		78.4 \pm 29.7	7.9 \pm 1.0	4.6 (68)	0.59 \pm 0.05

Values are the mean \pm SEM (n = 5).

^a Change was determined by subtracting the lowest LH level between pulses from the LH level at the peak of the next pulse.

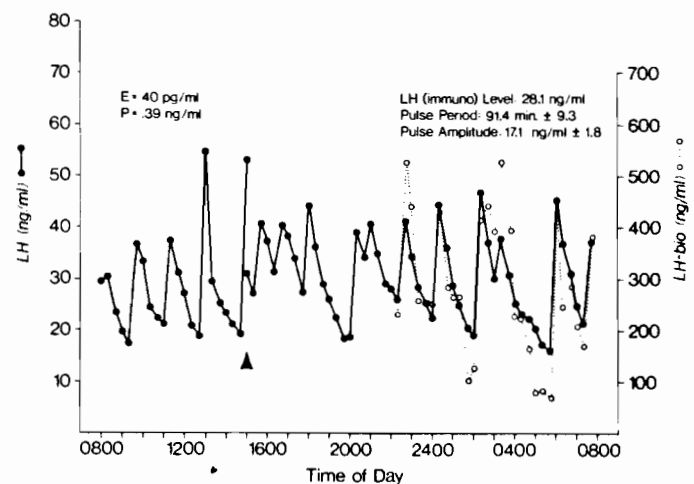


FIG. 1. LH pulse pattern; EF phase. The 24-h LH pulse pattern from a normal woman is shown. The time line has been normalized to begin at 0800 h; the actual clock time at which the sampling began and ended is indicated by the arrow. LH levels, as measured by bioassay, are indicated by the dotted line ($r = 0.87$). E, estradiol.

every 100 min [14.4 ± 1.0 (\pm SE) pulses over 24 h], which were relatively low in amplitude (15.0 ± 2.4 ng/ml), with a 24-h mean of 32.8 ± 8.2 ng/ml. LH bioactivity was measured in two women (A and B) during the EF phase and demonstrated correlations with LH immunoreactivity of 0.87 and 0.83, respectively [$P \leq 0.01$; see Fig. 1 for an example of the LH (immuno and bio) pattern in one individual].

Compared to the EF phase the LF phase was characterized by more frequent LH pulses (17.0 ± 0.9 pulses over 24 h; interpulse interval, 83 min; $P \leq 0.05$); the LH pulse amplitude tended to be of low amplitude (12.7 ± 1.7 ng/ml), with a similar mean LH level (36.6 ± 6.8 ng/ml). Figure 2 is an example of a LF secretory pattern of LH.

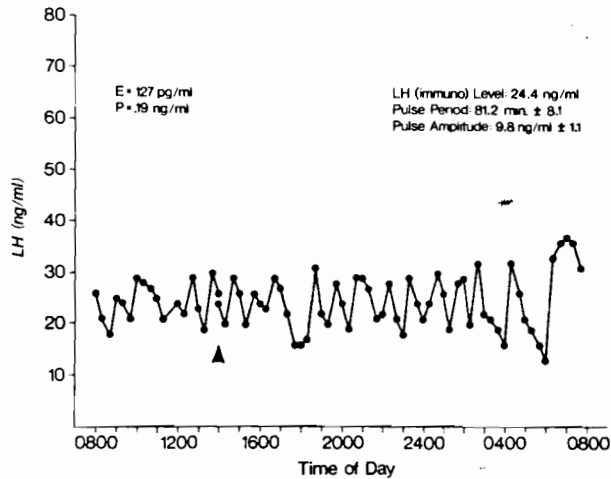


FIG. 2. LH pulse pattern; LF phase. The 24-h LH pulse pattern from a normal woman is shown. The time line has been normalized to begin at 0800 h; the actual clock time at which the sampling began and ended is indicated by the arrow. E, estradiol.

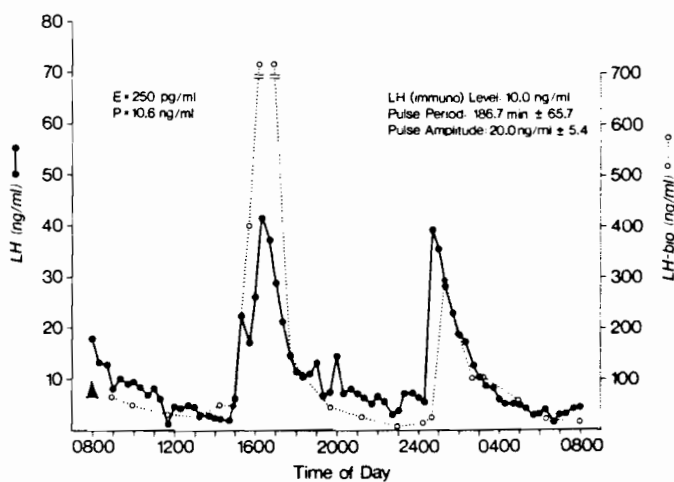


FIG. 3. LH pulse pattern; ML phase. The 24-h LH pulse pattern from a normal woman is shown. The time line has been normalized to begin at 0800 h; the actual clock time at which the sampling began and ended is indicated by the arrow. LH levels, as measured by bioassay, are indicated by the dotted line ($r = 0.88$). E, estradiol.

There was a distinct difference in the LH pulsatile pattern between the follicular and ML phases (Figs. 3 and 5). During the ML phase, compared to the EF and LF phases, a significant decrease was found in pulse frequency (4.4 ± 0.7 pulses over 24 h; interpulse interval, 272 min; $P \leq 0.001$); this difference was accompanied by a correspondingly lower mean LH level (15.0 ± 4.2 ng/ml) in the ML than in the EF and LF phases ($P \leq 0.02$); moreover, during the interval between pulses in the ML phase, the plasma LH levels fell to the lowest levels during the entire menstrual cycle. The LH pulse amplitude was 24.7 ± 6.6 ng/ml in the ML phase. The numbers of relatively large amplitude (≥ 20 ng/ml) LH pulses per 24 h were similar in the EF, LF, and ML phases. Fewer low amplitude (< 20 ng/ml) LH pulses occurred in the ML phase (Table 2). The correlations between the LH immunoassay and bioassay samples for the two women (A and B) during the ML phase were 0.88 and 0.92, respectively [$P \leq 0.01$; see Fig. 3 for an example of an LH (immuno and bio) secretory pattern].

P treatment in the LF phase compared to that in the untreated LF phase effected a marked decrease in LH pulse frequency (6.8 ± 1.6 pulses/24 h; $P \leq 0.002$), an increase in LH pulse amplitude (33.0 ± 5.4 ; $P \leq 0.02$), and a decrease in the mean LH level (29.0 ± 6.7 ; $P \leq 0.004$). (See Fig. 4 for an example; the findings are summarized in Fig. 5.) There was also an increase in the relative number of large pulses with P treatment (Table 2). That P treatment converted the LH secretory activity in the LF phase to a pattern that closely resembled that found in the ML phase is demonstrated when Figs. 2, 3, and 4 are compared. The correlations between the LH immunoassay and bioassay samples for the 2 women (A

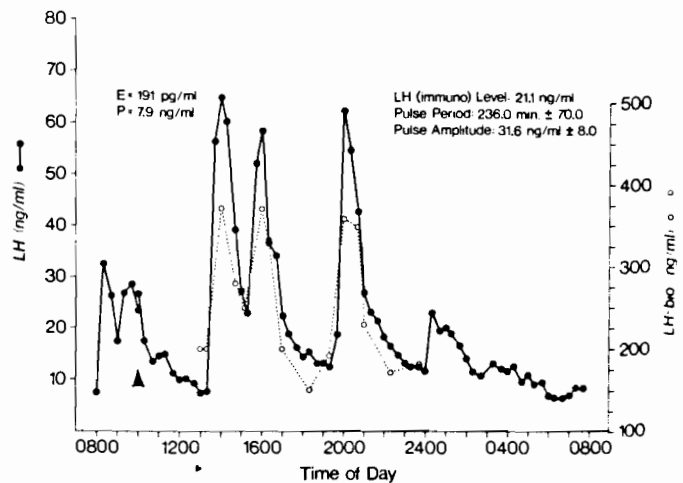


FIG. 4. LH pulse pattern; LF + P phase. The 24-h LH pulse pattern from a normal woman is shown. The time line has been normalized to begin at 0800 h; the actual clock time at which the sampling began and ended is indicated by the arrow. LH levels, as measured by bioassay, are indicated by the dotted line ($r = 0.93$). E, estradiol.

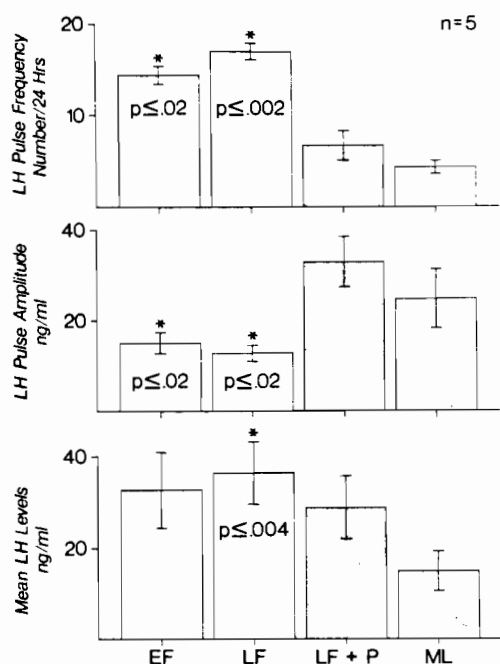


FIG. 5. The mean levels (\pm SEM) for three parameters of LH pulsatile activity throughout the menstrual cycle are indicated by the bar graphs. *, Significant comparisons with LF + P. Other significant comparisons: LH pulse frequency: EF vs. LF, $P \leq 0.05$; EF vs. ML, $P \leq 0.001$; LF vs. ML, $P \leq 0.0001$; mean LH level: EF vs. ML, $P \leq 0.02$; LF vs. ML, $P \leq 0.02$.

and B) were 0.93 and 0.95, respectively ($P \leq 0.01$; see Fig. 4). P induced a LH surge on the second or third day of treatment (midfollicular) in most of the individuals, but did not induce ovulation. P treatment delayed their next menses by 7–10 days.

Although episodic FSH activity was present in all cycle phases studied, the low amplitude fluctuations made formal FSH pulse analysis impossible; notwithstanding, plasma FSH levels demonstrated a significant ($P \leq 0.05$) positive correlation with LH pulse activity in all five individuals in the EF, ML, and LF + P phases and in two of the five volunteers in the LF phase. Figure 6 is an example of LH-FSH correlation in a volunteer; Table 2 lists the average r values by cycle phase.

Discussion

This study documented marked changes in the immuno- and bioassayable LH pulsatile patterns during the menstrual cycle in normal women. The LH secretory pattern in the follicular phase demonstrated more pulsatile activity (with a pulse occurring about every 90 min) than that in the ML phase (with an interpulse interval of 4–6 h), confirming previous observations in women (5–7). The LH pulsatile pattern appeared to be reproducible, in that individuals had statistically indistinguishable LH secretory patterns in the two separate

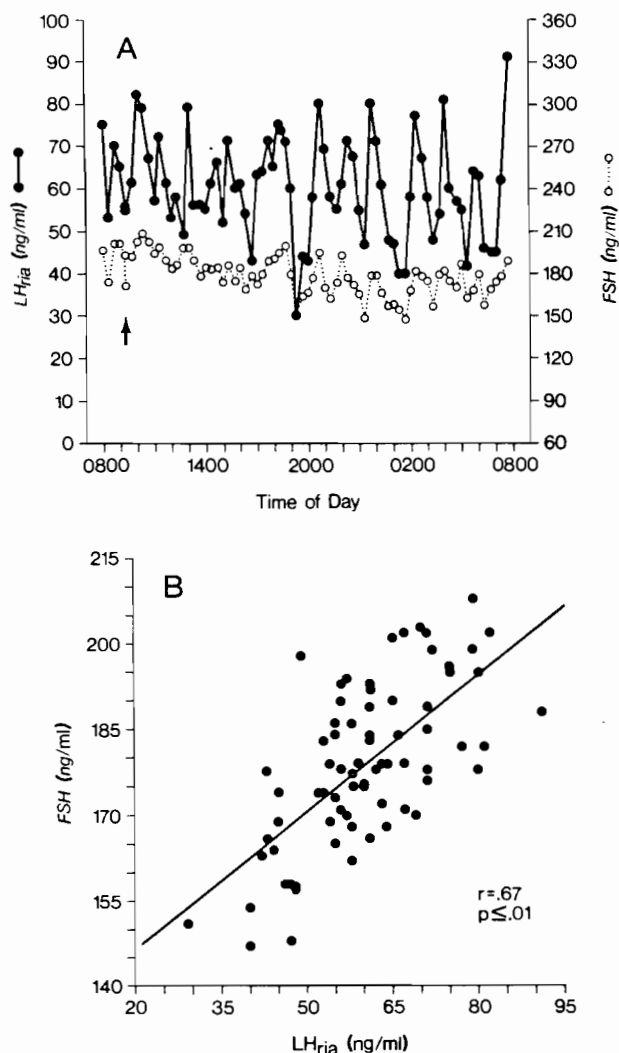


FIG. 6. A, The 24-h LH and FSH pulse patterns from a normal woman in the EF phase. B, LH is plotted vs. FSH for the same data as those shown in A ($r = 0.67$; $P \leq 0.01$).

EF phases studied. These changes in gonadotropin secretory activity may have differential effects on ovarian function (e.g. follicular development and the lifespan of the corpus luteum). This study also demonstrated that bioactive and immunoactive pulses of LH occurred synchronously. The concordance between LH immuno- and bioassays affirms the inference, based solely on immunoassay determinations, that these acute episodes of LH activity transmit biological signals.

There appeared to be two populations of LH pulses when pulse amplitudes were carefully examined throughout the menstrual cycle. When the data were superficially scanned, there was a tendency to focus only on the large amplitude (≥ 20 ng) LH pulses, especially in the ML phase graphs. While these large amplitude pulses were present throughout the cycle, a greater percentage of the total LH pulses were of large amplitude in the ML phase.

The 24-h mean LH level was significantly lower in the luteal than in the follicular phase. Yet, on daily samples, both the highest and lowest LH levels obtained throughout the cycle (excluding the midcycle LH surge) occurred during the luteal phase. Presumably, the high LH values occurred when daily samples were obtained during a pulse, with the low values obtained during the interpulse interval.

P treatment of normal women for 8 days in the follicular phase resulted in a LH secretory pattern indistinguishable from that of the ML phase. These findings are nearly identical to the LH secretory changes in P-treated oophorectomized ewes (15). These observations indicate that the change in the LH pulse pattern observed in the luteal phase is produced by the feedback effect(s) of P, rather than by a timing mechanism intrinsic to the brain.

The neuroendocrine locus of action for P's feedback effect in the human female is likely to include a hypothalamic site by inference, since frequency modulation is probably effected by brain-dependent mechanisms; however, the pituitary cannot be excluded as a possible site for P-induced amplitude modulation. Studies conducted in the female rhesus monkey implicate the central nervous system as a site of action for P-mediated feedback effects on gonadotropin secretion (16, 17). Wildt *et al.* (16) found that P failed to block the E-induced LH surge in monkeys with lesions of their arcuate nuclei. Terasawa *et al.* (17) found that pentobarbital was capable of blocking the P-induced LH surge in intact monkeys. Human studies by Ropert *et al.* (18) and Quigley and Yen (19) indicate that P modulation of luteal LH pulses may depend on inhibition of endogenous opiate activity. Their studies demonstrated that an opiate receptor antagonist (naloxone) could reverse the midluteal slowing of LH secretory activity. Together, these reports underscore the importance of the brain as well as the pituitary (20) in the physiological regulation of the primate menstrual cycle.

Changes in the frequency (and perhaps amplitude) of pulsatile LRH secretion during the menstrual cycle could represent an important regulatory component of the mechanisms governing pituitary and ovarian function. Relative to pituitary gonadotropin secretion, a recent study by Wildt *et al.* (21) demonstrated that LRH pulse frequency is a major determinant of LH/FSH secretion ratios, and, in particular, that slowing of LRH pulse frequency can effect selective and preferential rise in plasma FSH levels over those of LH. In women during the late stages of the luteal phase just preceding menses, baseline plasma levels of FSH are known to increase. It is possible that the action of P in slowing LRH pulse frequency during the ML phase is responsible for the rising tide of FSH soon to follow. LRH pulse frequency modulation may represent an integral part of physiolog-

ical control of gonadotropin secretion, which, in turn, may have cascading effects on the regulation of ovarian function. Changing frequencies of pulsatile LH secretion and alterations in the plasma ratios of LH to FSH may provide important signals to the ovary, influencing follicular development and luteal function. The results of a recent study in the female rhesus monkey by Schoonmaker and colleagues (22) underscore the importance of maintaining threshold levels of plasma LH to adequately support normal luteal function. We speculate that the slowing of LH pulses in the mid- to late luteal phase coupled with the exceedingly low LH bioactivity extant during the LH interpulse interval contribute to the natural demise of the corpus luteum in the nonpregnant cycle.

In agreement with others (7), our data suggest that FSH is released in an episodic manner. Although it was not possible to quantify pulse frequency due to the low amplitude nature of FSH fluctuations, LH and FSH were correlated during both the follicular and luteal phases of the cycle. We infer from this correlation that although there may not always be a one to one correspondence between LH and FSH pulses, they often occur in synchrony. Since intermittent LRH secretion is the probable cause of LH pulses (23, 24), and since it is well known that exogenous LRH administration releases FSH as well as LH in humans and other species, the correlation between LH and FSH release reported here concurs with our current understanding of neuroendocrine control of gonadotropin secretion.

Acknowledgments

The authors gratefully acknowledge the laboratory assistance of Patricia Gosciewski, Lorraine Shen, Julie Hazelton, Judy Tsoi, and Patricia Payne.

References

1. Dierschke DJ, Bhattacharya AN, Atkinson LE, Knobil E 1970 Circadian oscillations of plasma LH levels in the ovariectomized rhesus monkey. *Endocrinology* 87:850
2. Steiner RA, Peterson AP, Yu JYL, Conner H, Gilbert M, ter-Penning B, Bremner WJ 1980 Ultradian luteinizing hormone and testosterone rhythms in the adult male monkey, *Macaca fascicularis*. *Endocrinology* 107:1489
3. Gay VL, Sheth NA 1972 Evidence for a periodic release of LH in castrated male and female rats. *Endocrinology* 90:158
4. Katongole CB, Naftolin F, Short RV 1974 Seasonal variations in blood luteinizing hormone and testosterone levels in rams. *J Endocrinol* 60:101
5. Midgley Jr AR, Jaffe RB 1971 Regulation of human gonadotropins. X. Episodic fluctuation of LH during the menstrual cycle. *J Clin Endocrinol Metab* 33:962
6. Yen SSC, Tsai CC, Naftolin F, Vandenberg G, Ajabor L 1972 Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. *J Clin Endocrinol Metab* 34:671
7. Filicori M, Hoffman A, Crowley W, The frequency modulation of pulsatile release of gonadotropins in the human menstrual cycle. 64th Annual Meeting of The Endocrine Society, San Francisco CA, 1982 (Abstract 803)

8. Santen RJ, Bardin CW 1973 Episodic luteinizing hormone secretion in man. *J Clin Invest* 52:2617
9. Naftolin F, Yen SSC, Perlman D, Tsai CC, Parker DC, Vargo T 1973 Nocturnal patterns of serum gonadotropins during the menstrual cycle. *J Clin Endocrinol Metab* 37:6
10. Midgley AR 1966 Radioimmunoassay: a method for human chorionic gonadotropin and human luteinizing hormone. *Endocrinology* 79:10
11. Bremner WJ, Matsumoto AM, Sussman A, Paulsen CA 1981 Follicle-stimulating hormone and human spermatogenesis. *J Clin Invest* 68:1044
12. Diamond EJ, Aksel S, Hazelton JM, Abee CR, Serum concentrations of estradiol and progesterone in cycling and noncycling Bolivian squirrel monkeys. *Am J Primatol* in press
13. Van Damme MP, Robertson DM, Diczfalusy E 1974 An improved *in vitro* bioassay method for measuring luteinizing hormone (LH) activity using mouse Leydig cell preparations. *Acta Endocrinol (Copenh)* 77:655
14. Dufau ML, Hodgen GD, Goodman AL, Catt KJ 1977 Bioassays of circulating luteinizing hormone in the rhesus monkey: comparison with radioimmunoassay during physiological changes. *Endocrinology* 100:1557
15. Goodman RL, Bittman EL, Foster DL, Karsch FJ 1981 The endocrine basis of the synergistic suppression of luteinizing hormone by estradiol and progesterone. *Endocrinology* 109:1414
16. Wildt L, Hutchinson JS, Marshall G, Pohl CR, Knobil E 1981 On the site of action of progesterone in the blockade of the estradiol-induced gonadotropin discharge in the rhesus monkey. *Endocrinology* 109:1293
17. Terasawa E, Noonan J, Bridson WE 1982 Anesthesia with pentobarbitone blocks the progesterone-induced luteinizing hormone surge in the ovariectomized rhesus monkey. *J Endocrinol* 92:327
18. Ropert JF, Quigley ME, Yen SSC 1981 Endogenous opiates modulate pulsatile luteinizing hormone release in humans. *J Clin Endocrinol Metab* 52:583
19. Quigley ME, Yen SSC 1980 The role of endogenous opiates on luteinizing hormone secretion during the menstrual cycle. *J Clin Endocrinol Metab* 51:179
20. Knobil E 1980 The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res* 36:53
21. Wildt L, Hausler A, Marshall G, Hutchinson JS, Plant TM, Belchetz P, Knobil E 1981 Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology* 109:376
22. Schoonmaker JN, Bergman KS, Steiner RA, Karsch FJ 1982 Estradiol-induced luteal regression in the rhesus monkey: evidence for an extraovarian site of action. *Endocrinology* 110:1708
23. Clarke IJ, Cummins JT 1982 The temporal relationship between gonadotropin releasing hormone and luteinizing hormone secretion in ovariectomized ewes. *Endocrinology* 111:1737
24. Levine JE, Kwok-Yuen FP, Ramirez VD, Jackson GL 1982 Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* 111:1449