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Development of the first urinary reproductive hormone ranges referenced to independently determined ovulation day

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Abstract

Background: Urinary hormone level analysis provides valuable fertility status information; however, previous studies have not referenced levels to the ovulation day, or have used outdated methods. This study aimed to produce reproductive hormone ranges referenced to ovulation day determined by ultrasound.
Methods: Women aged 18–40 years (no reported infertility) collected daily urine samples for one complete menstrual cycle. Urinary luteinising hormone (LH), estrone-3-glucuronide (E3G, an estradiol metabolite), follicle stimulating hormone (FSH) and pregnanediol-3-glucuronide (P3G, a progesterone metabolite) were measured using previously validated assays. Volunteers underwent trans-vaginal ultrasound every 2 days until the dominant ovarian follicle size reached 16 mm, when daily scans were performed until ovulation was observed. Data were analysed to create hormone ranges referenced to the day of objective ovulation as determined by ultrasound.
Results: In 40 volunteers, mean age 28.9 years, urinary LH surge always preceded ovulation with a mean of 0.81 days; thus LH is an excellent assay-independent predictor of ovulation. The timing of peak LH was assay-dependent and could be post-ovulatory; therefore should no longer be used to predict/determine ovulation. Urinary P3G rose from baseline after ovulation in all volunteers, peaking a median of 7.5 days following ovulation. Median urinary peak E3G and FSH levels occurred 0.5 days prior to ovulation. A persistent rise in urinary E3G was observed from approximately 3 days pre- until 5 days post-ovulation.
Conclusions: This study provides reproductive hormone ranges referenced to the actual day of ovulation as determined by ultrasound, to facilitate examination of menstrual cycle endocrinology.

Keywords: estrone-3-glucuronide; follicle stimulating hormone; hormone ranges; luteinising hormone; menstrual cycle; ovulation; pregnanediol-3-glucuronide.

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Introduction

The path to pregnancy has changed in recent decades, with women delaying pregnancy until their 30s or even later [1, 2] when female fertility is known to decrease significantly [3], and often after many years of oral contraceptive use. Despite this, when the decision is taken to start a family, expectations are that it will happen quickly and many women wish to control the process. However, many women are unfamiliar with their ovulatory cycle, e.g., approximately 40% of women in a recent US study were unaware that ovulation usually occurs 14 days prior to menses or that clear mucous vaginal discharge is a sign of impending ovulation [4]. This lack of knowledge of personal ovulatory cycles is especially pertinent for women following discontinuation of oral contraceptives, which will have masked their natural cycles, sometimes for many years [5]. In addition, a third of US women, participating in this recent study, were unaware of the adverse effects of reproductive aging, sexually transmitted infections, obesity or irregular menses on fertility [4]. Furthermore, in a UK study of women trying to conceive, only 12.7% of women correctly estimated their day of ovulation, and only 55% estimated an ovulation day that fell within their fertile window [6].

There is considerable inter-cycle variability in the timing of ovulation observed both between women and

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between cycles in individual women. The mean individual range of cycle variability has been reported as 6.7 days [7], while another study found that 46% of women had cycles that varied by 7 days or more [8]. Therefore, data of previous cycle lengths alone is not at all sufficient to determine a woman's fertile period within any given cycle. This is further illustrated by the fact that anovular cycles in apparently normal women are reported to occur in between 2% and 9% of cycles in different studies [9–11].

Trans-vaginal ultrasound is an effective and standard method for the detection of the day of ovulation, as long as examinations are frequent enough (daily/every 2 days) [12]. Unfortunately, it is costly and partly invasive, and thus impractical for routine use by women trying to conceive. Serum levels of reproductive hormones can provide valuable information to women about their cycle and timing of fertility. Reference hormone ranges in women with natural menstrual cycles and no reported infertility provide a valuable tool for understanding the normal range of hormones in relation to day of cycle. Unfortunately, serum measurements need to be carried out sequentially to gain an understanding of the whole cycle, thus this is not usually warranted unless there is reason for concern. However, these reproductive hormones, or their metabolites, are also detectable in urine, providing a convenient and non-invasive method for repeated investigation.

Some previous studies have compared urinary hormone profiles relative to each other, but not relative to the objective day of ovulation [13, 14]. For example, a recent study by Blackwell et al. looked at urinary hormone profiles of estrone glucuronide (EG), pregnanediol glucuronide (PdG) and luteinising hormone (LH), using a mixture of laboratory and home-based monitoring, and concluded that urinary hormone monitoring was a useful tool for cycle examination [14]. Other studies analysing serum hormone profiles have used the day of the LH peak to establish reference ranges [15], but this introduces relevant imprecision into the profiles, as it makes assumptions in timing of peak LH levels relative to ovulation. This timing can be influenced by intra-individual variation in time from peak LH to ovulation (approx. 28–48 h) [16], occurrence of peak LH levels post-ovulation and variability in the sensitivity of the LH assays to the metabolites of LH (thus the reported timing of the LH peak can be assay-dependent). Aligning data to the first day of the cycle is not appropriate due to the inter-individual variability of the length of the follicular phase.

Direito et al. analysed hormone levels relative to ultrasound-identified ovulation day, using urinary samples, and corresponding hormone assays conducted in the 1990s [17]. In addition, a study by Ecochard et al. described the average range of follicle stimulating hormone (FSH) relative to ultrasound-observed ovulation, also using urinary analysis performed 15–20 years ago [18]. Studies conducted on urine samples from the 1990s, although of great interest, may not be truly representative of women approximately 20 years later, since factors known to affect fertility, such as alcohol consumption, smoking habits and body mass index (BMI), have increased in the last two decades (e.g., BMI has increased by 0.5 kg/m^2 per decade worldwide [19]) and menstrual cycle disturbances like polycystic ovary syndrome (PCOS) are also more common [20].

It is very desirable for women to have accurate information of their individual cycle and timing of ovulation in order to successfully plan or avoid a pregnancy, or to enable them to rapidly identify any possible abnormalities that may affect their fertility. Urinary hormone levels can provide this detail, but a revisitation of ranges is critical to reflect the endocrinology of women today. This study therefore sought to create new urinary reproductive hormone ranges in relation to the ultrasound-determined day of objective ovulation.

Materials and methods

Women aged 18–40 years with no reported infertility and a minimum of two natural cycles prior to the study start were recruited via local and in-clinic advertising in Grevenbroich, Germany. The study was approved by the Ethics Committee of the Chamber of Physicians, Duesseldorf, Germany (study NCT01802060), it was conducted from February to June 2013 and called the *Me*nstrual Cycle *Mo*nitoring *Study* (MeMo Study).

Study method

Women enrolled on the study were required to collect daily first morning void urine samples from the first day of their period (Day 1 of their menstrual cycle), until the first day of their next period, and recorded menses in a daily diary. During their cycle, women attended the study site (green-ivf, Center of Gynecological Endocrinology and Reproductive Medicine, Grevenbroich, Germany) to obtain serum samples and for trans-vaginal ultrasound to determine the day of ovulation. Trans-vaginal ultrasound was conducted every 2 days until the dominant follicle diameter reached 16 mm (folicules reach 17–27 mm in size just prior to ovulation), at which time the women were required to attend for daily ultrasound scans, with subsequent scans carried out on Days 7 and 9 following ovulation. Where ovulation occurred between visits, the day of ovulation was considered as 0.5 days following the last visit where a dominant follicle was observed. Ultrasound was conducted by two clinicians (JR and CG) and all images stored for central review. Daily urine samples were collected into sample pots

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containing the bacteriostatic sodium azide. Volunteers were required to refrigerate samples on collection and return them to the study site at each visit (every 1–2 days), where samples were frozen at −80 °C prior to analysis.

Urinary hormone measurement

Hormone analyses were conducted as batch analyses, ensuring complete cycles were analysed on single assay plates. Samples were brought to room temperature and mixed prior to analysis; it had previously been determined that up to five freeze-thaw cycles had no affect on analyte concentration.

Urinary LH, estrone-3-glucuronide (E3G, a metabolite of estradiol), FSH and pregnanediol-3-glucuronide (P3G) were measured using in-house assays on the AutoDELFIA platform (Perkin Elmer, Waltham, MA, USA). Levels of LH were also evaluated using the Perkin Elmer assay. All assays utilised monoclonal antibodies.

The Perkin Elmer LH assay employs a β subunit–β subunit sandwich assay and it is able to detect intact LH, free β LH (LH-β) and LH β core fragment (LH-βcf). This assay was validated for use in urine samples and demonstrated the following performance characteristics: sensitivity limit of 0.5 mIU/mL (two standard deviations above mean of zero measurement); intra- and inter-assay percentage confidence values (CV) were below 3% at all standards tested (28, 51 and 111 mIU/mL); linearity was seen on dilution of sample to a 1 in 20 dilution; no hook effect, a false negative test result with certain immunoassays due to very high concentrations of the analyte, was observed when testing at maximal concentration of 1000 mIU/mL.

The in-house LH assay consists of immobilised biotinylated antibody (antibody #2119; SPD Development Co., Ltd, Bedford, UK) that recognises the α LH subunit bound to streptavidin plates, and a second, europium-labelled antibody that recognises the β subunit (antibody #2301; SPD Development Co., Ltd, Bedford, UK), thus it is only able to measure intact LH. Assay sensitivity was 0.1 mIU/mL and inter- and intra-assay percentage CV were <5%; linearity was seen in dilutions up to 1 in 20 and no high-dose hook was observed when testing up to 1000 mIU/mL.

FSH was measured with an in-house sandwich assay consisting of europium-labelled anti-β subunit antibody (antibody #5948; SPD Development Co., Ltd, Bedford, UK) and biotinylated anti-α FSH (antibody #4882; SPD Development Co., Ltd, Bedford, UK) immobilised on streptavidin plates. The sensitivity of this assay was 0.136 mIU/mL; inter- and intra-assay percentage CV was <5% for standards tested (1.77, 8.2, 42.9, 219 mIU/mL); linearity was seen up to a 1 in 20 dilution and no high dose hook was observed when testing up to 1000 mIU/mL.

A competitive in-house immunoassay was used for measuring E3G, consisting of immobilised high affinity antibody for E3G (antibody #4155; SPD Development Co., Ltd, Bedford, UK), with competition for binding between sample and europium-labelled E3G. Validation of this assay for use in urine demonstrated the following performance: sensitivity was 0.5 ng/mL; intra- and inter-assay percentage CV was below 5% for all standards tested (3, 20, 37, 170 ng/mL); linearity was seen up to a 1 in 20 dilution of urine sample.

The P3G assay used was also an in-house competitive immunoassay based on competition between sample and europium-labelled P3G for binding by a high affinity antibody (antibody #5806; SPD Development Co., Ltd, Bedford, UK). Assay sensitivity was 0.021 μg/mL and intra- and inter-assay percentage CV was below 10% for standards tested (0.16, 0.8, 4.0, 20.0, 100.0 μg/mL); linearity was seen up to a 1 in 20 dilution of urine sample.

Data analysis

All results were entered into the study database using the Teleform system (Autonomy Inc, San Francico, CA, USA). Data were analysed using SAS version 9.2 to create hormone ranges referenced to the day of ovulation as determined by ultrasound. The median, 10th and 90th centiles of each hormone were determined using the day referenced to the day of ovulation. Day of urinary LH surge was defined as first rise from baseline by the interpretation of graphical data by the panel of authors.

Results

Volunteer characteristics

A total of 51 volunteers were recruited into the study on a first-come, first-in basis; 10 women withdrew or were withdrawn from the study, including two women who were found to have ovarian cysts at the time of their first scan (of which they were previously unaware). One woman was found to have had an anovular cycle. Thus data were available for analysis from 40 women; further details of study withdrawals are shown in Figure 1.

The mean age of women was 28.9 years and 95% were white; details of volunteer demographics and menstrual cycle characteristics are provided in Table 1. The mean cycle length of volunteers was 28 days and the mean day of ovulation was Day 15.

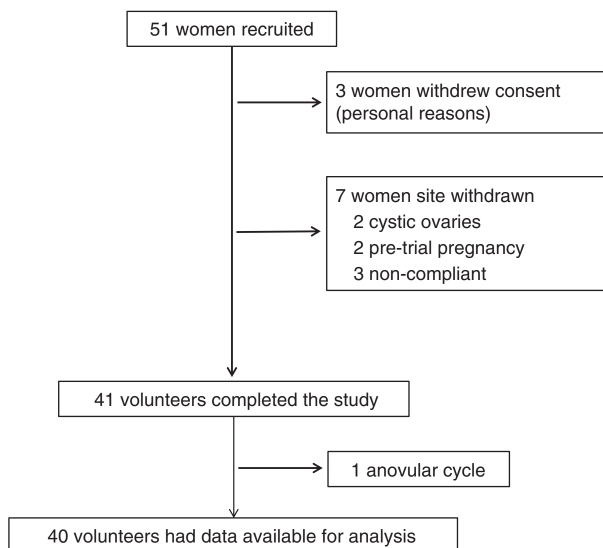

Figure 1 Flow diagram of volunteer participation.

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Table 1 Volunteer demographics.

	n	
Mean age, years (SD)	40	28.9 (4.8)
Age range, years	40	18.0–37.0
18–25, n (%)		11 (28.5)
26–30, n (%)		10 (25.0)
31–35, n (%)		16 (40.0)
36–40, n (%)		3 (7.5)
Ethnicity, n (%)	40	
White		38 (95.0)
Asian		2 (5.0)
Mean cycle length[a], days (SD)	37	27.7 (3.4)
Mean day of ovulation[b] (SD)	40	15.4 (3.8)
Mean length of luteal phase[a], days (SD)	37	12.3 (2.5)
Number of previous pregnancies, n (%)	40	
0		23 (57.5)
1		11 (27.5)
2		6 (15)
Number of previous live births, n (%)	40	
0		28 (70.0)
1		10 (25.0)
2		2 (5.0)
Number of previous miscarriages, n (%)	40	
0		34 (85.0)
1		6 (15.0)
2		0 (0.0)
Number of previous terminations, n (%)	40	
0		38 (95.0)
1		1 (2.5)
2		1 (2.5)

SD, standard deviation. [a]Excluding pregnant volunteers, [b]Also considered length of follicular phase.

Hormone study ranges

Study urinary hormone ranges consisting of the median urinary hormone levels and 10th–90th centile ranges by cycle day, relative to the day of objective ovulation as assessed by ultrasound, were derived for LH, E3G, FSH and P3G (Figure 2); Table 2 shows the corresponding values for the mean urinary level of each hormone relative to the day of ovulation.

Urinary LH surge preceded ovulation for most women (mean time from surge to ovulation 0.81 days, standard deviation [SD] 0.89). Peak urinary LH levels were seen a median of 0.5 days prior to ovulation (5th–95th centile: –1.5–+0.5 days). However, the timing of LH peak was dependent on whether the assay was measuring total or intact LH (Figure 3); peak LH was observed approximately 1 day later with the total LH assay (Perkin Elmer assay) compared with the intact LH assay (in-house assay system). The timing of the LH surge was the same irrespective of the assay used. Comparison of LH surge characteristics observed in individual volunteers is shown in Figure 4; these examples illustrate the influence of the assay used on the surge profile. Six volunteers had LH surge profiles that did not differ between assays (an example of one such volunteer is shown in Figure 4A). However, for most individuals, the total LH assay continued to detect LH for several days post-surge, with LH levels peaking later than that observed when using the intact LH assay (an example of one such volunteer is shown in Figure 4B). In six cases, a second peak in LH levels was seen with the total LH assay, whereas the intact LH assay only showed a single peak (an example of one such volunteer is shown in Figure 4C).

A rise in urinary P3G from baseline occurred after ovulation in all volunteers; levels peaked a median of 7.5 days following ovulation (5th–95th centile range: +4.5–+10.5 days). Median urinary peak E3G levels were also observed 0.5 days pre-ovulation (5th–95th centile: –2.5–+9.5 days) and the same median peak day was seen for FSH levels (–0.5 days, 5th–95th centile: –2.5–+0.5 days). There was a persistent and substantial rise in urinary E3G observed from approximately 3 days prior to ovulation until up to 5 days post-ovulation for the 90th centile.

This study did not aim to examine age-related hormonal changes, however, differences in the median levels of women aged <30 years (n=20) compared with those aged ≥30 years (n=20) were observed. It was found that median levels of several hormones were higher in women age ≥30 years, although numbers were too low for formal analysis (median level for <30 years vs. median level for ≥30 years: volunteers' peak intact LH=57.1 vs. 71.3 mIU/mL; peak FSH=19.8 vs. 22.6 mIU/mL; Day 3 FSH=4.9 vs. 6.7 mIU/mL; peak P3G=29.1 vs. 34.3 mIU/mL). Whereas no difference in peak E3G (59 vs. 60.3 mIU/mL) or peak total LH (69.9 vs. 69.8 mIU/mL) were observed between age groups.

Discussion

This study presents the first urinary reproductive hormone ranges referenced to the actual day of ovulation, thus providing ranges to examine menstrual cycle endocrinology.

The high level of agreement between the LH surge and day of ovulation observed in this study highlights that urinary LH measurements are a reliable and accurate predictor of ovulation. The LH surge causes the dominant follicle to rupture and release a mature ovum and ovulation typically occurs approximately 28–48 h after the LH surge [16] and will not occur in its absence [21]. In this study, however, identification of the LH peak was found to be assay-dependent and could occur post-ovulation,

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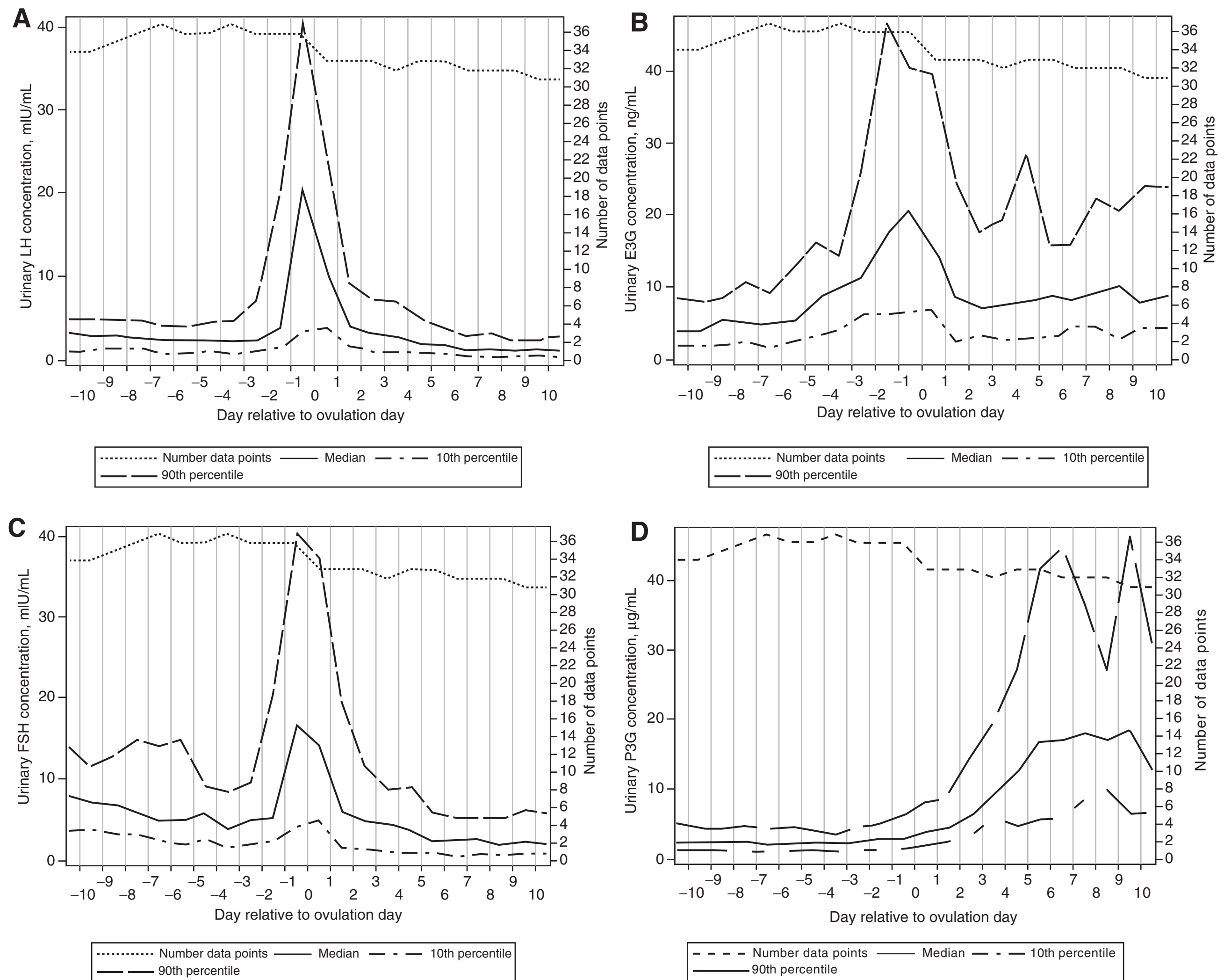

Figure 2 Reference ranges of urinary hormone levels relative to ovulation day (determined by trans-vaginal ultrasound) for: (A) LH (intact); (B) E3G; (C) FSH; (D) P3G.

therefore care must be employed when interpreting LH profiles. The difference in these profiles is most likely due to the recognition of LH-βcf in the urine samples. Human LH is a heterodimeric glycoprotein consisting of a smaller α subunit (LH-α) and a larger β subunit. Urinary LH-α and intact LH-β have been observed to show a similar pattern to that of complete LH during the menstrual cycle [22, 23]; however, LH-βcf material was observed to increase during and up to 3 days after the urinary LH surge [22]. LH-βcf is a fragment of LH produced by the degradation of LH, which most likely occurs in the kidneys [22]. LH-βcf was originally isolated from the human pituitary gland, and subsequently a urinary form (with minimal structural differences) was identified and characterised [24, 25]. LH-βcf has been shown to be the predominant form of LH in urine during the peri-ovulatory period and levels peak 1–3 days later than those of intact LH [23]. In contrast, no LH-βcf surge has been detected in serum. This supports the view that LH-βcf is a product of metabolic degradation, hence the lag period observed between the peak levels of intact LH and LH-βcf peak, as the process of degradation will extend the time taken to appear in urine [23]. A previous study by Park et al., which characterised the urinary LH surge in young women, utilised the LH assay recognising LH-βcf [26]. Thus, this finding that urinary LH surges are extremely variable in all aspects of configuration, amplitude and duration, is likely to be influenced by the detection of LH-βcf. Similarly, a study by Ecochard et al. observed two LH peaks in some cycles, and found the concentration of LH continued to increase post-ovulation [27]. From observations in other studies analysing the pattern of LH-βcf, these findings can be explained by the

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Table 2 Mean urinary levels of each hormone relative to the day of ovulation.

Day relative to ovulation[a]	n	Intact LH, mIU/mL Median (10–90 centiles)	Total LH, mIU/mL Median (10–90 centiles)	E3G, ng/mL Median (10–90 centiles)	FSH, mIU//mL Median (10–90 centiles)	P3G, μg/mL Median (10–90 centiles)
−16	13	6.3 (2.8–11.4)	5.1 (2.9–11.3)	7.5 (4.9–2.5)	5.4 (2.0–9.6)	3.4 (1.0–5.6)
−15	16	4.3 (2.1–12.0)	6.2 (2.3–15.8)	8.4 (4.0–29.1)	4.6 (2.6–15.5)	3.4 (0.8–10.4)
−14	23	6.8 (1.2–11.0)	6.9 (1.7–15.0)	7.7 (4.0–21.2)	6.0 (5.6–14.1)	2.9 (0.8–5.5)
−13	28	8.7 (1.8–18.1)	8.3 (1.9–21.1)	8.7 (5.3–18.9)	7.4 (2.2–16.3)	2.8 (0.9–5.4)
−12	34	5.5 (2.5–14.5)	5.9 (2.4–16.0)	8.8 (4.0–17.5)	7.1 (2.9–20.1)	2.1 (0.8–6.1)
−11	38	8.2 (2.2–12.6)	8.1 (2.5–14.9)	9.1 (4.7–18.9)	7.7 (2.5–16.1)	2.2 (1.0–4.9)
−10	38	6.9 (2.8–11.9)	7.7 (3.1–14.7)	8.9 (4.5–18.3)	7.0 (3.6–11.5)	2.1 (1.0–4.4)
−9	39	6.6 (3.4–12.0)	7.7 (2.4–19.1)	11.6 (4.4–19.2)	6.5 (3.2–12.8)	2.1 (0.9–4.2)
−8	40	6.5 (2.9–11.9)	7.3 (3.4–16.6)	11.6 (5.7–23.1)	5.8 (2.9–14.5)	2.2 (0.9–4.5)
−7	41	5.6 (2.3–9.7)	6.7 (2.7–19.5)	11.2 (4.2–19.8)	4.8 (2.4–11.7)	1.8 (0.7–3.8)
−6	40	6.2 (1.9–10.0)	7.9 (2.9–21.7)	12.0 (6.1–27.1)	5.1 (1.9–13.7)	1.8 (0.8–4.3)
−5	40	6.0 (2.9–11.5)	8.5 (3.4–17.8)	16.4 (7.7–35.2)	5.5 (2.3–9.7)	1.9 (0.9–3.9)
−4	41	5.3 (2.2–10.7)	7.5 (2.5–17.1)	22.0 (11.4–33.0)	3.78 (1.6–7.7)	1.9 (0.9–3.1)
−3	40	5.9 (2.8–15.6)	9.4 (3.2–22.6)	26.4 (14.0–53.6)	4.7 (1.8–9.3)	2.0 (1.0–4.1)
−2	40	8.5 (4.0–46.6)	13.5 (3.9–47.5)	37.3 (16.3–92.8)	5.3 (2.2–18.9)	2.6 (1.0–4.5)
−1	40	44.6 (6.5–101.0)	37.5 (7.7–90.8)	46.2 (17.1–85.6)	15.0 (3.9–35.9)	2.3 (1.1–5.5)
0	37	27.1 (9.9–67.9)	57.9 (23.5–110.0)	36.9 (14.9–84.9)	14.1 (4.9–37.4)	3.4 (1.6–7.7)
1	37	10.1 (4.1–23.5)	41.6 (11.4–97.9)	19.6 (6.1–53.1)	6.1 (1.6–19.6)	4.3 (1.7–8.5)
2	37	7.5 (1.9–18.2)	32.6 (8.5–88.9)	18.7 (7.5–38.6)	4.7 (1.3–12.0)	6.5 (2.7–14.7)
3	36	6.4 (1.8–17.2)	23.7 (6.5–49.4)	16.9 (6.5–42.3)	3.8 (1.0–13.1)	9.0 (4.0–25.8)
4	36	5.0 (2.1–12.8)	13.6 (3.1–48.1)	18.0 (6.6–61.6)	3.5 (0.7–9.1)	13.5 (4.6–32.1)
5	36	3.9 (1.3–9.1)	10.9 (3.6–23.8)	20.1 (7.3–48.2)	2.3 (0.8–6.5)	17.1 (5.3–53.6)
6	35	3.0 (0.9–7.1)	7.3 (2.1–20.5)	19.2 (10.2–49.1)	2.5 (0.4–6.4)	18.1 (5.7–59.0)
7	35	3.0 (1.0–8.20)	6.6 (1.8–17.9)	20.8 (10.3–48.2)	2.2 (0.6–5.3)	18.7 (8.2–41.0)
8	35	2.8 (0.9–6.1)	5.2 (1.4–16.6)	22.8 (6.3–45.04)	1.7 (0.5–6.0)	18.6 (9.7–35.9)
9	34	3.5 (1.6–6.2)	3.7 (2.3–13.0)	17.7 (10.0–52.3)	2.1 (0.7–6.1)	19.9 (6.2–47.3)
10	34	2.6 (1.2–7.2)	4.4 (1.4–13.3)	19.8 (6.7–51.7)	1.7 (0.7–5.7)	13.1 (6.5–36.3)
11	30	3.1 (1.0–13.8)	3.2 (1.3–14.6)	15.3 (7.5–37.8)	2.3 (0.5–8.7)	11.0 (4.4–29.9)
12	23	3.9 (1.5–7.2)	4.0 (1.7–7.0)	11.8 (3.2–23.2)	3.6 (0.7–5.0)	9.6 (4.5–19.9)
13	21	4.5 (2.5–10.1)	5.7 (2.3–10.9)	7.5 (4.9–21.5)	4.6 (2.0–9.1)	8.8 (3.8–18.6)

[a]Data is rounded to whole day.

presence of LH-βcf and its detection by the assay used in this study. Thus these and similar studies are not describing the endocrinologically relevant LH surge, but rather characterising biologically active intact LH and, to a greater extent, its metabolites. This differential detection of LH-βcf by different assays confounds the literature with conflicting descriptions of the LH surge, but these discrepancies are entirely due to assay specificity. It is important to emphasise that, in our study, the assay that recognises intact LH and the assay that recognises total LH are both equally able to define the day of the LH surge. Information regarding LH-βcf detection by assays is generally not available, as most quantitative assays are validated for serum use, where LH-βcf is not detectable.

Home-based ovulation tests are typically designed to identify ovulation by detection of this LH surge in urine. Studies have confirmed their accuracy in detecting the LH surge relative to serum hormone levels and in predicting ovulation relative to ultrasound-detected ovulation [28–32]. Thus, the results of this study confirm previous findings on the accuracy of urinary hormone testing to predict the onset of the fertile window in women and the application of urinary LH surge detection for home-based fertility testing [33]. The data shown here indicate that a LH cut-off value would be effective in predicting ovulation. However, as there is overlap between the population baseline value of intact LH (90th centile around 10–15 mIU/mL prior to surge) and surge level (10th centile for day of ovulation 9.9 mIU/mL), a single threshold would not provide 100% accurate prediction. In addition, the observed persistent and notable rise in urinary E3G from approximately 3 days prior to ovulation makes E3G a candidate marker for the onset of a woman's fertile window, as it is generally accepted that sperm can survive for up to 5 days in sperm-supportive, fertile cervical mucus [9]. More sophisticated versions of the home-based ovulation test detect both

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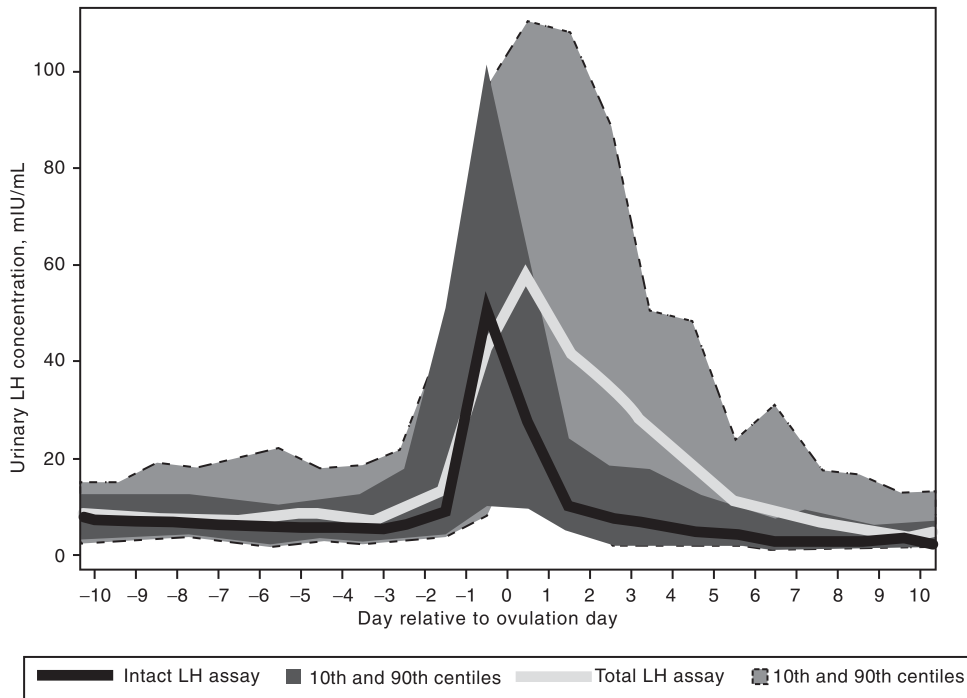

Figure 3 Level of urinary LH relative to ovulation day, as determined by ultrasound, measured using in-house assays (identifying intact LH) on AutoDELFIA platform (Perkin Elmer) and standard Perkin Elmer assay (identifying total LH).

urinary LH and E3G, to identify the earlier onset of the fertile window signified by the increase in E3G prior to the LH surge [34, 35]. A rise of urinary P3G above baseline is a consistent marker of luteinisation. The human ovum has a lifespan of <1 day, and as our data indicate that P3G rise is consistently >1 day after ovulation, this rise provides an excellent marker for closure of the fertile window.

The National Academy of Clinical Biochemistry in the USA states that point-of-care tests for the detection of urinary LH have excellent diagnostic sensitivity for the detection of ovulation [36]. In Guideline 176, they strongly recommend the use of such devices for the purpose of detecting ovulation, stating that urine LH tests are recommended to predict ovulation within 48 h of a positive test [36].

The use of home fertility monitoring is not only valuable in enabling women to identify their fertile days, but can also alert women to possible subfertility. For instance, persistent lack of an LH surge highlights a high proportion of anovulatory cycles and may be indicative of PCOS, for example. PCOS is found in up to 12% of the population and is often underdiagnosed [37–40], mainly because of differing, and sometimes inconsistent, diagnostic criteria.

One limitation of urinary testing is the variation in urine volume associated with sample collection, which is a potential source of error due to the effect of volume on concentration. Creatinine is a waste product of muscle metabolism, which is relatively constantly excreted in urine; characteristics that have led to it being utilised to normalise the quantity of a given analyte in urine samples. Thus creatinine adjustment is frequently used to correct for urinary volume effects, but this has been found to be unnecessary for the determination of specific hormonal parameters on a given day, e.g., LH peak [41]. Furthermore, in a study evaluating urinary and serum pregnanediol, the adjustment for creatinine introduced an error in older women due to an observed decline in creatinine clearance with age, and this adjustment is thus discouraged in such instances [42]. This study has found that urinary hormone analysis without the need for creatinine correction can provide all the necessary detail of menstrual cycle endocrinology. Other potential limitations of this study are the relatively small sample size and limited ethnicity representation (95% white). In a study by Marsh et al., higher estradiol levels were observed in African-American women compared with Caucasian women, thus the urinary ranges reported here may not be representative of women in all ethnic groups, although no differences in FSH or LH were observed in this study [43].

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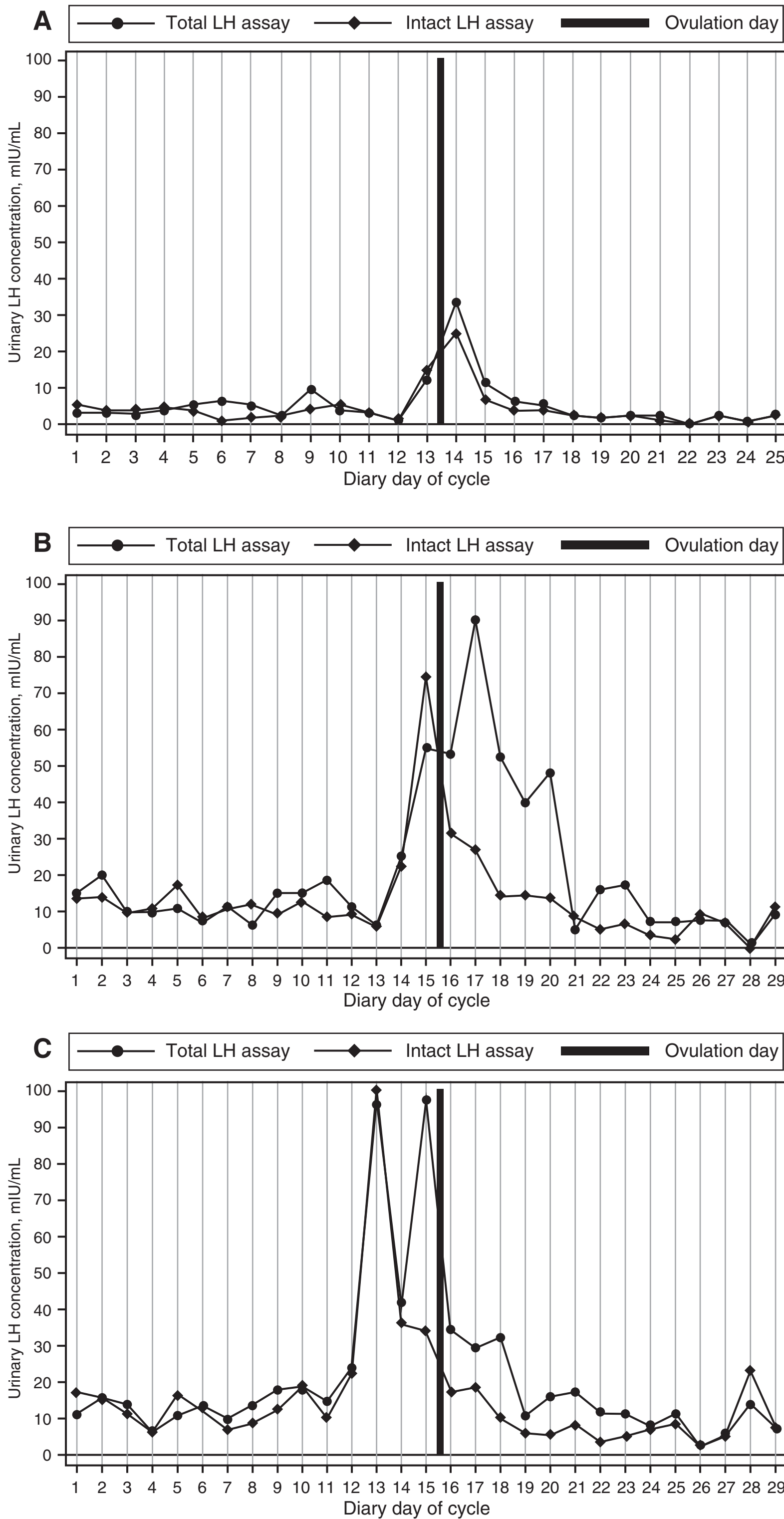

Figure 4 Individual profiles of volunteers of urinary LH relative to ovulation day, as determined by ultrasound, measured using in-house assays on AutoDELFIA platform (Perkin Elmer), measuring intact LH and standard Perkin Elmer assay, measuring total LH.
(A) A volunteer where both assays provided equivalent surge profiles; (B) A volunteer where peak LH concentration differed by 2 days between assay; (C) A volunteer where in-house assay showed single peak, whilst Perkin Elmer assay showed 2 peaks.

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In conclusion, this study highlights the accuracy and reliability of urinary hormone measurements for predicting and confirming ovulation, perhaps in some instances, replacing the need for blood sample analysis. Furthermore, it provides reproductive hormone ranges referenced to the actual day of ovulation, to give urinary hormone ranges for use in the examination of menstrual cycle endocrinology and for close cycle monitoring for timing of interventions.

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