ORIGINAL ARTICLE: REPRODUCTIVE ENDOCRINOLOGY

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;■:■–■. ©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 stimulus for the luteinization process (8); however, the pituitary support of the

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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 ≥ 10 μg/mg Cr. The progestation process included all days with PDG >10 μg/mg Cr. The luteolysis/