Steroids 101 (2015) 125–129

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Random serum progesterone threshold to confirm ovulation

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ARTICLE INFO

Article history:
Received 12 January 2015
Received in revised form 18 June 2015
Accepted 18 June 2015
Available online 22 June 2015

Keywords:
Menstrual cycle
Progesterone
Ovulation
Infertility
Fertility awareness methods
Ultrasound

ABSTRACT

Background: Serum progesterone (P) rises after ovulation in the luteinisation process.
Objective: To identify an accurate progesterone threshold to confirm ovulation in the assessment of a woman's fertility.
Methods: In a secondary analysis of an observational European multicentre study, this study included 107 women over 326 menstrual cycles and tracked daily first morning urine (FMU), changes in observed cervical mucus discharge, serum progesterone, and ultrasonography to identify the day of ovulation. A serum progesterone level was available for 102 women over a total 260 cycles with one or two P levels per cycle.
Results: It was found that a single serum P $\geqslant$ 5 ng/ml is highly specific with a specificity of 98.4 (95% CI 96.0–99.5), with a sensitivity of 89.6 (95% CI 85.2–92.9).
Conclusion: A random serum progesterone level $\geqslant$5 ng/ml confirms ovulation. This may be of use for clinicians wanting to confirm that ovulation has occurred.

1. Introduction

Confirmation of ovulation is an essential component in the evaluation of women experiencing fertility problems. Chronic anovulation is associated with increased risks of infertility, endometrial cancer, and osteoporosis [1]. While daily transvaginal ultrasound is the gold standard for documenting ovulation, it is too invasive or expensive to be used on a routine basis. Alternatively, a single mid-luteal phase serum progesterone level greater than 3 ng/ml has long been proposed as the second best indication of ovulation [2,3]. Nevertheless, it is well known that there is variability in random single blood samples for progesterone because levels can increase in response to the LH pulsations occurring after ovulation, which in turn is determined by the pulsatility of gonadotropin-releasing hormone (GnRH) secretion by the hypothalamus [8]. For this reason, we attempted to determine the lowest progesterone threshold to identify ultrasound-confirmed ovulation.

In a previous study, we identified a method to confirm ovulation using urinary pregnanediol measurements [4]. While urinary measurements can be done in a home setting, a single random urine test may not be as accurate as a single random serum measurement in confirming ovulation. Thus, in the present study we identify a single random serum progesterone threshold to confirm ovulation. A single value that would minimize the misclassification of ovulation would be clinically useful in the assessment of a woman's fertility. The main goal of this study was thus to achieve a low false positive rate, that is, highest specificity, to ensure the confirmation of ovulation.

2. Methods

2.1. Patients

Patients were recruited from 1996 to 1997 from eight natural family planning clinics in France, Italy, Germany, Belgium, and Spain as previously reported [4]. A database of information was created but due to legal–commercial disclosure agreements with the funding company (Quidel Corporation), the results regarding

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http://dx.doi.org/10.1016/j.steroids.2015.06.013
0039-128X/

the role of serum progesterone in confirming post-ovulatory infertility could not be published until now. The inclusion criteria consisted of women aged 19–45 years with previous menstrual cycle lengths of 24–34 days. Exclusion criteria consisted of women with a consistent history of anovulatory cycles, infertility or active hormonal treatment of infertility in the past 3 months, use of hormonal contraception or hormonal replacement in the past 3 months, abnormal cycles (polycystic ovary syndrome or luteal defect), hysterectomy, tubal ligation(s), or pelvic inflammatory disease. In addition, runners and breastfeeding or postpartum mothers (<3 months) were excluded. A total of 107 women were finally included, contributing an average of three cycles. The study examined 326 cycles which have been analyzed in other studies [5]. The study was approved by the local Ethics Committee (Comite Consultatif de Protection des Personnes dans la Recherche Biomedicale de Lyon). Each of 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.

2.2. Demographics

Data collected from patients included information on age, age at menarche, parity, past oral contraceptive use, lifestyle habits, such as smoking, diet, and physical activity (h/week), sleep duration (h/-day), and stress levels (subjective assessment). Height and weight were measured and body mass index (BMI) calculated.

2.3. Hormonal investigations

The women collected first morning urine samples daily, which were stored frozen until assayed in the laboratory for quantitative hormone detection of estrone-3-glucuronide (E3G), pregnanediol-3a-glucuronide (PDG), FSH, and LH with the use of time-resolved fluorometric immunosorbent assays (Delfia). All samples from each woman were tested in duplicate in the same assay and the results were adjusted for creatinine (Cr). Venous samples for progesterone were collected on two defined occasions during each participant's menstrual cycle in order to capture both the pre and post ovulatory phases. The first sample was collected at any point during a 7-day period following the last day of menses to establish a baseline progesterone value. The second sample was collected at any point during a 7-day period following the end of the fertile phase as defined by a rise (more than 3 days) in basal body temperature. If the participant was unable to interpret her temperature, the study site coordinator was responsible for determining the appropriate day based on the participant's usual mid-luteal cycle length. Each blood draw consisted of 20 ml of whole blood and it was collected in 10 ml Vacutainer tubes (without anticoagulant). Serum separation filters were provided to facilitate the separation of the serum from the clotted whole blood without the need for centrifugation. The serum was aliquoted into 2 ml volumes in storage tubes and frozen at less than -20° Celsius. All samples from each of the participating women were then frozen and shipped to the laboratory for testing. Serum samples were then assayed for quantitative hormone detection of progesterone using a time-resolved Europium based fluorometric immunosorbent assays (Delfia; Perkin Elmer-Wallac, Waltham, MA, USA). Samples were thawed and then tested in triplicates in the same assay. Briefly, antibodies to progesterone were immobilized in 96-well ELISA plates followed by a blocking step. Samples and standards were mixed with a fixed amount of Europium labeled progesterone, added to the wells of the microtiter plates, and incubated for 1–2 h. Unbound progesterone, labeled and unlabeled, was then washed off and progesterone levels in the wells were determined using a time resolved fluorescence reader measuring the amount of Europium labeled progesterone in the wells. Interassay variations were negligible.

2.4. 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 Corp.), whichever came first. Scanning was performed every other day until a follicle reached 16 mm and then daily until evidence of ovulation (the ultrasound day of ovulation, US-DO). Details regarding ultrasound investigations were previously published [6].

2.5. Phases of the cycle

The first day of the menstrual cycle was self-reported by the women. This was defined as the first day of the menstrual period where the woman observed bright red blood. Brown spotting was not considered to be a menstrual period. For the purposes of this study, the menstrual cycle was divided into three phases: the latent phase, the fertile window, and the luteal phase. The latent phase is from the first day of the cycle to the day before the fertile window. The biological fertile window based on pregnancy probabilities has been shown to be a 6-day period ending on the day of ovulation [7]. The luteal phase is from the day after the US-DO until the day before the first day of the next menses. Cycles with a luteal phase of >17 days were considered to be possible pregnancies. Proven pregnancies were defined by a positive urine beta-hCG test.

2.6. Statistical analysis

The characteristics of the women who participated in the study and provided blood samples to measure progesterone were described by the mean, standard error of the mean and range, or proportion for binary variables. The specificity was calculated as the proportion of cycles with a negative progesterone test, i.e., $P < 5$ ng/ml, during the potentially fertile phase, i.e., from the first day of menses up to and including the ovulation day as defined by ultrasound. The sensitivity was calculated as the proportion of cycles with a positive progesterone test, i.e., $P \geqslant 5$ ng/ml, during the post-ovulatory infertile phase. Specificity and sensitivity were provided as well as their 95% confidence interval. A Receiver Operating Characteristic (ROC) curve was drawn to exhibit the relationship between false positives, in the *x*-axis, i.e., 1-specificity, and true positives *y*-axis, i.e., sensitivity. All statistical analyses were performed using R® software (R Version 3.0.0, 2013 The R Foundation for Statistical Computing).

3. Results

The characteristics of women who participated in the study and provided results for this analysis are shown in Table 1. Serum progesterone values are shown in Table 2 and Fig. 1. The serum values were available in 260 of the 283 cycles (92%) with a known ovulation day established using ultrasound (US-DO): 2 days of values for 250 cycles and 1 day of values for 10 cycles for a total of 510 days with results. These measurements were obtained throughout the menstrual cycle as follows: 128 (25%) during latency phase, 131 (26%) during the biological fertile window and 251 (49%) during the post-ovulatory phase. The specificity and sensitivity of the P test in a range from 0.15 to 51.71 ng/mL is depicted in the ROC curves in Fig. 2. The ideal concentration threshold in a serum sample for P positivity was found to be $\geqslant 5$ ng/ml.