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Use of urinary pregnanediol 3-glucuronide to confirm ovulation ☆

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ABSTRACT

Objective: Urinary hormonal markers may assist in increasing the efficacy of Fertility Awareness Based Methods (FABM). This study uses urinary pregnanediol-3a-glucuronide (PDG) testing to more accurately identify the infertile phase of the menstrual cycle in the setting of FABM.
Methods: Secondary analysis of an observational and simulation study, multicentre, European study. The study includes 107 women and tracks daily first morning urine (FMU), observed the changes in cervical mucus discharge, and ultrasonography to identify the day of ovulation over 326 menstrual cycles. The following three scenarios were tested: (A) use of the daily pregnandiol-3a-glucuronide (PDG) test alone; (B) use of the PDG test after the first positive urine luteinizing hormone (LH) kit result; (C) use of the PDG test after the disappearance of fertile type mucus. Two models were used: (1) one day of PDG positivity; or (2) waiting for three days of PDG positivity before declaring infertility.
Results: After the first positivity of a LH test or the end of fertile mucus, three consecutive days of PDG testing over a threshold of 5 μg/mL resulted in a 100% specificity for ovulation confirmation. They were respectively associated an identification of an average of 6.1 and 7.6 recognized infertile days.
Conclusions: The results demonstrate a clinical scenario with 100% specificity for ovulation confirmation and provide the theoretical background for a future development of a competitive lateral flow assay for the detection of PDG in the urine.

1. Introduction

Since the mid-20th century, urinary hormone assays have been proposed to help identify the fertile phase of the menstrual cycle [1,2]. These assays can be used by women wishing to postpone pregnancy by using Fertility Awareness Based Methods (FABM). Three urinary hormonal testing methods have long been proposed in scientific literature to help identify the ovulatory period: oestrone-3-glucuronide (E1G), pregnanediol-3a-glucuronide (PDG), and luteinizing hormone (LH) [3,4]. In addition to urinary markers, cervical mucus is one of the most widely used biological markers for self-estimating the beginning and end of the fertile phase in a menstrual cycle [5–7]. Furthermore, two clinical indicators of ovulation are broadly known, the mucus peak symptom [6,8–11] and the basal body temperature (BBT) rise. Instead of mucus or BBT as indicators, a hormonal marker of ovulation would be useful. Some home-based ovulation predictor kits based on LH identification in the urine have been marketed for this purpose [12,13]. However, in a previous study, it was discovered that ovulation may sometimes be missed with LH kits if their threshold are above 20 mIU/mL [11]. Furthermore, there are many different amplitudes, configurations, and durations of the LH surge that might erroneously predict ovulation [14,15].

A more direct and objective measure to confirm ovulation is the urinary measure of the metabolite of post ovulatory progesterone. Several authors have suggested that the use of single morning urinary samples of PDG above a threshold would be a better indicator of ovulation [16–18]. Even more, devices using this concept were

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at one time considered for marketing [19]. However, this approach was vulnerable to error due to the nature of the assays of urinary PDG and the variability in PDG concentration thought out the menstrual cycle [20]. Traditionally, PDG concentrations have been corrected for creatinine to avoid these problems; however, this correction adds a technical difficulty to develop simple-to-use, home-based-point of care devices. As a result, other methods combining electronic urinary monitors are being studied to address this problem [21], although they are likely to be cost prohibitive for many women. Despite the latter looking very promising, it is clear that other more affordable, easy to use, and versatile methods would be welcomed by FABM users.

The combination of robust markers of ovulation, namely urinary hormones and cervical mucus, could synergistically improve the identification of the fertile and infertile phases. In the mid-nineteen nineties, researchers collected information from normally ovulating women regarding daily urinary hormone measures, recordings of basal body temperature, cervical mucus observations, and serial ovarian ultrasound in order to study the possibility for a PDG urine hormonal assay [11]. A database of information was created but due to legal-commercial disclosure agreements, the results regarding the role of PDG in confirming post-ovulatory infertility were not published until now; this paper will present these results. In this study we assessed the potential diagnostic qualities of these markers, focusing on a given urinary PDG concentration threshold to identify the post-ovulatory infertile phase of the cycle.

2. Experimental

2.1. Subjects

This European prospective study was conducted between 1996 and 1997 in eight fertility centers: Aix-en-Provence, Dijon, and Lyon (France), Milano and Verona (Italy), Düsseldorf (Germany), Liège (Belgium), and Madrid (Spain). It included healthy menstruating women aged 18–45 years with previous menstrual cycles of 24–34 days who had experience recording basal body temperature and monitoring cervical mucus. However, for the purpose of the current analysis, no women were excluded based on the duration of the cycle.

Women with a history of infertility, pelvic inflammatory disease, cycle disturbances, disturbed follicular development, or current hormone therapy were excluded from the study. We also excluded women who had had gynaecological surgery, a delivery within the last three months, women who were breastfeeding, and competitive athletes.

The study included 107 women and analyzed an average of three cycles per woman for a total of 326 cycles. The original study that collected the data [11] was approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Lyon). All participants gave their written informed consent. The study procedures were carried out in accordance with the Ethical Standards for Human Experimentation established by the Declaration of Helsinki.

2.2. Methods

2.2.1. Mucus symptoms

Study participants checked for changes in cervical mucus two or three times daily, recording the sensation (dry, moist, wet, or slippery) and the consistency (tacky, creamy, or stretchy) of the mucus. This information allowed for the ability to distinguish between (i) days with no mucus felt or seen; (ii) days with mucus felt or seen but not having the characteristics of high fertility; and (iii) days with mucus that felt wet or slippery or that resembled an egg-white and had a stretchy appearance. The last day of clear, stretchy and/or lubricative mucus discharge was called the peak symptom [9,22]

2.2.2. Hormone assessments

Assays were carried out on the first morning urine (FMU) with two 10–12 mL aliquots frozen on the day of collection at −20 °C in tubes containing gentamicin sulphate. On the day of analysis, the aliquots were thawed in a single laboratory and tested in duplicates for quantitative detection of oestrone-3-glucuronide (E1G-ng/mL), pregnanediol-3a-glucuronide (PDG-μg/mL), follicle stimulating hormone (FSH-mIU/mL), and luteinizing hormone (LH-mIU/mL) using time-resolved fluorometric immunosorbent assays (Delfia). Each hormonal sample was repeated twice: the relative difference (i.e. CV) was respectively 5.96%, 10.79%, 8.66% and 7.17% for PDG, FSH, LH and E1G. We cannot provide detailed information on assay performance except the intra-assay CV's. This data remains within the property of the funding company.

2.2.3. Ultrasound scans

The serial transvaginal ovarian ultrasounds (with follicle measurements) started either at the onset of the fertile cervical mucus or at the detection of the LH surge by the home test. These scans were carried out every other day until the largest follicle reached 16 mm, then every day until evidence of ovulation. To note that while there is increasing evidence to indicate that multiple ovarian follicular waves develop during the human menstrual cycle [22], the evidence always point towards the last wave being the ovulatory single event of a given cycle [23].The same physician at each centre performed the scans. The ultrasound-determined day of ovulation (US-DO) was the 24-h period that separated the sight of a mature follicle on one scan and either of the following on the second scan: (i) a change in the follicle size, shape, or sonographic density; (ii) follicle rupture; (iii) the presence of an early corpus luteum; (iv) the presence of free fluid in the cul-de-sac. If a woman missed an ultrasound examination, the US-DO was the first day after the last pre-ovulatory ultrasound with a follicle $\geqslant$18 mm or the second day with a follicle <18 mm.

2.3. Measured outcomes

2.3.1. Fertility definitions

The *fertile phase* was estimated during the pre-ovulatory phase as the period stretching from the first day of menses to the end of the US-DO. The *infertile phase* was defined as the day after ovulation day, up to the following menses.

Positive PDG test was defined as a test result above a defined concentration threshold. A *negative PDG test* was defined as a test result below that threshold. Different PDG concentration thresholds in FMU samples were analyzed for specificity, sensitivity, true negative and true positive cycles. A cycle with at least one day with a positive PDG test in the fertile phase was classified as a *false positive:* i.e. PDG concentration was high despite being during the potentially fertile phase. A cycle with all days with negative PDG tests in the fertile phase was classified as a *true negative*: i.e. PDG was appropriately low during the potentially fertile phase. A cycle with days in the infertile phase with positive PDG tests was classified as a *true positive*: i.e. PDG was appropriately high during the infertile phase. A cycle without at least one day in the infertile phase with a positive PDG test was classified as a *false negative*: i.e. PDG was always low despite being in the infertile phase.

The *sensitivity* was estimated as the proportion of true positives, that is, cycles with appropriate recognition of the post-ovulatory infertile phase. The *specificity* was estimated as the proportion of true negatives, that is, cycles with appropriate recognition of the