Characterization of hormonal profiles during the luteal phase in regularly menstruating women

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Objective: To characterize the variability of hormonal profiles during the luteal phase in normal cycles.
Design: Observational study.
Setting: Not applicable.
Patient(s): Ninety-nine women contributing 266 menstrual cycles.
Intervention(s): The women collected first morning urine samples that were analyzed for estrone-3-glucuronide, pregnanediol-3-alpha-glucuronide (PDG), FSH, and LH. The women had serum P tests (twice per cycle) and underwent ultrasonography to identify the day of ovulation.
Main Outcome Measure(s): The luteal phase was divided into three parts: the early luteal phase with increasing PDG (luteinization), the midluteal phase with PDG ≥ 10 μg/mg Cr (progestation), and the late luteal phase (luteolysis) when PDG fell below 10 μg/mg Cr.
Result(s): Long luteal phases begin with long luteinization processes. The early luteal phase is marked by low PDG and high LH levels. Long luteinization phases were correlated with low E1G and low PDG levels at day 3. The length of the early luteal phase is highly variable between cycles of the same woman. The duration and hormonal levels during the rest of the luteal phase were less correlated with other characteristics of the cycle.
Conclusion(s): The study showed the presence of a prolonged pituitary activity during the luteinization process, which seems to be modulated by an interaction between P and LH. This supports a luteal phase model with three distinct processes: the first is a modulated luteinization process, whereas the second and the third are relatively less modulated processes of progestation and luteolysis. (Fertil Steril® 2017;108:175–82. ©2017 by American Society for Reproductive Medicine.)
Key Words: Luteal phase, menstrual cycle, luteinization, luteal deficiency

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There has been a recent emphasis on the continuum (1) that exists in hormonal profiles during the menstrual cycle. Given this spectrum of menstrual cycle variability, there seems to be no clear demarcation between the so-called normal and abnormal cycles. In addition, individual hormonal profiles in women of proven fertility are not uniform but differ considerably between women and depart from the standard hormone curve (2–6). Thus, further insights into the menstrual cycle physiology may be gained from observing the diversity of hormonal profiles and examining the reasons for ovulatory dysfunction, which may assist in managing infertility.

The present study focuses on the spectrum of hormonal profiles during the luteal phase. The quality of the luteinization process is essential for a successful implantation and for the maintenance of early pregnancy (7). The preovulatory LH surge is the

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stimulus for the luteinization process (8); however, the pituitary support of the luteal function is not limited to the preovulatory LH surge because LH continues to act through an endocrine feedback during the entire luteinization process (9). Despite the important implications of this mechanism in fertility, few studies have examined the hormonal profiles during the luteinization process in regularly menstruating women (10). Moreover, most published studies used the LH peak as reference day, which does not allow a full assessment of changes in LH levels during the early luteal phase.

Clinical and biochemical luteal phase deficiencies (11) are not uncommon among regularly menstruating women (12). Here, the definition of luteal phase deficiency is based on two criteria: a shortened luteal phase duration and a suboptimal luteal P level. Schliep et al. (12) found significantly lower LH and FSH levels across the cycle in women with luteal phase duration <10 days, while other investigators (13), with fewer cycles analyzed, reported lower midfollicular FSH levels but no difference in LH levels. We thus aimed to analyze, in our data set, the correlation between the length of the luteal phase and the levels of P, FSH, and LH.

Many investigators have correlated the length of the preovulatory phase with that of the luteal phase (14, 15). This is important because the treatment of a luteal phase deficiency might start by addressing the proper development of the follicle. This motivated our analysis of the relationship between the preovulatory phase and the luteal phase in normally menstruating women.

In the midnineties, a large observational study was carried out on normally fertile women; it included ultrasound-confirmed ovulation, daily urine hormone measurements, and self-assessment of cervical mucus and basal body temperature. Due to commercial disclosure agreements, the results regarding the luteal phase could not be published before the present study.

This study is a secondary analysis of a previously published report. It describes the diversity of hormonal profiles during the luteal phase and considers the following covariates: age, length of the preovulatory phase, day 3 hormonal levels, diameter of the preovulatory follicle, and length of the luteal phase. Moreover, special attention was given to the evolution of LH after the day of ovulation (as determined by ultrasound) to clarify the results presented in an earlier analysis or the same data (4).

MATERIALS AND METHODS

Subjects

The women were recruited between 1996 and 1997 from eight natural family planning clinics located in France, Italy, Germany, Belgium, and Spain. The inclusion criteria were women ages 19–45, with previous menstrual cycle lengths of 24–34 days. The exclusion criteria were a consistent history of anovulatory cycles, infertility or active hormonal treatment for infertility in the past 3 months, use of hormonal contraception or hormone therapy in the past 3 months, abnormal cycles (polycystic ovarian syndrome or luteal phase defect), hysterectomy, tubal ligation(s), and pelvic inflammatory disease. In addition, the study excluded runners and breastfeeding or postpartum mothers (<3 months). In the end, the study included 107 women who contributed 326 cycles (i.e., three cycles per woman, on average).

The study was approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Lyon). All the participants gave their written informed consent, and the study procedures were carried out in accordance with the Ethical Standards for Human Experimentation established by the Declaration of Helsinki.

Assessments

Demographics. The data collected included age, age at menarche, parity, past oral contraceptive use, and lifestyle habits such as smoking, diet, and physical activity (hours/wk), sleep duration (hours/d), and stress levels (subjective assessment). Height and weight were measured and the body mass index calculated.

Hormonal assays. The women collected daily samples of early morning urine (16) for quantitative analyses of estrone-3-glucuronide (E1G), pregnanediol-3-alpha-glucuronide (PDG), LH, and FSH. The aliquots were frozen at −20°C on the day of collection and assayed later for hormone detection using time-resolved fluorometric immunosorbent assays (Delfia, PerkinElmer). All the assays were run in duplicates, averaged, and adjusted for creatinine. As suggested by Collins et al. (17), the ratio of E1G to PDG was calculated; this ratio controls for the negative effect of urine concentration variability on hormone test results.

Serum P had to be collected on two defined occasions of each cycle (18): during the follicular phase, within a week after the end of menses and during the week that follows the end of the fertile phase as defined by a rise in basal body temperature. However, due to the practical difficulties in obtaining the samples within these timeframes, the samples were obtained at various points during early, mid, or late luteal phase. These samples were assayed for quantitative P detection using a time-resolved Europium-based fluorometric immunosorbent assay (Delfia, Perkin Elmer Wallac).

Ultrasound investigations. Serial transvaginal ovarian ultrasounds with follicle measurement were performed by a single physician per center. Ovarian scanning started on the first day women observed cervical mucus or when an LH surge was detected by LH home tests (Quidel Corporation), whichever came first. Scanning was performed every other day until a follicle reached 16 mm, then daily until evidence of ovulation (see further details in a previous publication [19]). The estimated day of ovulation as determined by ultrasound (USDO) was defined as the day of maximum follicular enlargement followed the next day by evidence of rupture.

Early, mid, and late luteal phase. The luteal phase was divided into three parts: the early luteal phase with increasing PDG (the luteinization process), the midluteal phase (the progestation process), and the late luteal phase (the luteolysis process). The threshold to separate these processes was a PDG = 10 μg/mg creatinine (Cr). Precisely, the luteinization lasted from the USDO (excluded) to the first day (excluded) with a PDG level $\geq$10 μg/mg Cr. The progestation process

included all days with PDG >10 μg/mg Cr. The luteolysis/regression process lasted from the first day at which PDG fell <10 μg/mg Cr to the last day of the cycle. No evidence-based threshold has been proposed in the literature to define a sufficient luteal phase. The threshold of 10 μg/mg Cr was arbitrarily chosen, this value being the average PDG value during the luteal phase in our data set and also the value close to the thresholds used by other authors.

Studied factors. The following factors were chosen for a study of their correlations with hormonal profiles during the postovulatory phase: age, length of the preovulatory (follicular) phase (from the first day of menses to the USDO included), early follicular phase hormonal data (represented by the average hormonal levels estimated at days 2, 3, and 4 of the cycle), diameter of the preovulatory follicle (the maximum diameter of the largest follicle observed by ultrasonography during the preovulatory–follicular–phase), and length of the postovulatory–luteal–phase (from the day after the USDO to the day before the next menses).

Outcome criteria. The following factors were chosen to represent the hormonal profiles during the postovulatory phase: the lengths of early, mid, and late luteal phase and the average hormonal levels during each of these three phases.

Evolution of LH after ovulation. In a previous analysis of the same data set, LH level was shown to be stable or even sometimes increasing after ovulation (as determined by ultrasound). To clarify this result, we estimated the trend in LH after ovulation in each cycle and defined a "relative rise" in LH after ovulation as the difference between LH levels 3 days after ovulation and on the USDO divided by the latter and multiplied by 100, that is, $[(LH_{USDO+3} - LH_{USDO})/LH_{USDO}] * 100$. This quantity is negative when LH decreases after the USDO and positive when it increases.

Statistical Analyses

To describe the luteal phase, the mean and SD of each of its characteristics was calculated. Hormonal profiles were graphically represented where each line represents the geometric average of hormonal levels calculated over the cycles belonging to each of the tercile groups: high, medium, and low values of the studied factor.

To assess the correlation between each studied factor and the outcome criteria, the following process was followed for each factor: [1] each cycle was classified into one of the three tercile groups according to the value of the studied factor; [2] the within-group geometric average of each outcome factor was calculated as well as the SEM; [3] the prediction of the outcome by the studied factor was tested using a linear mixed model to take into account the hierarchy (cycles within women). In the latter tests, the outcome criteria as well as hormonal predictors were log-transformed before the analysis of variance to achieve near-normal distributions.

Then, to assess the relationship between LH and the evolution of PDG during the early follicular phase, we plotted the evolution of the "relative rise" in LH according to the length of the early follicular phase. The statistical significance of the relationship between LH and the evolution of PDG during the early follicular phase was tested using a linear mixed regression.

Finally, we have calculated the portion of the variability explained by the between-subjects variability, using the R^2, known as a relevant summarizing statistic of mixed-effects models (20).

All statistical analyses were performed using the R software (R Version 3.2.3, 2015, The R Foundation for Statistical Computing). $P<.05$ was considered statistically significant.

To describe the luteal phase, the mean and the standard deviation of each of its characteristics was calculated. Hormonal profiles were graphically represented where each line represents the geometric average of hormonal levels calculated over the cycles belonging to each of the tercile groups: high, medium, and low values of the studied factor.

To assess the correlation between each studied factor and the outcome criteria, the following process was followed for each factor: i) each cycle was classified into one of the three tercile groups according to the value of the studied factor; ii) the within-group geometric average of each outcome factor was calculated as well as the standard error of the mean; iii) the prediction of the outcome by the studied factor was tested using a linear mixed model to take into account the hierarchy (cycles within women). In the latter tests, the outcome criteria as well as hormonal predictors were log-transformed before the analysis of variance to achieve near-normal distributions.

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All statistical analyses were performed using the R® software (R Version 3.2.3, 2015 The R Foundation for Statistical Computing). A P-value $<.05$ was considered for statistical significance.

RESULTS

Ovulation was confirmed by ultrasound in 283 out of all 326 cycles (87%) of the 107 women. The 17 cycles with luteal phases >17 days were considered as possible pregnancies and excluded. Thus, 266 cycles out of 326 (82%) of 99 women out of 107 (93%) were kept for the present analysis. The average length of these 266 cycles was 28.1 days (range, 22–44).

Demographic and Cycle Characteristics

The mean age of the 99 women was 32.5 years (range, 19–44). The women had a median of two previous pregnancies (range, 0–6). Their mean body mass index was 21.2 kg/m^2 (range, 17.1–28.3), and their first menses occurred on average at age 13.2 years (range, 9–17). Among these women, 11%

were current smokers and 14% had ≥3 h/wk physical activity.

Luteal Phase Description

Supplemental Table 1 shows the characteristics of the luteal phase. In only 3% of the cycles, the length of the luteal phase (as defined using the USDO) was <10 days. In only 8% of cycles, the maximum PDG level was below 10 μg/mg Cr. In 244 out of the 266 cycles (92%), PDG was ≥10 μg/mg Cr for at least 1 day.

In the 244 cycles where the maximum PDG level was ≥10 μg/mg Cr, the average ± SD length of the luteinization process (delay between the day after the USDO and the day before the first day with PDG level ≥10 μg/mg Cr) was 4.1 ± 1.7 days (range, 1–9). This process lasted 1–3 days in 38%, 4–5 days in 40%, and >5 days in 21% of the cycles.

The progestation process (days during which P was ≥10 μg/mg Cr) lasted 5.6 ± 3 days (range, 0–12).

The luteolysis/regression process (i.e., delay between the first day with PDG <10 μg/mg Cr and the last day of the cycle) lasted 3.2 ± 1.5 days (range, 1–6).

Correlations between Cycle Characteristics and Hormonal Profiles during the Postovulatory Phase

The supplemental tables provide all the results of the correlations between each studied factor and each outcome (i.e., characteristic of the postovulatory phase; Supplemental Tables 1–10). For simplicity, we have presented the main statistically significant correlations, as shown in Table 1 (results for age, preovulatory length, maximum follicle size, and luteal phase duration) and Table 2 (results for day 3 hormonal levels).

Age. Age did not significantly predict any luteal phase characteristic in our population.

Preovulatory phase length. The length of the preovulatory phase did not predict the length of the early, mid, or late-luteal phase. The significant differences for longer preovulatory phase length were a lower E1G value and a higher PDG value at ovulation, a lower E1G value during the early luteal phase, and a higher LH value during the periovulatory period and the midluteal phase.

Maximum follicle size. A high maximum follicle size was correlated with a high E1G level and a low PDG level at ovulation, and thus an almost doubled E1G/PDG ratio (Supplemental Fig. 1), and with a slightly higher LH level during the midluteal phase. Thus, the maximum follicle size was significantly associated with the hormonal level during the periovulatory period but did not predict the characteristics of the postovulatory phase. To be precise, early, mid, and late luteal phase lengths, urinary E1G and PDG levels, and serum P profiles were all similar in case of small, medium, or large preovulatory follicle size.

Luteal phase length. A long luteal phase was clearly correlated with a long early luteal phase (additional 2 days) but

TABLE 1

Some luteal phase characteristics according to preovulatory phase length, maximum follicle size, and luteal phase length.

		Preovulatory phase length			
Phase	**Variable**	**< 13 d**	**13 d to 16 d**	**≥ 17 d**	***P* value**
Periovulatory	E1G (ng/mg Cr)	47.4 (0.22)	43.66 (0.15)	38.7 (0.25)	.01
Periovulatory	PDG (μg/mg Cr)	2.11 (0.21)	2.7 (0.13)	3.07 (0.23)	<.01
Periovulatory	LH (mIU/mg Cr)	10.06 (0.32)	11.74 (0.18)	12.95 (0.3)	.02
Early luteal	E1G (ng/mg Cr)	27.25 (0.22)	25.02 (0.15)	24.15 (0.3)	.05
Midluteal	LH (mIU/mg Cr)	2.41 (0.28)	2.77 (0.18)	3.32 (0.35)	.02
		Maximum follicle size			
		< 20 mm	**20 mm–23 mm**	**≥ 24 mm**	
Periovulatory	E1G (ng/mg Cr)	35.24 (0.23)	43.86 (0.16)	49.53 (0.21)	<.01
Periovulatory	PDG (μg/mg Cr)	3.02 (0.2)	2.44 (0.16)	2.58 (0.18)	.03
Midluteal	LH (mIU/mg Cr)	2.64 (0.27)	2.86 (0.22)	2.87 (0.26)	.05
		Luteal phase length			
		< 13 d	**13 d or 14 d**	**15 d or 16 d**	
Periovulatory	PDG (μg/mg Cr)	3.32 (0.2)	2.61 (0.13)	2.12 (0.22)	<.01
Early luteal	Length (days)	2.73 (0.23)	3.83 (0.13)	4.83 (0.2)	<.01
Early luteal	PDG (μg/mg Cr)	5.17 (0.19)	4.96 (0.11)	4.3 (0.18)	<.01
Early luteal	LH (mIU/mg Cr)	7.37 (0.31)	10.19 (0.18)	13.07 (0.28)	<.01
Midluteal	Length (days)	4.58 (0.23)	5.38 (0.16)	5.78 (0.24)	.02
Midluteal	PDG (μg/mg Cr)	13.31 (0.15)	13.83 (0.11)	13.93 (0.15)	.03
Midluteal	LH (mIU/mg Cr)	2.31 (0.33)	2.85 (0.18)	3.31 (0.29)	.01
Late luteal	PDG (μg/mg Cr)	6.04 (0.17)	6.04 (0.12)	5.72 (0.17)	.01
Late luteal	FSH (mIU/mg Cr)	0.85 (0.37)	1.05 (0.28)	1.23 (0.41)	.01
Late luteal	LH (mIU/mg Cr)	1.29 (0.32)	1.73 (0.19)	2.21 (0.32)	<.01

Note: Values are geometric averages, with SEM in parentheses. *P* values show the results of mixed models. Only the significant results are presented.

Ecochard. Hormonal profiles during the luteal phase. Fertil Steril 2017.

TABLE 2

Some luteal phase characteristics according to day 3 hormonal levels.

Phase	Variable	< 7.5 ng/mg Cr	7.5 to 11.4 ng/mg Cr	≥11.5 ng/mg Cr	*P* value
			E1G on day 3 of the cycle		
Periovulatory	E1G (ng/mg Cr)	28.42 (0.21)	43.4 (0.16)	62.06 (0.18)	< .01
Periovulatory	PDG (μg/mg Cr)	2.19 (0.2)	2.65 (0.17)	3.1 (0.16)	< .01
Periovulatory	FSH (mIU/mg Cr)	3.05 (0.29)	3.38 (0.33)	3.98 (0.28)	.04
Periovulatory	LH (mIU/mg Cr)	10.63 (0.24)	11.53 (0.25)	12.65 (0.23)	.05
Early luteal	Length (days)	4.31 (0.18)	3.73 (0.17)	3.35 (0.19)	.01
Early luteal	E1G (ng/mg Cr)	16.9 (0.23)	25.21 (0.16)	35.81 (0.17)	< .01
Early luteal	PDG (μg/mg Cr)	4.24 (0.17)	5.01 (0.14)	5.25 (0.14)	< .01
Midluteal	E1G (ng/mg Cr)	18.77 (0.22)	24.67 (0.16)	33.75 (0.17)	< .01
Late luteal	E1G (ng/mg Cr)	12.95 (0.21)	17.93 (0.15)	23.32 (0.18)	< .01
Late luteal	PDG (μg/mg Cr)	5.44 (0.16)	6.2 (0.14)	6.24 (0.14)	.05
			PDG on day 3 of the cycle		
		< 1.7 μg/mg Cr	**1.7 to 2.4 μg/mg Cr**	**≥2.5 μg/mg Cr**	
Periovulatory	E1G (ng/mg Cr)	34.53 (0.19)	48.08 (0.19)	49.49 (0.2)	< .01
Periovulatory	PDG (μg/mg Cr)	1.93 (0.17)	2.67 (0.17)	3.64 (0.16)	< .01
Periovulatory	FSH (mIU/mg Cr)	3.14 (0.32)	3.39 (0.26)	3.95 (0.33)	.01
Periovulatory	LH (mIU/mg Cr)	10.76 (0.23)	12.44 (0.22)	11.79 (0.27)	.03
Early luteal	Length (days)	4.49 (0.18)	3.65 (0.16)	3.26 (0.19)	< .01
Early luteal	E1G (ng/mg Cr)	20.34 (0.21)	26.82 (0.18)	29.97 (0.19)	.01
Early luteal	PDG (μg/mg Cr)	4.36 (0.15)	4.84 (0.14)	5.41 (0.16)	< .01
Midluteal	Length (days)	4.11 (0.22)	5.57 (0.18)	6.19 (0.19)	< .01
Midluteal	PDG (μg/mg Cr)	13.08 (0.13)	13.67 (0.14)	14.36 (0.13)	.01
Late luteal	Length (days)	3.57 (0.18)	2.82 (0.2)	2.33 (0.2)	< .01
Late luteal	PDG (μg/mg Cr)	5.2 (0.14)	6.17 (0.14)	6.71 (0.14)	< .01
			FSH on day 3 of the cycle		
		< 2 mIU/mg Cr	**2–4.1 mIU/mg Cr**	**≥4.2 mIU/mg Cr**	
Periovulatory	FSH (mIU/mg Cr)	1.89 (0.31)	3.49 (0.27)	6.18 (0.23)	< .01
Periovulatory	LH (mIU/mg Cr)	8.89 (0.25)	11.5 (0.22)	15.16 (0.23)	< .01
Early luteal	FSH (mIU/mg Cr)	0.92 (0.33)	1.85 (0.28)	2.7 (0.25)	< .01
Midluteal	FSH (mIU/mg Cr)	0.55 (0.37)	0.9 (0.26)	1.43 (0.25)	< .01
Late luteal	FSH (mIU/mg Cr)	0.64 (0.37)	1 (0.31)	1.69 (0.27)	< .01
Late luteal	LH (mIU/mg Cr)	1.34 (0.27)	1.67 (0.25)	2.17 (0.25)	.01
			LH on day 3 of the cycle		
		< 2.2 mIU/mg Cr	**2.2–3.9 mIU/mg Cr**	**≥4 mIU/mg Cr**	
Periovulatory	FSH (mIU/mg Cr)	2.46 (0.32)	3.5 (0.3)	4.86 (0.25)	< .01
Periovulatory	LH (mIU/mg Cr)	7.76 (0.25)	11.85 (0.22)	17.05 (0.2)	< .01
Early luteal	FSH (mIU/mg Cr)	1.2 (0.33)	1.81 (0.29)	2.17 (0.3)	< .01
Early luteal	LH (mIU/mg Cr)	6.33 (0.23)	11.22 (0.2)	13.92 (0.24)	< .01
Midluteal	FSH (mIU/mg Cr)	0.67 (0.34)	0.91 (0.29)	1.17 (0.29)	< .01
Midluteal	LH (mIU/mg Cr)	1.77 (0.23)	3.05 (0.22)	4.04 (0.23)	< .01
Late luteal	FSH (mIU/mg Cr)	0.74 (0.38)	1.06 (0.31)	1.41 (0.3)	< .01
Late luteal	LH (mIU/mg Cr)	1.04 (0.26)	1.9 (0.22)	2.51 (0.25)	< .01

Note: Values are geometric averages, with SEM in parentheses. *P* values show the results of mixed models. Only the significant results are presented.

Ecochard. Hormonal profiles during the luteal phase. Fertil Steril 2017.

less clearly correlated with a long midluteal phase (+1.2 days). It was also correlated with a low PDG level at ovulation, a low PDG level during the early luteal phase, a high LH level (almost doubled) during the early luteal phase, and a high LH level during the mid and late luteal phases. Thus, a long luteal phase was clearly associated with both a delayed increase of PDG and a higher LH level during the luteal phase (Fig. 1).

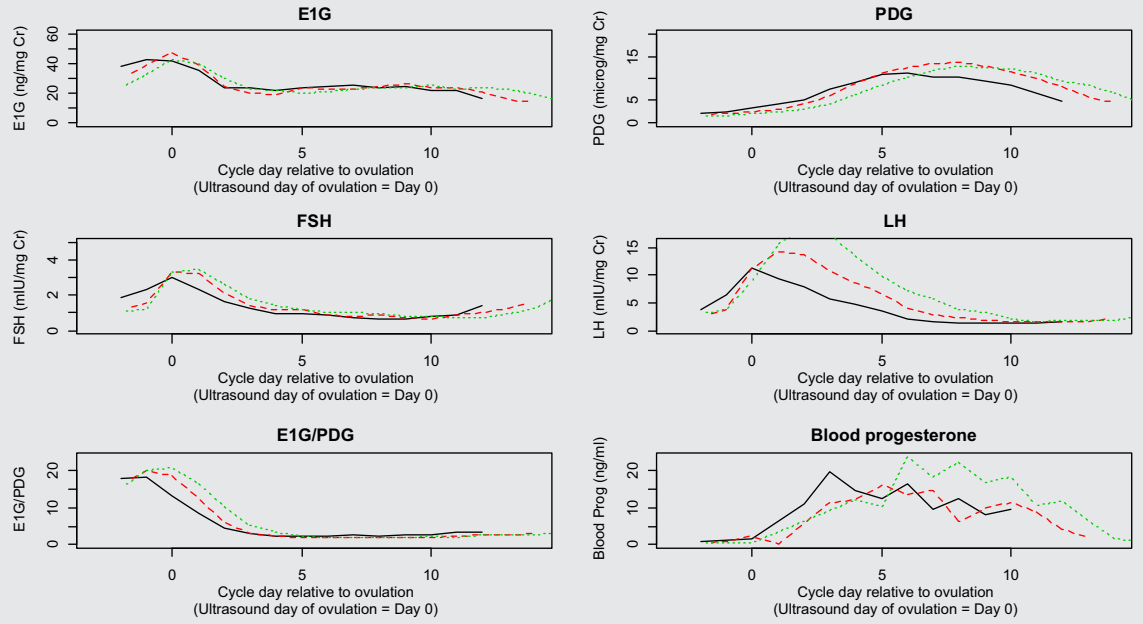
Day 3 hormonal levels. High day 3 level of E1G, PDG, FSH, and LH were all predictors of high FSH and LH at ovulation.

Moreover, high E1G on day 3 of the cycle was a predictor of [1] high E1G and PDG at ovulation; [2] high E1G during early, mid, and late luteal phases; [3] high PDG during early and late luteal phases; and [4] short early luteal phase.

High PDG on day 3 of the cycle was a predictor of [1] high E1G and PDG at ovulation; [2] high PDG during early, mid, and late luteal phases; and [3] long midluteal phase but short early and late luteal phase.

High FSH on day 3 of the cycle was a predictor of [1] high FSH during early, mid, and late luteal phases; and [2] high LH during the late luteal phase.

FIGURE 1



Variations in hormonal profiles according to the length of the luteal phase (*continuous black line*, <13 days; *red dashed line*, 13–15 days; *green dotted line*, 15 or 16 days). Each line represents the geometric average of hormonal levels calculated over, respectively, 70, 132, and 64 cycles.

Ecochard. Hormonal profiles during the luteal phase. Fertil Steril 2017.

High LH on day 3 of the cycle was a predictor of high FSH and LH during early, mid, and late luteal phases.

LH during the Early Luteal Phase

Three days after ovulation, the LH level was lower than that measured on the USDO in 52% of the cycles. In the remaining 48%, LH level increased after the USDO. This increase was significantly ($P<.01$) more frequent in cycles with long early luteal phase (luteinization process). As shown in Figure 2, there is a linear correlation between the pattern of LH increase (as measured by the "relative rise" defined above) and the length of the luteinization process.

Intra- and Interwoman Variability of the Luteal Phase

Supplemental Table 10 presents the R^2 coefficient for each characteristic of the luteal phase. This coefficient represents the proportion of interwoman variability. It is noticeable that the minimum is observed for early luteal phase length (0.12, i.e., 12% of the variability of this quantity is observed between women). Conversely, it can be said that 88% of the variability of early luteal phase length is reflected in the cycles of the same woman. This suggests that the length of the luteinization process may vary substantially within a particular woman's cycles. The PDG variability follows the same pattern: the PDG is highly variable between cycles of a particular woman (80% of the variability is observed between cycles of a particular woman, and only 20% of the variability is represented between women).

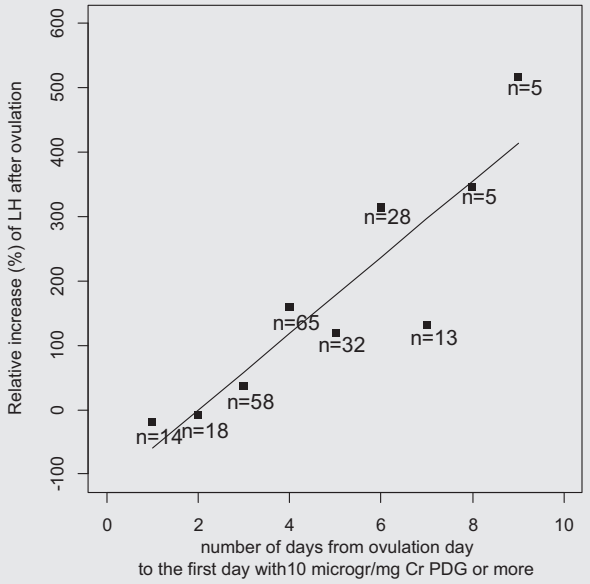
DISCUSSION

The present characterization of the hormonal profiles during the luteal phase provides a better insight into the physiologic dynamics of normal cycles. Long preovulatory (follicular) phases were not significantly correlated with short luteal phases nor with low PDG levels or a long early luteal phase (i.e., a slow luteinization process). High E1G or PDG levels on day 3 of the cycle were good predictors of a short early luteal phase (i.e., a fast luteinization process) and high E1G and PDG levels during the periovulatory phase. LH increased after ovulation in about half the cycles.

There are few predictors of the length of the luteal phase in the present data set. Longer early luteal phases (slow luteinization) were strongly correlated with higher increases of LH, and longer luteal phases had slower luteinization processes (i.e., longer early luteal phase), frequently marked by clear increases of LH after ovulation and a delayed and protracted increase of PDG. This trend was observed not only in urine (PDG) but also in blood (P). Most of the variability of the length of the early luteal phase is observed between cycles; the variability between women is lower than the variability observed between cycles of a same woman. Larger follicles at ovulation were associated with higher E1G levels at ovulation but did not predict the characteristics of the postovulatory phase.

The higher frequency of short luteal phases in cycles with long preovulatory (follicular) phases is a well-known observation. It is interesting to note that although this was observed within the context of ovulatory dysfunction (21, 22), it is also present in regularly menstruating women (23).

FIGURE 2



Relative increase of LH after ovulation according to the number of days from the ovulation day (excluded) to the first day with PDG $\geq$10 μg/mg Cr. Each point represents the average of LH over all cycles having the same number of days from the ovulation day to the first day with $\geq$10 μg/mg Cr PDG.

Ecochard. Hormonal profiles during the luteal phase. Fertil Steril 2017.

This reflects the so-called continuum of variability of hormonal profiles (1). The length of the menstrual cycle has some utility as an indicator of cumulative hormonal exposure (24). Nevertheless, it would not be clinically relevant to make assumptions about the "normality" of the menstrual cycle simply on cycle length observations because both the follicular and the luteal phase do vary. Age-related changes may also create confusion: in many women approaching menopause, the follicular phase lengthens and the luteal phase shows deficiency, without clear causal effect of inappropriate follicular development on the luteal phase. Finally, in a pathological context, such as polycystic ovary syndrome, both a long preovulatory phase and a short luteal phase are common, again, without clear causal relationship (25). In our data, advanced age was not a predictor of shorter luteal phase but only, as expected, a predictor of higher FSH levels during the late luteal phase. In these data, the absence of correlation between age and the length of the luteal phase may be easily explained by the rather young ages of the women.

Our results regarding the good predictive values of day 3 E1G and PDG levels on the characteristics of the luteal phase are important: high E1G or high PDG on the third day of the cycle predicted a significantly faster rise in PDG during the early luteal phase (i.e., a faster luteinization process). This finding was not expected; it needs to be confirmed by further studies. If confirmed, this finding will have some implications for potential fertility evaluation and treatments.

We observed only short luteal phases (<10 days) in 3% of the cycles analyzed. In their recent study on luteal phase deficiency in regularly menstruating women, Schliep et al. (12) observed 9% of cycles with a clinical luteal phase deficiency (<10 days). Luteal deficiency, based on clinical diagnostic without a precise definition, was one of the exclusion criteria of our study. Thus, we cannot assume our numbers reflect the frequency of short luteal phases in the general population. This is a significant limitation of our data set. We also note that we excluded 17 women for presumed pregnancy (luteal phase longer than 17 days): this is another limitation of our study because some of these cycles could be outliers without representing pregnancy. Thus, our study does not describe two of the most relevant groups of patients, those with an exceptionally long luteal phase, who were excluded as potentially pregnant, as well as those that had a "luteal phase defect."

Our results suggest that the maximum follicle diameter may be considered as an indirect indicator of the follicle development process. In our sample, larger follicles were associated with higher periovulatory levels of E1G. These results are similar to those of other investigators (21) who also observed smaller follicles in case of luteal phase deficiency. Higher E1G may reflect the activity of a larger follicle. However, in a physiological context, follicle size may not have an impact on the outcome of the luteinization process because our data showed no significant differences in hormonal profiles between the mid and the late luteal phase and, specifically, no difference in PDG levels, despite different follicle sizes. This would lead us to conclude that follicle size itself does not have a great impact on the quality of the corpus luteum in physiological contexts. It remains to be shown whether improving the follicular processes would affect corpus luteum activity. Nevertheless, our observations on normal cycles may not be as relevant to pathological ovarian dysfunction or cycle abnormalities.

In agreement with previous results obtained on the same database (4), LH increased after ovulation in nearly one out of two cycles. Longer early luteal phases (slow luteinization) were strongly correlated with higher increases of LH. Our findings support a luteal phase model that includes three processes: [1] a luteinization process during the early luteal phase in which LH and PDG interact in the development of the corpus luteum; [2] a progestation process during the midluteal phase in which the corpus luteum produces P in amounts >10 μg/mg Cr to support a potential pregnancy; and [3] a regression or luteolysis process during the late luteal phase in which the corpus luteum regresses, P levels decrease, and the endometrial lining is sloughed off, leading to menses. We have shown that the luteinization process has a variable length and may be predicted by day 3 hormonal levels, whereas the progestation and luteolysis processes have relatively fixed lengths and are independent of the characteristics of the preovulatory phase. Generally, when the luteinization process is long, PDG begins at a low level and LH remains high. It is possible that this high LH represents the central control required for promoting adequate luteinization, but this may require a longer luteinization period. Also, in the progestation and regression processes, PDG has high maximal values when the overall luteal phase is long. Comparing the latter length with the estimated day of implantation (day 8 to 10, on average) (26), we may speculate that, in most cases,

PDG reaches 10 μg/mg Cr or more before the implantation window.

Although less variable in length than the follicular phase, the variability of the luteal phase seems to be stemming from the variability of the early luteal phase, or luteinization process. When the corpus luteum does not produce enough P, a negative feedback to the hypothalamus may cause an increase in LH to further luteinize the corpus luteum; then P increases further. After this first luteinization process, the rather fixed lengths of the other two phases are compatible with the assumption that the functional lifespan of the corpus luteum may depend on paracrine and autocrine mechanisms rather than on the pituitary-ovarian axis alone (10).

Conclusions

Our results provide some evidence in favor of a prolonged pituitary activity during the luteinization process in which LH changes in the early luteal phase are likely modulated by P levels. There is utility in conceptualizing the luteal phase as three distinct phases. Our results are compatible with a luteal phase model that includes three processes: the first is a modulated luteinization process, whereas the second and the third are relatively less modulated processes of progestation and luteolysis.

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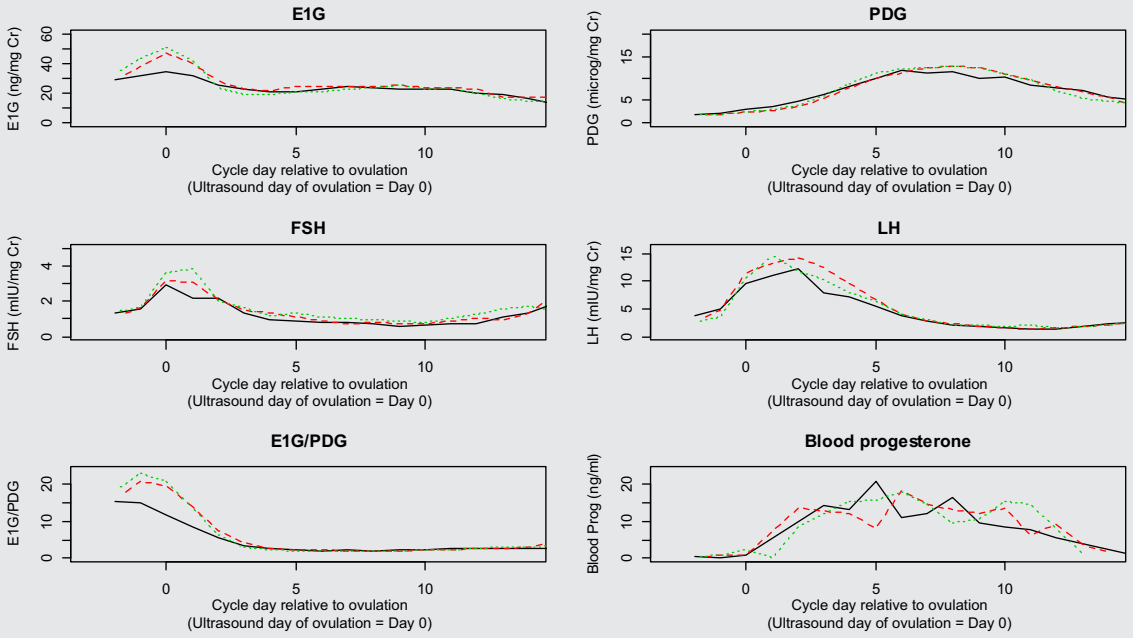
SUPPLEMENTAL FIGURE 1



Variations in hormonal profiles according to the maximum follicle size at ovulation (*continuous black line*: <20 mm, *red dashed line*: 20–24 mm, *green dotted line*: >24 mm). Each line represents the geometric average of hormonal levels calculated over respectively 71, 105, and 88 cycles.

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