

# Nocturnal Slowing of Pulsatile Luteinizing Hormone Secretion in Women during the Follicular Phase of the Menstrual Cycle\*

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**ABSTRACT.** The gonadotropin secretion pattern in normal reproductive age women ( $n = 5$ ) was evaluated for the presence of a circadian rhythm. The women volunteered for a series of 24-h admissions in different phases of their menstrual cycles (early follicular, late follicular, and midluteal). Plasma LH and FSH levels were determined by RIA in blood samples drawn through indwelling venous catheters at 20-min intervals throughout a normal 24-h sleep-wake cycle. The gonadotropin secretory pattern was subjected to cosine analysis for identifying rhythmicity. The LH interpulse interval fluctuated with a significant 24-h rhythm during the early follicular phase in four of

the five women. The maximum interpulse intervals occurred during the early morning between 0100 and 0500 h (mean, 0250 h), with a corresponding increase in LH pulse amplitude occurring within the same time interval (mean, 0320 h). We found no consistent 24-h rhythms in overall mean plasma LH levels during any phase of the menstrual cycle, nor did we find a significant rhythmicity in either LH interpulse interval or LH pulse amplitude during the late follicular or luteal phase. These results demonstrate that the LH pulse-generating system is frequency modulated on a circadian basis during the early follicular phase of the human menstrual cycle. (*J Clin Endocrinol Metab* 61: 43, 1985)

**D**ETAILED analysis of gonadotropin secretion patterns throughout the menstrual cycle reveal a broad spectrum of complex rhythms. At one extreme is the midcycle gonadotropin surge that occurs at approximately 28-day intervals and whose onset appears to be linked to the time of day (1, 2). At the other extreme are the rapid (ultradian) pulses of LH that occur at 1- to 4-h intervals with a frequency that is dependent on the phase of the menstrual cycle (3, 4). The existence of a gonadotropin rhythm with a frequency that lies between these two extremes (*i.e.* a daily or circadian rhythm) has also been postulated. It is well known that the secretion of other pituitary hormones occurs on a nycterohemeral basis (*e.g.* nocturnal increases in GH, PRL, and ACTH). In early pubescent children, gonadotropin secretion increases markedly during sleep (5), whereas in adults, reports of a circadian gonadotropin rhythm have been conflicting (6-10); however, in most earlier studies, no attempt was made to take into account the relatively

high amplitude, high frequency pulsatile release of gonadotropins, which could mask a more subtle circadian variation.

We report here the results of a detailed investigation into the secretory patterns of LH and FSH in normal women over the course of 24 h during certain phases of the menstrual cycle and document the presence of a pronounced 24-h rhythmicity in the frequency of LH pulses during the follicular phase of the cycle.

## Materials and Methods

### *Subject and blood collection*

The subjects were five normal women (aged 24-34 yr) of normal weight for height and with a history of normal menstrual cycles. All had similar life styles; they were active during the day and slept 6-8 h from late evening to morning. All subjects had normal plasma levels of LH, FSH, estradiol (E) and progesterone (P), measured in daily venous blood samples obtained in the first study cycle.

The pulsatile patterns of LH and FSH were determined during four admissions (24 h each) to the Clinical Research Center at the University of Washington during five menstrual cycles in each woman. The cycle phases studied were early follicular (EF; days 1-4; twice, admissions in first and fifth cycles), late follicular (LF; days 9-12; third cycle), and midluteal

Received October 17, 1984.

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\* This work was supported by Parent NIH Grants BSRG 2-507-RR-05432, P50-HD-12629-04, and HD-12625-05, CRC Grant RR-37, and the V.A.

(ML; days 21–24; first cycle). The second and fourth menstrual cycles were rest cycles. The cycle phases were confirmed by measuring plasma E and P levels at the beginning and end of each admission (Table 1).

During each admission, blood samples were obtained every 20 min for 24 h through an indwelling iv line while the subject was kept at bedrest. The presence of apparent sleep was noted by the attending staff, but no formal sleep studies were performed.

### Hormone assays

Plasma samples were analyzed for LH and FSH using double antibody RIA techniques (11, 12). Standard NIH reagents were used, including the LER-907 reference preparation. The sensitivity of the LH assay was 6 ng/ml; intra- and interassay variabilities were 5.5% and 8.4%, respectively. The sensitivity of the FSH assay was 25 ng/ml; intra- and interassay variabilities were 7.3% and 9.7%, respectively. The E and P assays used were previously described (4).

### Pulse analysis

Pulsatile LH patterns were analyzed by applying a modification of the Santen and Bardin method (13). For each sampling series, measurement error was assessed based on assay replicate variability. A pulse was defined as an increase from nadir to peak that was 2 SD greater than the assay variability. Overlapping 10- and 20-min sampling intervals were employed for 16 h in the EF phase (4-h segments in four women) and for 16 h in the LF phase (8-h segments in two women). When these data were subjected to pulse analysis, there were no differences in LH pulse frequency between the 10- and 20-min data.

### Diurnal rhythm analysis

For each subject, the number and amplitude of discrete LH pulses during each 24-h sampling interval (admission) were determined by the above methods. To define the 24-h secretion profile, an interpulse interval value was determined for each hour of the 24 h during each admission. If the hour mark fell between two LH pulse peaks, the interpulse interval value assigned to that hour was the length of time between the two peaks; if a peak fell exactly on the hour, that hour was assigned the average of the interpulse intervals on either side of the pulse peak (see Fig. 1 for an illustration of this procedure). Likewise, a LH pulse amplitude was determined for each of the 24 h during each admission. Hourly amplitudes were calculated by a weighted average. For example, when an hour occurred

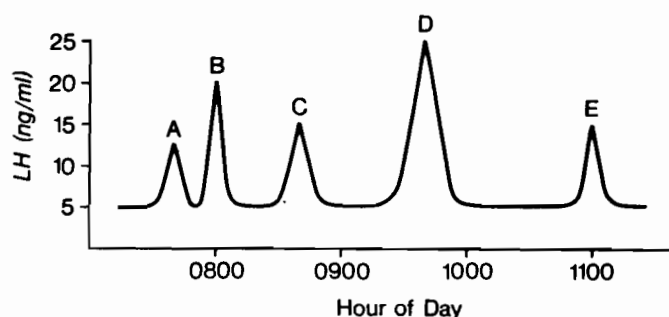


FIG. 1. Hypothetical pulsatile LH data to illustrate methods used to assess LH interpulse interval (II) and peak amplitude (amp) at hourly intervals. Hourly LH interpulse intervals are calculated as follows:

$$\text{at 0800 h, assigned interpulse interval} = \frac{(\text{II A to B}) + (\text{II B to C})}{2} \\ = (20 + 40)/2 = 30 \text{ min}$$

At 0900 and 1000 h, assigned interpulse intervals are II C to D and II D to E, respectively; II at 0900 h = 60 min; II at 1000 h = 80 min. Hourly LH pulse amplitudes are calculated as follows: at 0800 h, assigned amplitude is the amplitude of peak B; amplitude at 0800 h = 15 ng/ml; at 0900 h, assigned amplitude is calculated by weighted average as follows

$$= \frac{(\text{amp of D}) (\text{time from C to 0900 h}) + (\text{amp of C}) (\text{time from 0900 h to D})}{(\text{II C to D})} \\ = [(20 \text{ ng/ml}) (20 \text{ min}) + (10 \text{ ng/ml}) (40 \text{ min})]/60 \text{ min} \\ = (400 + 400)/60 = 13.33 \text{ ng/ml}$$

At 1000 h, assigned amplitude is calculated by weighted average as follows:

$$= \frac{(\text{amp of E}) (\text{time from D to 1000 h}) + (\text{amp of D}) (\text{time from 1000 h to E})}{(\text{II D to E})} \\ = [(10 \text{ ng/ml}) (20 \text{ min}) + (20 \text{ ng/ml}) (60 \text{ min})]/80 \text{ min} \\ = (200 + 1200)/80 = 17.5 \text{ ng/ml}$$

between two LH peaks, the peak closer to a given hour had its amplitude weighted more heavily than the amplitude of the peak farther away from that hour. If a peak fell exactly on the hour, that hour was assigned that pulse's amplitude, and no average was performed (Fig. 1).

A 24-h cosine regression was used to analyze individual variations in overall (mean) plasma LH concentration, LH interpulse interval, and LH pulse amplitude over time. The regression provided estimates of the amount of fluctuation (amplitude of the cosine) and the time of the maximum fluctuation (phase of the cosine). Since each subject had two separate EF phase admissions, the two 24-h sets of data were merged to facilitate data analysis. The data were arranged (merged) as if the EF admission had been for 48 consecutive h.

Cosinor analysis was used to evaluate rhythmicity in the group as a whole (14). This analysis provided an estimate of the 95% confidence limits for the amplitude and the phase of each parameter (*i.e.* for overall mean LH, interpulse interval, and pulse amplitude). The hypothesis that there was no consistent circadian rhythm in the group was rejected if the probability of the group amplitude being zero was less than 0.05 (*i.e.* zero amplitude fell outside of the 95% confidence limits).

## Results

A summary of the LH secretory data for pulse frequency, pulse amplitude, and mean level is presented in

TABLE 1. Characterization of menstrual cycle phases

	Range of cycle days relative to LH surge	E (pg/ml)	P (ng/ml)
EF (n = 5)	-8 to -15	57.5 ± 5.2 <sup>a</sup>	0.29 ± 0.02 <sup>a</sup>
LF (n = 5)	-1 to -5	121.1 ± 17.8	0.30 ± 0.03
ML (n = 5)	+7 to +9	156.6 ± 29.0	7.5 ± 1.7

<sup>a</sup> Mean ± SEM.

## NOCTURNAL SLOWING OF LH PULSE FREQUENCY

Fig. 2. The mean levels for the five normal women are indicated for the three cycle phases studied.

### *EF phase*

A representative graph of the LH secretory activity during the EF phase in one woman is shown in Fig. 3. The LH data from this woman had a significant 24-h periodicity of both LH interpulse interval and pulse amplitude.

Four of the five volunteers had a significant 24-h periodicity in LH interpulse interval during the EF phase (Table 2). Analysis of the group data revealed a significant degree of synchrony in the LH interpulse interval

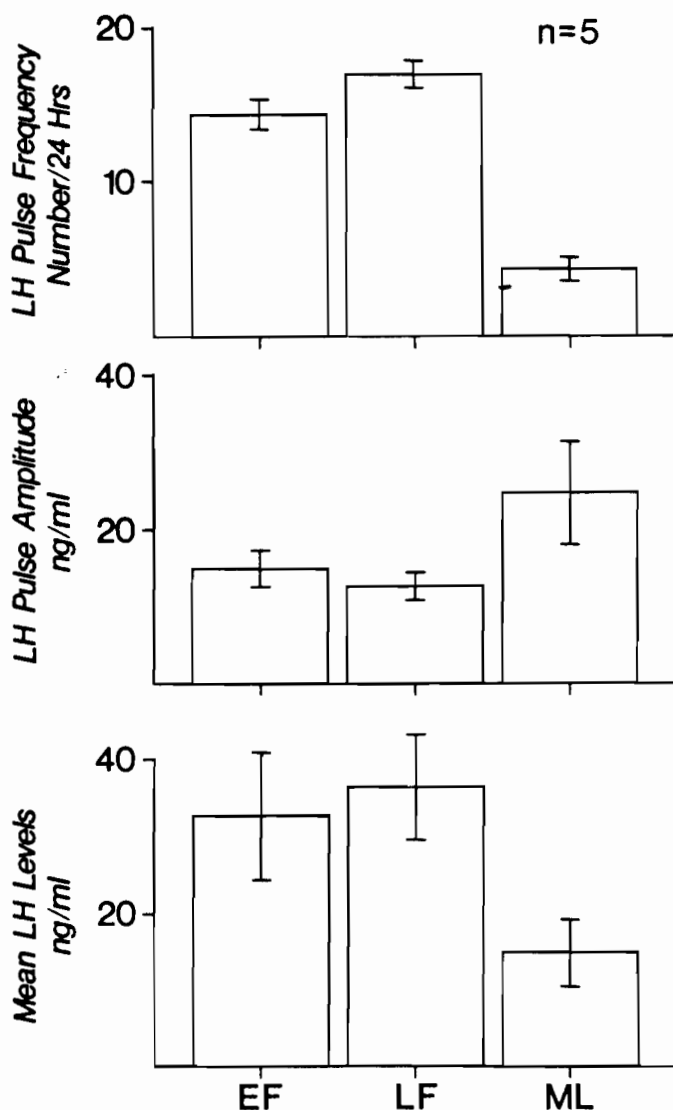


FIG. 2. The mean ( $\pm$ SEM) levels for three parameters of LH pulsatile activity throughout the menstrual cycle. Significant comparisons: LH pulse frequency: EF vs. LF,  $P \leq 0.05$ ; EF vs. ML,  $P \leq 0.001$ ; LF vs. ML,  $P \leq 0.001$ ; mean LH level: EF vs. ML,  $P \leq 0.02$ ; LF vs. ML,  $P \leq 0.02$ .

rhythm among individuals ( $P \leq 0.05$ ). The average interpulse intervals across all individuals are shown in Fig. 4, top panel.

LH pulse amplitude was also associated with a 24-h rhythm during the EF phase in three of the five women studied. However, even though the time of maximum pulse amplitude was similar in all five women (ranging from 0205–0450 h), statistically significant synchrony in the pulse amplitude rhythm among individuals was not demonstrated for the group data (Fig. 4, bottom panel).

No convincing evidence was found for the presence of a 24-h rhythm in overall (mean) LH levels during the EF phase when the grouped data were analyzed (Table 2); using the merged data from two separate 24-h admissions, a significant rhythm for overall LH levels was detected in only two of the five women studied.

No clear relationship was found between apparent sleep and changes in LH secretory profiles; all of the women slept for a variable number of hours during the 2300–0700 h segment of the sampling interval.

### *LF phase*

Changes in LH interpulse interval and amplitude over 24 h in the LF phase in the five women were not as pronounced as in the EF phase. Two of the women had significant increases in LH interpulse interval, which occurred at 0630 and 1925 h, and two had significant changes in the LH pulse amplitude, which occurred at 0455 and 0040 h (see Table 2). Analysis of the group data for LH interpulse interval and amplitude demonstrated no significant rhythm. The maximum LH interpulse interval and amplitude tended to occur early in the day in this LF phase group (0000–0700 h); however, there were several individuals in whom these maxima occurred at other times during the day.

Significant 24-h variations in overall (mean) LH levels were detected in three of the five women during the LF phase, with the maximum plasma levels occurring between 0720 and 1140 h (Table 2). There was no evidence for a consistent rhythm in overall LH levels in the women as a group.

### *ML phase and FSH patterns*

Due to the infrequent occurrence of LH pulses during the luteal phase of the menstrual cycle, we were unable to calculate cosine regressions for our ML data. We did not find a significant diurnal rhythm in FSH secretory activity during any of the cycle phases studied.

## Discussion

This report describes circadian changes in LH interpulse interval and amplitude during the follicular phase

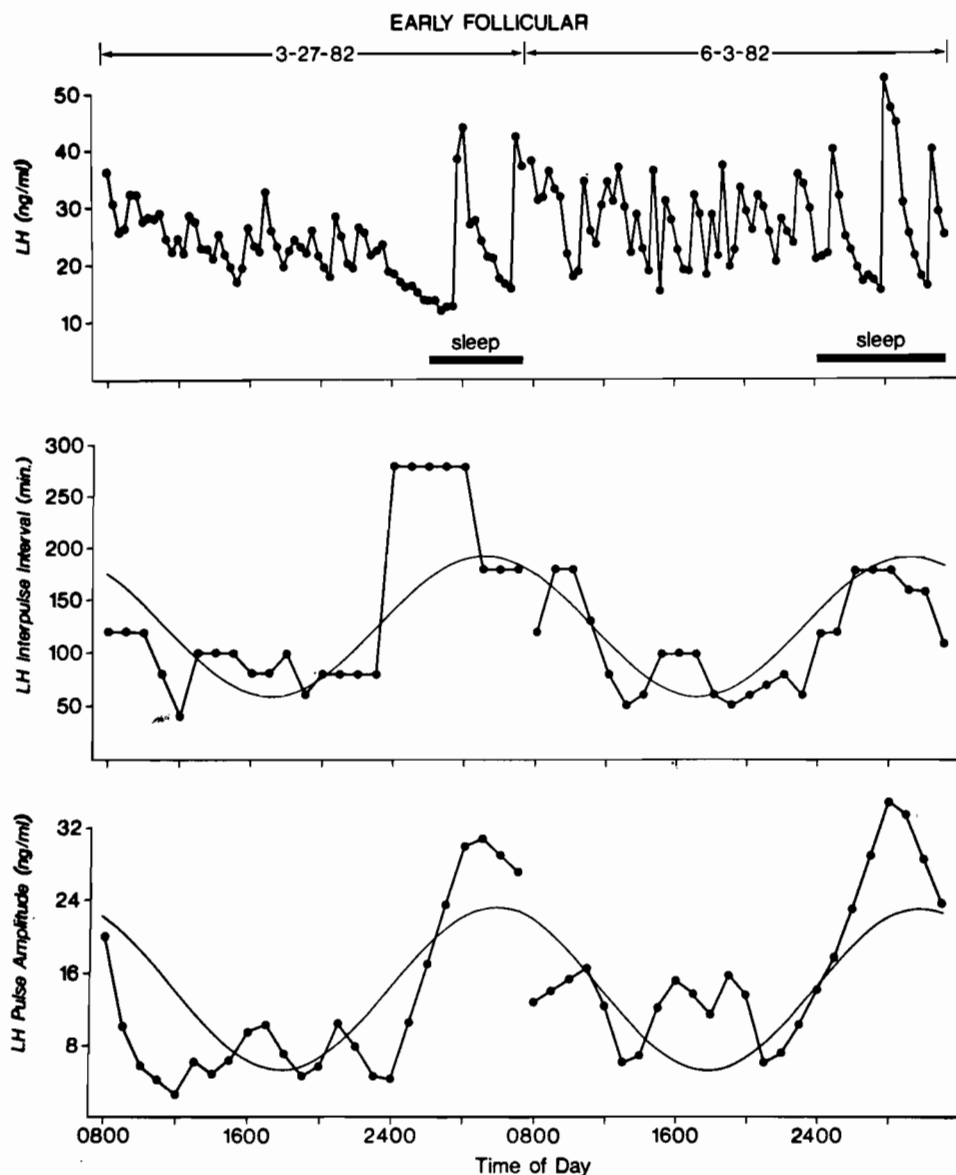


FIG. 3. *Top panel*, The merged LH data for 48 h in the EF phase of two cycles in subject E. ■, Observed sleep. A decrease in pulse frequency and an increase in pulse amplitude are apparent between 2300 and 0700 h in each sampling interval. *Middle panel*, The LH interpulse interval for subject E over 48 h. The cosine curve has been drawn. *Bottom panel*, The LH pulse amplitude for subject E over 48 h. The cosine curve has been drawn.

of the menstrual cycle in normal women. There was a subtle, but definite, nocturnal slowing of LH secretory activity with a concomitant increase in LH pulse amplitude which occurred in most of these women between 0300 and 0500 h in the EF phase. These same changes in LH secretion occurred in some women in the LF phase. That circadian changes in LH secretion were noted in all women studied in the EF phase and in only some of the women in the LF phase would imply that this nocturnal variation becomes less pronounced as women approach ovulation. We were unable to document a significant 24-h rhythm in LH interpulse interval, pulse amplitude, or overall mean plasma LH levels during the luteal phase of the menstrual cycle. The low frequency of LH pulses that occur during the luteal phase (4) would obscure statistical confirmation of a putative circadian

frequency oscillation, if one were to exist.

There is an established relationship between the sampling interval and pulse frequency. Other investigators have reported an increased LH pulse frequency when the sampling interval was decreased from 20 min to 10 min (15, 16). With the pulse detection technique used for this study, we did not find an increase in the number of pulses when selected 10- and 20-min sampling intervals were compared in the EF and LF phases. Although a significant increase in pulse frequency was detected in moving from the EF to the LF phase in this study, more frequent sampling of all subjects, especially in the LF phase, may have detected a greater difference. Furthermore, it is conceivable that more frequent sampling in the LF phase may have resulted in the detection of significant circadian changes in more subjects in the LF phase.

TABLE 2. Cosine analysis of LH secretory pattern

Patients	LH II			LH pulse amp			Overall mean plasma LH data		
	Excursion of cosine (min)	Max II (time of day)	Mean II (min)	Excursion of cosine (ng/ml)	Max amp (time of day)	Mean amp (ng/ml)	Excursion of cosine (ng/ml)	Max LH (time of day)	Mean LH (ng/ml)
EF (2 admissions, 48 h merged)									
A	12.3	0220	99.6	2.41	0310	16.0	1.08	1705	28.0
B	34.2 <sup>a</sup>	0120	123.8	3.57	0205	11.1	2.69 <sup>a</sup>	1300	23.6
C	43.9 <sup>b</sup>	0230	137.3	6.44 <sup>b</sup>	0320	15.5	1.49	0305	21.5
D	43.2 <sup>b</sup>	0225	102.5	10.8 <sup>b</sup>	0225	27.1	2.08	1740	65.1
E	67.4 <sup>b</sup>	0405	126.0	9.13 <sup>b</sup>	0450	14.3	2.39 <sup>a</sup>	0940	25.4
EF Group mean	38.9 <sup>a</sup>	0250	117.8	6.24	0320	16.8	0.84	1320	32.7
LF (single admission, 24 h)									
A	26.5 <sup>a</sup>	0630	93.8	3.60 <sup>a</sup>	0455	10.8	0.99	0805	24.4
B	16.5 <sup>a</sup>	1925	85.4	0.09	0925	8.3	0.65	1140	30.2
C	17.8	0110	100.0	7.51	0655	13.9	4.51 <sup>a</sup>	0720	23.4
D	17.1	0150	105.0	7.86 <sup>b</sup>	0040	14.9	6.60 <sup>b</sup>	2340	47.9
E	9.9	0055	78.3	4.79	2220	19.8	6.70 <sup>b</sup>	2330	57.0
LF Group mean	10.4	0205	92.5	3.02	0250	13.5	2.23	0120	36.6

II, Interpulse interval; max, maximum; amp, amplitude.

<sup>a</sup>  $P \leq 0.05$ .

<sup>b</sup>  $P \leq 0.001$ .

To our knowledge, this is the first report documenting a significant 24-h periodicity in the pattern of pulsatile LH release during the menstrual cycle. Our results are consistent with the observation of Kapen *et al.* (7), who found a suggestive, though not statistically significant, nocturnal increase in LH interpulse interval during the periovulatory period of the menstrual cycle. The same group was unable to confirm a consistent difference in the LH interpulse interval between night and day periods during the EF phase (9); however, rigorous analysis of the interpulse interval rhythm was not conducted in either the periovulatory or EF phase study groups.

The apparent absence of 24-h rhythmicity in overall (mean) LH levels in our subjects is in general agreement with several other studies published within the last decade. No circadian changes in LH and FSH secretion over 48 h (30- and 60-min sampling intervals) were found when normal men were studied by Krieger and her associates (6). Alford *et al.* (8), using a continuous withdrawal sampling technique, found no evidence for circadian changes in integrated (4-h) plasma levels of LH and FSH in a mixed study group of healthy men and women. When nocturnal LH secretory activity was studied in 12 normal women during the periovulatory interval of the menstrual cycle, neither nocturnal changes nor a relationship with sleep stages was found (7). However, a consistent reduction of overall LH levels associated with the onset of sleep was reported to occur in 5 normal women during the EF phase (9). Although we did not conduct a formal sleep study, we found no evidence that

a similar reduction of mean plasma LH levels occurred during sleep in our subjects.

The fact that the circadian LH pulse frequency rhythm occurred in synchrony among the volunteers studied during EF, plus the fact that this rhythm was consistent across two entirely different menstrual cycles for four of the five women, strongly suggest the presence of a subtle underlying circadian mechanism for the control of LH pulse frequency during the EF phase. The evidence for a similar circadian control of LH pulse amplitude is not as strong; nevertheless, we did find a close phase relationship between the LH interpulse interval regressions and those for LH pulse amplitude which suggests an interaction between these two parameters. In fact, our inability to discern a 24-h rhythm in overall mean plasma LH levels is consistent with a direct relationship between interpulse interval and pulse amplitude; if LH pulse amplitude were to increase as LH pulses become less frequent, then one would expect overall mean LH plasma levels to remain relatively constant.

Specific nocturnal (sleep-entrained) increases in secretory activity have been well described for some anterior pituitary hormones. Distinct independent secretory episodes for GH and PRL occur within 2 h of the onset of sleep in normal subjects of both sexes (17). In contrast, the major episodic secretory episodes of cortisol and ACTH occur toward the end of the sleep interval (17). Prominent sleep-related episodes of increased LH secretion have been described during puberty, but become obscured as boys and girls achieve sexual maturity (5).

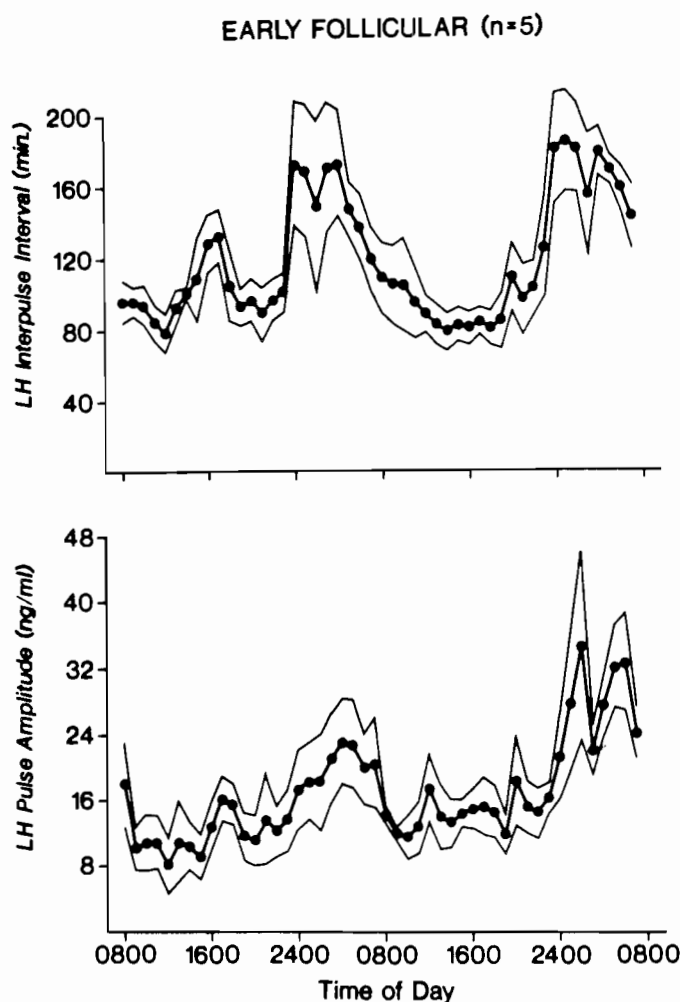


FIG. 4. The mean LH interpulse interval (top panel) and mean LH pulse amplitude (bottom panel) over 24 h in five women studied in the EF phase of two separate menstrual cycles. The data have been merged into 48 h. The SEM is indicated by the thinner lines.

The nocturnal LH secretory patterns in the adult women described in our study were different in character and more protracted than the nocturnal secretory patterns for pubertal LH and the other anterior pituitary hormones. Whether these observed changes in LH secretion were due to sleep is uncertain, as our study was not designed to address this specific issue. Because all of the subjects appeared to sleep during at least part of the nocturnal interval studied, sleep could be the trigger for the nocturnal slowing of pulsatile LH activity, but this remains to be documented by a study designed to address this issue.

The mechanisms governing this observed circadian shift in LH pulse frequency remain a matter of speculation; however, the pineal gland and melatonin secretion should be considered among the possible factors involved in this process. Pineal melatonin secretion is recognized to signal changes in day length and thereby to regulate

the activity of the reproductive axis in seasonally breeding animals (18). Although melatonin's role in primate species is less well understood, it is, nevertheless, clear that in humans of both sexes, melatonin secretion is tightly coupled to both the light-dark and sleep-wake cycles, with secretion being increased during darkness and sleep (19, 20). In a preliminary study of melatonin secretion patterns over the course of the human menstrual cycle, Wetterberg *et al.* (21) found that plasma melatonin concentrations were markedly elevated during the mornings of the EF phase, the same phase in which we now document that LH pulse frequency slows most dramatically during sleep (and darkness). Whereas melatonin secretion and gonadotropin pulse patterns may be only coincidentally related, the subject bears further investigation.

### Acknowledgments

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

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