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Corpus Luteum Insufficiency Induced by a Rapid Gonadotropin-Releasing Hormone-Induced Gonadotropin Secretion Pattern in the Follicular Phase*

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ABSTRACT. The pulse frequency of LH and FSH (and by inference, GnRH) is a major determinant of the relative baseline plasma levels of LH and FSH. Luteal phase deficiency has been reported to be associated with increased gonadotropin pulse frequency and inadequate preovulatory follicular development. In this study we induced in normal women a supraphysiological gonadotropin pulse frequency in the follicular phase to determine its effect on follicular development and corpus luteum function. Specifically, we tested the hypothesis that a supraphysiological GnRH pulse frequency would result in deficient luteal phase production of progesterone. The subjects were six normal ovulatory women (age range, 23–35 yr). They were initially studied during a control cycle (cycle 1). Then, 25 ng/kg GnRH was administered iv every 30 min from the early follicular phase of the next cycle (cycle 2) until ovulation occurred. GnRH administration resulted in increased follicular phase plasma LH and FSH levels and LH to FSH ratios, multiple preovulatory follicles (mean, 2.8) with increased mean integrated estradiol [1302 (pg/mL)day (cycle 1) *vs.* 2550 (pg/mL)day (cycle 2); $P < 0.05$; 4780 *vs.* 9360 (pmol/L)day, Systeme International units], spontaneous ovulation, decreased luteal phase plasma immunoreactive and bioactive LH levels, decreased luteal phase length [13.5 days (cycle 1) *vs.* 8.8 days (cycle 2); $P < 0.05$], and decreased mean integrated progesterone secretion [152 (ng/mL)day (cycle 1) *vs.* 66 (ng/mL)day (cycle 2); $P < 0.01$; 482 *vs.* 209 (nmol/L)day, Systeme International units]. We conclude that high frequency LH and FSH secretion during the follicular phase can induce inadequate progesterone secretion during the subsequent luteal phase, and we infer that the pathophysiological basis for this induced luteal phase deficiency is decreased LH support of corpus luteum function. (*J Clin Endocrinol Metab* **65:** 457, 1987)

DURING the course of the menstrual cycle in normal women the ultradian rhythm of pulsatile LH secretion undergoes a predictable shift in both frequency and amplitude (1–4). This change in the LH secretory pattern is thought to reflect the coordinated interplay between ovarian hormones and the brain-pituitary axis (3). Even within a given phase of the menstrual cycle, there are significant variations in the frequency and amplitude of LH pulses among women. For example, in normal women during the early follicular (EF) phase, LH pulse frequency may range from one pulse every 70 min to one every 120 min (3, 5).

Despite the normal variance of LH pulse frequency within the follicular phase, we now appreciate that some women with luteal phase deficiency (LPD) have an inappropriately high LH pulse frequency throughout their follicular phase, and this, in turn, is associated with inadequate progesterone (P) production during their subsequent luteal phase (6). It is not known whether the accelerated follicular phase LH pulse frequency and the LPD are causally related or are merely associated, but unrelated, phenomena. However, observations in women and studies in animals have found an association among decreased preovulatory FSH levels, inadequate follicular development, and LPD (7–9). Furthermore, gonadotropin pulse frequency has been demonstrated to be an important determinant of baseline levels of LH and FSH in a nonhuman primate model (10). In agonadal monkeys with arcuate nucleus lesions, an increase in GnRH-induced gonadotropin pulse frequency is associated with an increase in baseline LH and a decrease in baseline FSH (increased LH to FSH ratio) (10). We hypothesized that an inappropriately high LH pulse frequency in the follicular phase causes LPD by effecting a decrease in FSH levels and/or an alteration in the LH to FSH ratio in the follicular phase. To test this hypothesis, we increased the gonadotropin pulse frequency in normal women throughout their follicular phase by administering exogenous GnRH at an accelerated rate.

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Materials and Methods

Subjects

Six normal women, between the ages of 23 and 35 yr, participated in this study (Table 1), which was approved by the Human Subjects Committee of the University of Washington. These women were within ±10% of ideal body weight (Metropolitan Life Insurance Co. Tables, 1980) and had regular menstrual cycles, normal basal body temperature charts, and normal plasma levels of testosterone (T), PRL, and P (>12 ng/mL) in a menstrual cycle preceding the study. They were taking no medications and had not received any hormone therapy for the previous 12 months.

Protocol

The women served as their own controls. The first menstrual cycle of the study was only monitored (cycle 1/control); GnRH was administered in the second cycle (cycle 2/GnRH). Basal body temperature charts were completed for each cycle. Venous blood samples were obtained every morning throughout both study cycles. These blood samples were analyzed for LH, FSH, estradiol (E), P, and PRL by RIA and for LH bioactivity (LH-bio) by mouse interstitial cell bioassay. At midcycle during the study, daily morning pelvic sonograms of the ovaries were performed until two of the following three sonographic criteria for ovulation were observed: 1) an abrupt decrease in size or disappearance of a preovulatory size follicle, 2) a hyperechoic border or increased echoes in a follicle-like structure, and/or 3) an acute increase in free ip fluid. Mechanical real-time sector scanners were used, each having both 3- and 5-MHz transducers.

Women were admitted to the Clinical Research Center (University of Washington) in the EF phase of their second menstrual cycle (cycle day 2, 3, or 4). Their endogenous gonadotropin secretion pattern was determined for 12 h (the time span of each woman's 12-h admission was variable) by obtaining 5-mL blood samples every 20 min through a heparinized indwelling iv line. Serum was separated, frozen at −20 C, and stored until analysis for LH and FSH concentration. After the 12-h sampling interval was complete, each woman was fitted with an intermittent infusion pump (model A6H, Autosyringe, Hookset, NH). GnRH (Factrel, Ayerst Laboratories, New York, NY) in a heparinized solution was administered iv by the pump at a dose of 25 ng/kg given every 30 min. The GnRH dose varied from 1.3–1.7 μg/pulse and from 64–82 μg/day, depending on the subject's weight (Table 1). Frequent blood sampling (every 10 min) was continued for 2 more h after GnRH administration was initiated. GnRH was continued at the same dose and frequency throughout the follicular phase. Each subject underwent a second 12-h admission 4–7 days after initiation of GnRH. The induced gonadotropin pulse pattern was determined by obtaining 5-mL blood samples every 10 min for 12 h during this second admission. GnRH administration was terminated when two or more of the multiple follicles that developed were determined to have undergone ovulation by sonographic criteria. GnRH was administered between 9 and 13 days in the six volunteers (Table 1).

The ensuing luteal phase of the second study cycle was monitored by measuring daily hormone levels in the first four subjects (A–D). In addition to the daily hormone determinations, the last two subjects (E and F) to complete the study received hCG injections (5000 IU, im), beginning on the day the pump was removed and continued every 4 days for a total of four doses. hCG was administered to these two subjects to test the functional capability of their respective corpora lutea.

Hormone assays

Serum hormone concentrations were determined in blood samples obtained on a daily basis and during the two admissions. All the samples from an individual woman were analyzed in one hormone assay. Serum samples were analyzed in duplicate for LH and FSH by double antibody RIA (11, 12). Standard NIH reagents were used, including the LER-907 reference preparation. The sensitivity of the LH assay was 6 ng/mL [6 μg/L, Systeme International (SI) units] at a volume of 200 μL; intra- and interassay coefficients of variation were 5.5% and 8.4%, respectively. The sensitivity of the FSH assay was 25 ng/mL (25 μg/L, SI units) at a volume of 200 μL; intra- and interassay coefficients of variation were 7.3% and 9.7%, respectively.

Serum PRL concentrations were determined in duplicate by a double antibody RIA (13) with the NIADK human PRL kit (RP-1 standard, hPRL-3 anti-PRL serum). The intraassay coefficient of variation, determined by analysis of replicate variability in the assay samples was 6.5%. The interassay coefficient of variation was 14.8%. The sensitivity of the assay was 1 ng/mL (1 μg/L, SI units) at a serum volume of 200 μL.

The RIA for serum E was performed in duplicate using

TABLE 1. Clinical characteristics of study subjects

Patient	Age (yr)	%IBW	Cycle 1		Cycle 2		GnRH administration			
			Luteal length (days)	Total cycle length (days)	Luteal length (days)	Total cycle length (days)	GnRH dose (μg) Per pulse	GnRH dose (μg) Per day	Cycle days (by LH surge)	Menstrual cycle day began
A	31	104	14	26	10	20	1.7	82	−6 to 1	3
B	23	100	13	25	9	18	1.7	82	−6 to 4	2
C	33	92	13	27	12	26	1.5	72	−10 to 1	3
D	31	90	14	32	4	15	1.3	64	−7 to 3	3
E	35	94	15	25	21	31	1.7	82	−6 to 4	3
F	29	101	12	26	18	31	1.7	82	−8 to 1	4

IBW, Ideal body weight.

methodology described previously (14). The sensitivity of the E assay was 12 pg/mL (44 pmol/L, SI units) at a serum volume of 150 μL; intra- and interassay coefficients of variation were 8.2% and 8.8%, respectively.

Serum P was measured in duplicate with reagents supplied by Diagnostic Products Corp. (Los Angeles, CA). Cross-reactivity data supplied by the producer indicated less than 2.4% cross-reaction with all steroids tested, including 0.3% with 17 α-hydroxy-progesterone and less than 0.01% with T, E, pregnenolone, and cortisol. Sensitivity in our laboratory was less than 0.5 ng/mL (1.6 nmol/L, SI units) at a serum volume of 100 μL. Intra- and interassay coefficients of variation were 9% and 12%, respectively. External quality control used the WHO program, which involves monthly measurements of unknown samples; no significant bias (>10%) was detected, and variabilities were comparable to those described above for internal quality controls.

Serum LH-bio levels were measured in triplicate by using a modification (3) of procedures described by Van Damme *et al.* (15) and Dufau *et al.* (16). The mean intra- and interassay coefficients of variation were 8% and 13%, respectively.

Pulse analysis

An adaptive threshold method was used to determine the time and amplitude of hormone pulses. A pulse was defined as an increase from local minimum to local maximum that was greater than a threshold value. The correct threshold was determined in an iterative manner. Initially, the threshold was set at 2.5 times the SD of the sample replicates, and the number of pulses in the data set was determined. Based on the estimated number of pulses, the threshold was readjusted according to the following formula:

$$T = S^*(5.518 + F^*[-0.3519 + F^*(0.01339-0.0002478^*F)])$$

where T is the threshold, S is the SD of the replicates, and F is 100 × (number of pulses detected last time)/(number of samples in the data set). The analysis was then repeated with the new threshold. If the number of pulses detected was different from the number found on the previous pass, a new threshold was calculated according to the above formula, and the procedure was repeated. This iterative procedure was continued until the number of pulses detected stabilized. The formula for threshold was determined empirically based on computer simulations.

Data analysis and statistics

The daily hormone and sonographic data were arranged relative to the day of the peak LH value (surge), which was labeled day zero. Paired data from each subject obtained on the same cycle day relative to the LH surge were compared between cycles 1 and 2 for LH, FSH, LH to FSH ratio, and LH-bio by two-way analysis of variance with repeated measures. The data were expressed as the mean ± SEM unless otherwise indicated. Integrated values for follicular E secretion were determined from cycle days −6 to 0, and for luteal P secretion from cycle day 0 to the first day of the following menstrual period. These integrated values were determined by multiplying the mean daily hormone concentration by the number of days for each individual. Integrated E and P values, follicular and luteal phase lengths, and follicle number were compared between cycles 1 and 2 by paired *t* tests. The LH pulse parameters, *i.e.* frequency and amplitude, and mean plasma LH levels were compared between cycle 2 (GnRH) and control cycles (3) by Student's *t* test. [Note that the study subjects initiated GnRH administration on different cycle days (Table 1), and the subsequent menstrual period in cycle 2 started on varying days relative to the LH surge. Subsequently, in the follicular phase, the common days for GnRH administration were cycle days −6 to −1; in the luteal phase the common days before the next menstrual period for the four subjects who did not receive hCG were cycle days 1–4. Therefore, these were the common days (follicular −6 to −1; luteal 1–4) that were used for paired comparisons of data (by analysis of variance) for the follicular and luteal phases, respectively.]

Results

Follicular phase gonadotropin secretion patterns

A more rapid LH secretory pattern was successfully induced throughout the follicular phase in all volunteers. Figure 1 illustrates the endogenous EF phase LH pulse pattern in subject C, followed by the GnRH-induced pattern. The endogenous LH pulse pattern on cycle days −11 to −7 in these women was not different from previously published EF phase values in other normal women (3). While receiving GnRH, the LH pulse frequency, LH pulse amplitude, and mean plasma LH and FSH levels over each sampling period (admission) were increased (Table 2). The induced LH pulse frequency was approximately 26 pulses/24 h (extrapolated from the 12-h sampling period) compared to 17.6 pulses/24 h for the late follicular (LF) phase controls. There was not always a discernible change in LH secretion associated with every GnRH dose. This apparent absence of LH response was attributed to transient pituitary insensitivity and/or in-

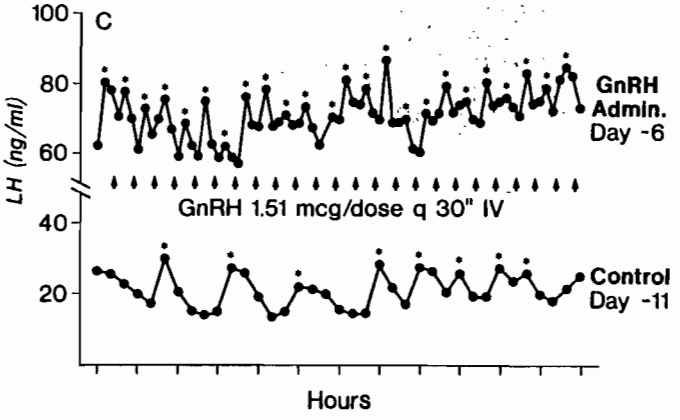

FIG. 1. The 12-h endogenous secretory pattern on day −11 of cycle 2 for subject C. The LH secretory pattern during iv GnRH on day −6 (dose given at *arrow*) of cycle 2 is illustrated in the *upper panel.* An *asterisk above a data point* indicates a detected pulse. SI unit conversion, 1 ng/mL = 1 μg/L.

TABLE 2. Exogenous GnRH-induced pulse parameters

Patient	Day from LH surge	Cycle day	LH pulse frequency (no./24 h)	LH pulse amplitude (ng/mL)[a]	Mean LH (ng/mL)[a]	Mean FSH (ng/mL)[a]
A	0	10	22	31.9	194.6	312.0
B	−3	6	22	19.3	140.0	169.3
C	−6	8	48	9.4	71.1	96.1
D	−3	8	16	99.8	211.2	186.1
E	−3	7	18	21.6	139.5	151.2
F	−5	8	32	7.0	54.6	179.2
Mean ± SEM			26.3 ± 4.9	31.5 ± 14.1	135.2 ± 25.8[b]	182.3 ± 29.1
Normal EF[c]			14.6 ± 1.5	16.4 ± 1.9	35.1 ± 8.8	164.0 ± 10.9
Normal LF[c]			17.6 ± 0.9	12.5 ± 1.8	36.6 ± 6.8	127.3 ± 16.1

[a] SI unit conversion, 1 ng/mL = 1 μg/L.
[b] $P \leq 0.01$ compared to EF and LF normal.
[c] EF, n = 5; LF, n = 5. These gonadotropin pulse parameters were obtained from previously reported data from normal women and are provided here for reference purposes (3). Values given are the mean ± SEM.

ability to detect subtle changes in LH secretion by the pulse analysis methodology.

In terms of LH pulse frequency, the induced gonadotropin secretion pattern was in the frequency range described for women with LPD (6). However, the pulse amplitude and mean LH and FSH levels were increased over these same parameters observed in women with LPD, despite the use of quite small GnRH doses.

Baseline gonadotropin levels

GnRH administration effected significant changes in baseline gonadotropin levels during cycle days −6 to −1 of cycle 2. The 6 day mean plasma LH level increased from 53.4 ± 6.0 ng/mL in the control cycle to 153.1 ± 24.0 ng/mL in cycle 2 ($P < 0.01$; 53 *vs.* 153 μg/L, SI units). The 6 day mean peak LH levels in the follicular phase were 131.5 ± 27.6 and 263.3 ± 36.9 ng/mL in the control and GnRH cycles, respectively ($P < 0.05$; 132 *vs.* 263 μg/L, SI units). There was also an increase in FSH, although it was less dramatic; the mean plasma FSH value on cycle days −6 to −1 was 159.7 ± 11.8 ng/mL (control) *vs.* 195.2 ± 20.0 ng/mL (GnRH; $P < 0.05$; 160 *vs.* 195 μg/L, SI units). This resulted in a major change in the mean LH to FSH ratio from 0.33 ± 0.04 (control) to 0.79 ± 0.07 (GnRH; $P < 0.001$). In conjunction with these changes in gonadotropin immunoactivity there was an increase in the mean 6 day plasma LH-bio during GnRH administration as well [0.38 ± 0.04 μg/mL (control) *vs.* 0.75 ± 0.10 μg/mL (GnRH); $P < 0.02$]. The changes in immunoactive LH and FSH during GnRH administration are illustrated in Fig. 2.

Plasma gonadotropin levels were analyzed over the 4 common luteal phase days in the four subjects who did not receive hCG. There were significantly lower plasma levels of LH, FSH, and LH-bio in cycle 2 compared to cycle 1. The 4 day mean plasma LH decreased from 64.9 ± 10.0 (control) to 35.0 ± 4.7 ng/mL (GnRH; $P < 0.05$; 65 *vs.* 35 μg/L, SI units), FSH from 186.0 ± 19.5 (control) to 86.3 ± 13.9 ng/mL (GnRH; $P < 0.01$; 186 *vs.* 86 μg/L, SI units), and LH-bio from 0.41 ± 0.09 (control) to 0.13 ± 0.03 μg/mL (GnRH; $P < 0.05$; Figs. 2A and 3).

Follicular development

GnRH administration had a pronounced effect on ovarian follicular development and activity. In the mid- to late follicular phase (cycle days −6 to 0) there was a significant increase in E secretion (Fig. 4) secondary to GnRH administration. Integrated E secretion was 1302.2 ± 193.8 in the control cycle, whereas it increased to 2549.9 ± 543.7 (pg/mL)day in the GnRH cycle [$P < 0.05$; 4780 *vs.* 9360 (pmol/L)day, SI units]. Besides increased plasma E, there were more ovarian follicles during GnRH administration, as reflected by the number of preovulatory size follicles on the cycle day preceding ovulation (Table 3). On the day before ovulation in the control cycles, there was only one follicle with a mean diameter of 14 mm or more in five of the six subjects; in the GnRH cycles there were between one and five follicles (average of three) of preovulatory size. There was no difference in mean follicular size between cycles. There was a trend toward a shorter follicular phase in the GnRH cycles [12.3 ± 1.2 days (control) *vs.* 10.2 ± 0.8 days (GnRH)], which was not statistically significant. There was a spontaneous LH surge followed by ovulation in all cycles studied. The overall effect of GnRH administration was to increase the number of ovarian follicles that developed, with corresponding increases in E levels.

PRL levels

Plasma PRL levels were measured in samples obtained on a daily basis and during admissions. In cycle 1 (con-

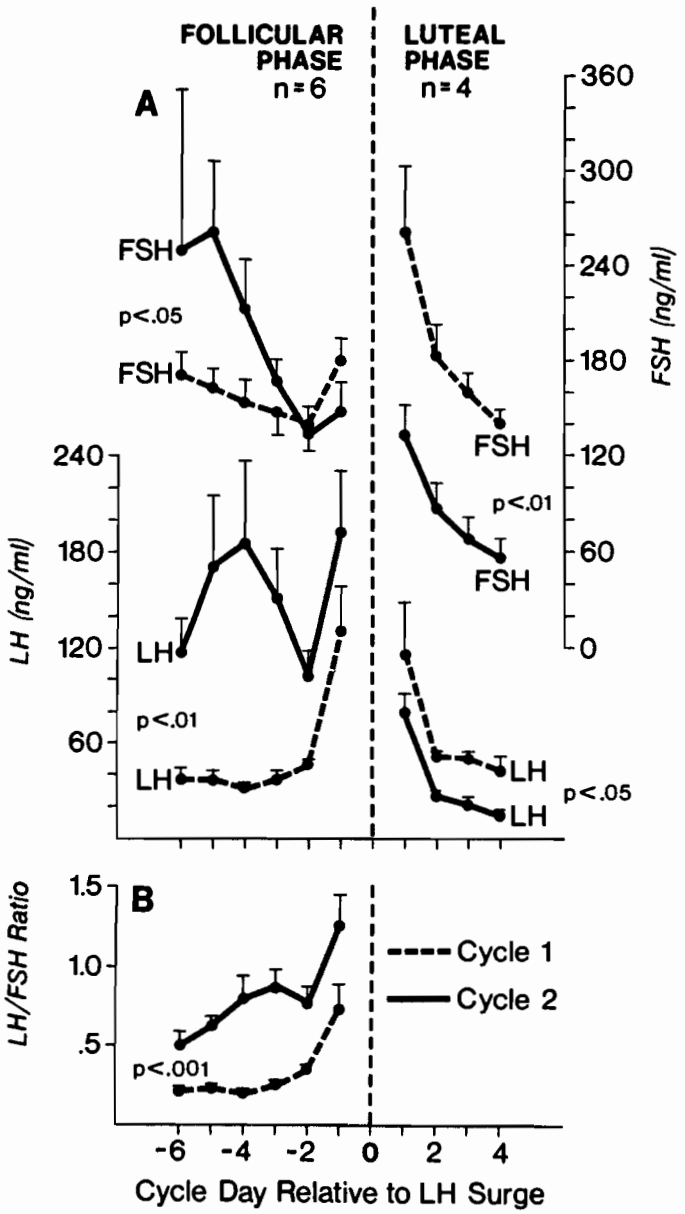

FIG. 2. A, Mean (± SEM) daily plasma LH and FSH levels during cycle 1 (control; - - -) and cycle 2 (GnRH; ——). SI unit conversion, 1 ng/mL = 1 μg/L. B, Mean (± SEM) daily plasma LH to FSH ratios in the follicular phase are indicated for cycle 1 (control; - - -) and cycle 2 (GnRH; ——).

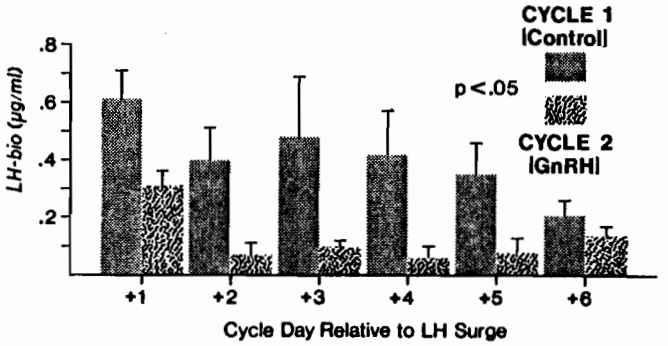

FIG. 3. Mean (± SEM) plasma LH-bio during the first 6 days of the luteal phase for cycle 1 (control) and cycle 2 (GnRH).

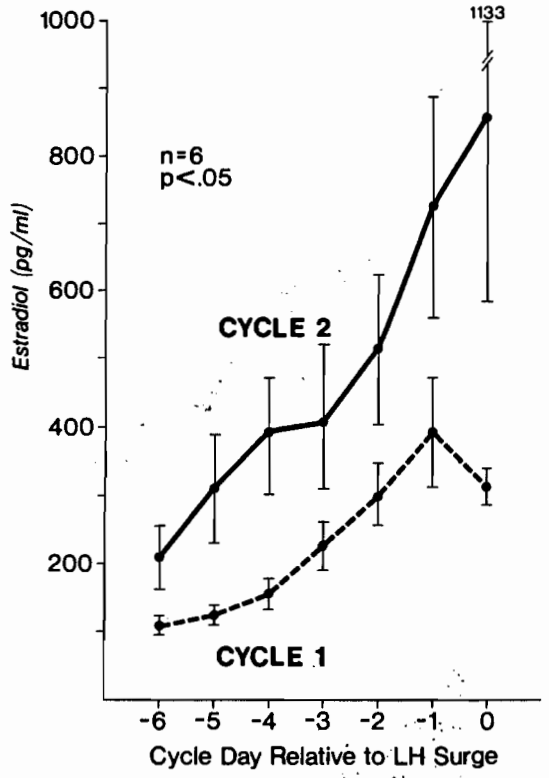

FIG. 4. Mean (± SEM) daily plasma E levels in cycle 1 (control) and cycle 2 (GnRH). SI unit conversion, 1 pg/mL = 3.67 pmol/L.

trol) the mean daily PRL level was 14.5 ± 0.7 ng/mL (15 μg/L, SI units), compatible with previously determined normal PRL levels in our laboratory. During GnRH administration in cycle 2, the mean daily PRL level increased to 19.4 ± 2.1 ng/mL ($P < 0.05$; 19 μg/L, SI units). The mean PRL level during the 12-h admission while receiving GnRH was increased as well (22.5 ng/mL; 22 μg/L, SI units). There were no differences in PRL levels in the respective luteal phases between control and GnRH cycles after GnRH administration was terminated (15.4 ± 2.0 *vs.* 16.8 ± 2.2 ng/mL; 15 *vs.* 17 μg/L, SI units).

Corpus luteum function

GnRH administration in the follicular phase led to changes in corpus luteum function in the ensuing luteal phase. GnRH administration was discontinued after the LH surge, when signs of ovulation were found in two or more follicles. The length of the ensuing luteal phase in the four subjects who did not receive hCG injections was reduced from 13.5 ± 0.3 days in the control cycle to 8.8 ± 1.7 days in the GnRH cycle ($P < 0.05$). Integrated luteal P levels in these same subjects were also reduced

TABLE 3. Number of preovulatory ovarian follicles

Patient	No. of follicles >14 mm in average diameter[a]	
	Cycle 1 (control)	Cycle 2 (GnRH)
A	1	2
B	2	5
C	1	2
D	1	4
E	1	3
F	1	1
Mean ± SEM	1.2 ± 0.2	2.8 ± 0.6[b]

[a] The reference day for follicle number for each woman was the day before ovulation (follicular collapse).

[b] $P < 0.05$ compared to control cycle.

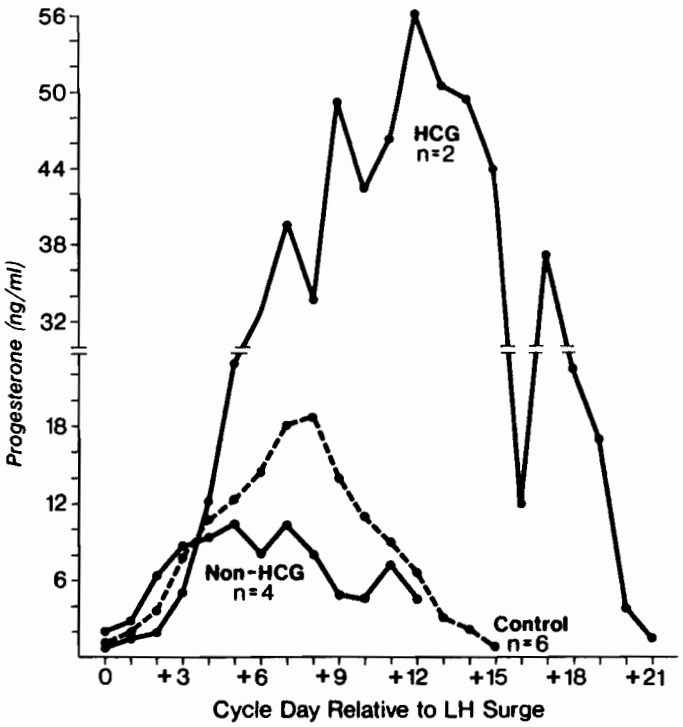

FIG. 5. Daily mean plasma P concentrations during the luteal phase in the control (cycle 1) and GnRH (cycle 2) cycles. The mean P curve in the two women who received luteal hCG treatment is presented as well. SI unit conversion, 1 ng/mL = 3.18 nmol/L.

from 151.6 ± 9.6 (ng/mL)day (control) to 65.7 ± 16.7 (ng/mL)day [GnRH; $P < 0.01$; Fig. 5; 482 *vs.* 209 (nmol/L)day, SI units]. Therefore, a shortened luteal phase with decreased P secretion resulted from the supraphysiological gonadotropin pulse frequency in the preceding follicular phase.

The two women who received supplemental hCG injections in the luteal phase of cycle 2 (GnRH) had luteal lengths of 18 and 21 days. The peak and integrated P levels were increased in the luteal phases exposed to hCG over levels observed in control cycles (Fig. 5).

Discussion

In this study, a relatively small GnRH dose administered at an increased frequency in the follicular phase resulted in marked changes in gonadotropin levels, follicular development, and corpus luteum function in normal women. The induced gonadotropin secretion pattern consisted of increased LH pulse frequency and LH pulse amplitude. This gonadotropin secretion pattern led to increases in baseline mean plasma LH and FSH levels, and an increase in the LH to FSH ratio in the mid- and late follicular phases of the GnRH cycles compared to control values. Folliculogenesis was increased subsequent to these gonadotropin changes; the ovaries of the women receiving GnRH demonstrated increased numbers of preovulatory follicles with corresponding increases in plasma E levels. A spontaneous gonadotropin surge occurred in all subjects, followed by ovulation. Despite multiple preovulatory follicles, a marked reduction in P secretion occurred in the luteal phase of these GnRH cycles. The basis for this profound decrease in P secretion was a marked decrease in LH levels after GnRH administration was terminated.

After ovulation of multiple follicles there are usually increased plasma levels of P from multiple corpora lutea throughout the luteal phase (17, 18). Plasma P levels in the luteal phase after ovulation induction with human menopausal gonadotropins are generally 2–4 times elevated over P levels found in spontaneous cycles. Considering that GnRH administration during this study induced the development of multiple preovulatory follicles with corresponding high plasma E levels, we expected to find increased plasma P levels in the GnRH cycles compared to those in spontaneous cycles. However, in the study subjects (after GnRH was discontinued), there was a significant decrease in luteal P secretion and the duration of the luteal phase compared to even spontaneous control cycles.

We previously reported that follicular phase LH pulse frequency is significantly increased in infertile women with out of phase endometrial biopsies in two spontaneous cycles (LPD) (6). Therefore, it appears there is an association between a rapid LH secretion pattern in the follicular phase and LPD. However, there were differences in the endocrine findings associated with decreased P secretion in spontaneous LPD compared to GnRH-induced LPD.

In spontaneous LPD, increased LH and PRL secretion rates with a decreased pulse amplitude have been noted throughout the follicular phase (19). Only a single follicle of normal dimensions with a corresponding normal preovulatory E level has been found in spontaneous LPD cycles (19). The decrease in P secretion after ovulation in spontaneous LPD is subtle but significant. The length

of the luteal phase is significantly decreased. Integrated P secretion is decreased, and while P continues to be secreted in a normal pulsatile fashion, the P pulses have decreased amplitude (19).

In GnRH-induced LPD the LH pulsatile secretion rate was accelerated in the follicular phase to a rate similar to that found in spontaneous LPD (EF, 30 pulses/24 h; LF, 27 pulses/24 h). In contrast to spontaneous LPD, in GnRH-induced LPD there were changes in follicular phase baseline LH and FSH as well as multiple preovulatory follicles. After ovulation the decrease in P secretion in the women receiving GnRH was more dramatic (less than half the normal P secretion), with a luteal phase duration of less than 10 days. In the induced LPD cycles, premature luteolysis could be attributed to decreased plasma LH levels, which may be the case in spontaneous LPD.

Perhaps more similarities in endocrine findings between spontaneous and induced LPD would have been found if the study design would have allowed GnRH administration at a rapid rate over several cycles or if the GnRH-induced pulse amplitude would have been lower. Nevertheless, despite the different findings in spontaneous and induced LPD, the association between a rapid LH secretion rate during follicular development and decreased P secretion after ovulation in both types of LPD remains a compelling finding. The fact that GnRH-induced LH secretion at a supraphysiological rate caused LPD in normal women still suggests that the rapid follicular LH pulse frequency in spontaneous LPD is part of the pathophysiology of this endocrine disease. Before this finding, the rapid follicular LH pulse rate in spontaneous LPD could easily have been dismissed as simply an effect of decreased P secretion in the previous cycle (3). Perhaps the more subtle decrease in P secretion in spontaneous LPD is associated with more subtle changes in luteal LH levels.

The overall effect of the induced gonadotropin secretion pattern was to elevate the baseline plasma concentrations of LH and FSH throughout the follicular phase. The increase in LH was greater than that in FSH. In the castrated rhesus macaque model of Wildt *et al.* (10) there was an increase in baseline LH and a decrease in baseline FSH with an increased GnRH pulse frequency. While the study we report here did not demonstrate a decrease in FSH, there was a highly significant increase in the LH to FSH ratio, as occurred in the nonhuman primate model of Wildt *et al.* (10). The failure to decrease baseline FSH with an induced increase in the gonadotropin pulse frequency also occurred when GnRH was administered to T-treated castrated male macaques (20). Therefore, the prevailing endocrine milieu of an animal (or person) may influence the nature of the pituitary response to changing GnRH pulse frequencies. An intact animal (or human) is even more complex, with multiple gonadal hormone feedback loops present; this may account for the different findings in relation to shifts in baseline gonadotropin levels among the various experimental models.

There was an abrupt decrease in pituitary LH secretion after a spontaneous LH surge and the subsequent discontinuation of GnRH administration. The physiological mechanism for the decreased gonadotropin secretion in the luteal phase remains speculative. GnRH administration at a relatively high frequency may have caused down-regulation of GnRH receptors on the pituitary gonadotropes (21). Another possibility for the decrease in luteal gonadotropin levels could be a temporary decrease in LH and FSH reserves secondary to depletion of the synthesis and storage capacity of the pituitary gonadotropes. A third possibility would be a temporary decrease in hypothalamic GnRH secretion in the luteal phase after discontinuation of exogenous GnRH exposure, this occurring secondary to the negative feedback effect of high levels of E in the follicular phase. Regardless of the mechanism, the acute decrease in immunoactive and bioactive LH in the luteal phase appeared to be the basis for the decrease in P secretion. In support of the concept that induced LPD was secondary to decreased LH support of the corpora lutea was the demonstration that exogenous hCG treatment in the luteal phase of subjects E and F resulted in increased levels of P secretion. The response of the women treated with hCG does not establish LH deficiency as the pathogenesis of LPD (hCG could have overridden more subtle defects), but it is consistent with this interpretation.

The effects of the changes in follicular gonadotropin levels on ovarian function were also notable. In the GnRH cycles, five of the six volunteers had two or more ovarian follicles develop with corresponding increases in plasma E concentrations. The increased plasma levels of gonadotropins, FSH in particular, over that which would be expected to occur in spontaneous cycles were the probable cause of multiple follicular development. Daily plasma FSH levels decreased in the LF phase during GnRH administration and thereby achieved a preovulatory nadir similar to the control cycles. The ovarian follicles developed rather quickly, as GnRH administration did not begin until several days into the follicular phase, yet there was a trend toward a short follicular phase length. The follicles that developed during GnRH administration appeared to be normal by the criteria of size and the quantity of E secreted; however, defects in follicular development, undetectable by the monitoring techniques engaged here, may have been present and caused the decreased P secretion after ovulation.

In this study, the normal gonadotropin secretory pattern was increased beyond the physiological range with

exogenous GnRH throughout most of the follicular phase. The dose of GnRH per pulse was relatively low (25 ng/kg); however, the increased frequency of administration resulted in the total daily dose falling within the normal GnRH dose range (36–80 μg/day) used for ovulation induction with iv GnRH (22–24). A total daily GnRH dose of 36–80 μg, administered at a physiological frequency (every 60–90 min) almost invariably leads to the ovulation of a single preovulatory size follicle in women undergoing ovulation induction. Despite the putative normal GnRH dose administered during this study, the response of the pituitary-ovarian axis in women receiving this regimen differed markedly from their normal menstrual cycle. These findings reaffirm that the frequency of pulsatile GnRH (with corresponding gonadotropin pulsatile secretion) is an important determinant of gonadal response *per se*, and the response is not simply a function of the GnRH dose. Reproductive abnormalities such as LPD can occur when the gonadotropin pulse frequency is outside the normal range.

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