Human Reproduction vol.13 no.1 pp.15–21, 1998

Detecting pre-ovulatory luteinizing hormone surges in urine

James S.Kesner[1,5], Edwin A.Knecht[1], Edward F.Krieg Jr[2], Allen J.Wilcox[3] and John F.O'Connor[4]

[1]Experimental Toxicology Branch and [2]Statistics Activity, Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, OH 45226, [3]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and [4]Irving Center for Clinical Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA

[5]To whom correspondence should be addressed at: Experimental Toxicology Branch, Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, MS-C23, Cincinnati, OH 45226, USA

The study objectives were to determine (i) if pre-ovulatory luteinizing hormone (LH) surges, undetected in urine by two immunoradiometric assays (IRMA), were detectable by an ultrasensitive immunofluorometric assay (IFMA) and (ii) the influence of creatinine adjustment on the detection and timing of the urinary LH surges. Daily urine specimens were contributed by healthy 25–36 year old volunteers during 14 ovulatory menstrual cycles for an epidemiological study conducted in 1983–1985. Specimens were selected as having been previously assayed by two IRMA without consistently detecting LH surges. These urine specimens were remeasured using an IFMA and adjusted for creatinine concentration. IFMA measurements revealed unambiguous LH surges in all cycles. Adjusting IRMA urinary LH values for creatinine concentrations revealed previously undetected LH surges in four of eight cycles. Creatinine adjustment also altered the timing of IRMA and IFMA LH surges by 1–5 days. These results demonstrate an IFMA that detects pre-ovulatory LH surges in unpreserved, frozen urine from cycles where such surges were previously undetectable. Further, creatinine adjustment can markedly affect detection and timing of the onset and peak of the urinary LH surge. While our analysis suggests that this adjustment improves the validity of the LH measure, this requires further investigation.

Key words: creatinine/epidemiology/menstrual cycle/two-site immunofluorometric assay/women

Introduction

Detection of the midcycle surge release of luteinizing hormone (LH) is critical clinically and epidemiologically for determining the time of ovulation *per se*, for evaluating the normality of menstrual cycle function, and as an anchor for determining the preimplantation period. Urine is a convenient and valuable medium for repetitive measurements of various biological markers including LH and other menstrual cycle hormones or their metabolites (Kesner *et al*., 1992b; Lasley and Shideler, 1994). Some reports, however, indicate that the pre-ovulatory LH surge is not reliably measurable in urine specimens. For example, home diagnostic products failed to detect LH surges in up to 40% of ovulatory cycles, especially if urine specimens had been previously frozen or collected only once per day (Nulsen *et al*., 1987; Kesner *et al*., 1992b). In another report, LH surges were not detected in ~30% of menstrual cycles when urine specimens had been stored frozen and then measured by a radioimmunoassay and two immunoradiometric assays (IRMA). Most of those cycles which lacked detectable LH surges exhibited oestrogen and progestin patterns consistent with ovulation; some yielded pregnancies (Wilcox *et al*., 1987).

Towards developing assays to measure LH more reliably in urine, Clough *et al*. (1992) have described an enzyme immunoassay that detects intact LH and the free glycoprotein α-subunit, while Cano and Aliaga (1995) have reported that a commercial IRMA that principally measures intact LH reliably detects pre-ovulatory-like LH profiles in urine. Both assays work without the need to add preservative.

Our laboratory has modified, characterized, and validated a time-resolved immunofluorometric assay (IFMA) to LH for application on urine specimens (Kesner *et al*., 1994a). Since the assay detects intact LH with strong cross-reactivity to the LH β-subunit, it would seem to be a viable candidate to detect intact and dissociated LH in urine. Our experience confirms this idea. LH surges have been detected in the appropriate time-window for ovulation in frozen urine specimens from >99% of a sample of >450 menstrual cycles judged to be ovulatory by steroid secretion patterns (unpublished data). The present report extends these observations by describing in detail a sample of cycles with unambiguous midcycle surges detected by the IFMA after previous LH assays had failed to detect a surge (Wilcox *et al*., 1987).

Urinary concentrations of substances analysed are often divided by urinary creatinine concentrations to standardize urine flow rate (Landy *et al*., 1990; Baird *et al*., 1991; Munro *et al*., 1991; Kesner *et al*., 1992a; Taylor *et al*., 1992; Keely and Faiman, 1994; Saketos *et al*., 1994). Some reports, however, suggest that creatinine adjustment neither reduces day-to-day variability of serial hormone concentrations nor improves the correlation between paired urinary and circulating concentrations (Chatterton *et al*., 1982; Boeniger *et al*., 1993; Demir *et al*., 1994; Hakim *et al*., 1994). Thus a second objective of this report is to demonstrate the impact of

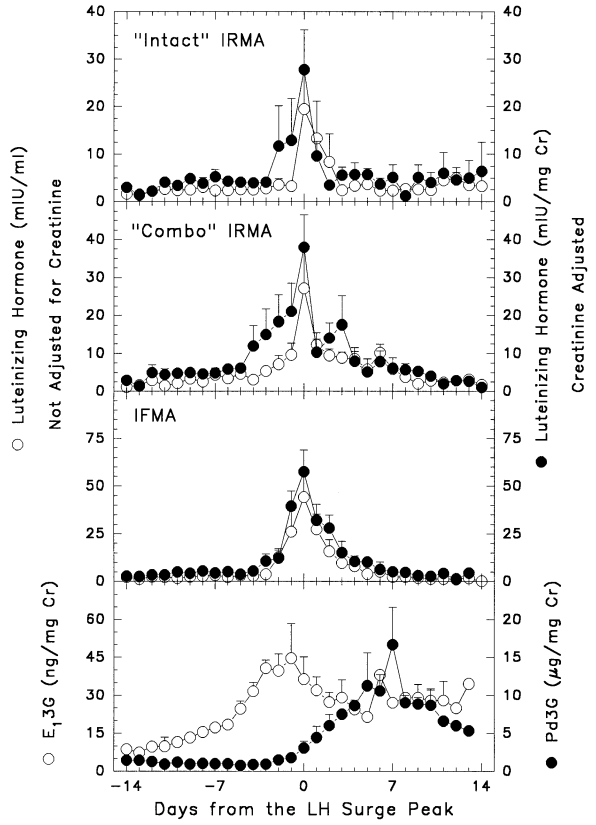

Figure 1. LH profiles for six menstrual cycles (group 1, Table I) during which surges were identified by the intact-immunoradiometric assay (IRMA), combo-IRMA and immunofluorometric assay (IFMA). Each LH profile is centred graphically about its own surge peak, i.e. within each cycle the day of the LH surge peak may vary among immunoassays and after creatinine adjustment. (See Table I for the timings of LH surge peaks and day of ovarian steroidogenesis transition.) Steroid values in the bottom panel are centred about the creatinine-adjusted IFMA LH surge peak. Profiles depict arithmetic means + SE for the six cycles. $E_1$3G = oestrone 3-glucuronide; Pd3G = pregnanediol 3-glucuronide.

creatinine adjustment on the detection and timing of the LH surge in urine.

Materials and methods

Subjects and specimens

Specimens described in this report are a subset of those collected during a study conducted in 1983–1985 (Wilcox *et al.*, 1988). Subjects were healthy volunteers who had discontinued using birth control for the purpose of achieving pregnancy. Subjects collected and froze their daily first morning urine specimens. Specimens were subsequently stored without preservative at –25°C, assayed for LH in 1985–1987, and then reassayed for this analysis in 1995.

The basis of specimen selection was as follows: 63 cycles which had previously been analysed for LH using two different IRMA and one radioimmunoassay (Wilcox *et al.*, 1987) were reviewed. All of these cycles were judged to be ovulatory, as shown by a rise of pregnanediol 3-glucuronide (Pd3G; the primary urinary metabolite of progesterone) during the luteal phase. Among the ovulatory cycles, we looked for cycles in which neither of the IRMA showed clear evidence of an LH surge, giving preference to cycles in which live births were achieved, which were irrefutably ovulatory. We selected 14 cycles contributed by nine women aged 25–36 years old. Of these, a urinary LH surge had not been detected by either IRMA in six cycles, of which five were conception cycles and four culminated in live births; an LH surge had not been detected by one of the IRMA in two cycles; and an LH surge had been detected by both IRMA in the remaining six cycles.

Assays

The two assays originally used to measure urinary LH in these specimens were two-site, non-competitive IRMA (Wilcox *et al.*, 1987). One IRMA employed a capture monoclonal antibody (A105) that recognized the gonadotrophin α-subunit. The other IRMA used two capture monoclonal antibodies: A105 and another (B201) that recognized fragments of human chorionic gonadotrophin (HCG) β-subunit with strong cross-reactivity with LH β-subunit and LH β-subunit fragments. For both IRMA, intact LH and its β-subunit and β-subunit fragments were detected using a monoclonal antibody (B105) labelled with ^{125}I (Wilcox *et al.*, 1987). Thus, one IRMA detected primarily intact LH (intact-IRMA), while the second IRMA detected intact LH, LH β-subunit, and LH β-subunit fragments (combo-IRMA).

Cross-reactivity for the intact-IRMA was 100% for intact HCG, 53% for intact LH, and <1% for HCG β-subunit, LH β-subunit, and HCG β-subunit fragment. Cross-reactivity for the combo-IRMA was 100% for intact HCG, 73% for intact LH, and 81% for HCG β-subunit, 47% for LH β-subunit, and 40% for HCG β-subunit fragment. Dilution curve slopes for three or four urine samples from normally cycling women were similar to those for the standard curves for both the intact-IRMA and combo-IRMA. Limits of detection for the intact-IRMA and combo-IRMA were 1.5–3.1 and 0.4–0.6 mIU/ml, respectively. Intra- and interassay coefficients of variation were 7.1 and 24.3% for the combo-IRMA and 4.8 and 19.7% for the intact-IRMA, respectively. All samples from a given menstrual cycle were measured within the same assay. Both assays used intact HCG CR119 as a standard preparation.

A two-site, non-competitive IFMA was used to reassay the urine specimens (Kesner *et al.*, 1994a). The capture antibody and europium-labelled detection antibody were both monoclonal and directed against distinct epitopes on the LH β-subunit. Cross-reactivity of this IFMA was 100% for intact LH, 66% for LH β-subunit, 1.0% for HCG, 4.7% for HCG β-subunit, <0.01% for α-subunit, and ≤0.003% for follicle stimulating hormone (FSH) and thyrotrophin (TSH) (Pettersson and Söderholm, 1990; Jaakkola *et al.*, 1990). IFMA LH measurements were performed blind to the previous IRMA and day of ovarian steroidogenesis transition (DOST; described below) results.

Oestrone 3-glucuronide ($E_1$3G; the primary urinary metabolite of oestradiol) and Pd3G were measured by competitive fluoroimmunoassays (Kesner *et al.*, 1994b). LH, $E_1$3G and Pd3G values were divided by urinary creatinine concentrations to adjust for urine flow rate. Urinary creatinine concentrations were measured using a modification of the Jaffe reaction (Jaffe, 1886; Taussky, 1954). In brief, creatinine and picric acid react in an alkaline environment to yield a red-orange tautomer, creatinine picrate, to be measured by spectrophotometry. Intra- and interassay coefficients of variation for the creatinine assay were 1.89 and 2.92%, respectively. In some cases, creatinine was not measured in specimens collected on the first and last few days of the menstrual cycle, precluding the calculation of creatinine-adjusted endocrine values for these days.

Detection criteria and statistical analysis

The onset and peak of the LH surge were defined as follows. The surge peak was the highest value occurring >5 days after menses

Table I. Detection of pre-ovulatory luteinizing hormone (LH) surges, pregnancy status, and day of ovarian steroidogenesis transition (DOST) for 14 ovulatory cycles. Timing of LH surges and DOST are relative to the first day of the previous menstruation. An arrow (→) indicates a change in timing after creatinine adjustment

Subject:cycle	Day of the LH surge peak or onset				Conceived	DOST
	Intact-IRMA	Combo-IRMA	IFMA			
	Peak	Peak	Peak	Onset		
Group 1[a]						
1:1	15	17 → 18	17 → 18	14 → 15	no	15
1:2	12	13 → 16	14 → 15	12	no	12
2:1	14 → 16	12 → 16	14 → 15	14	yes	13
3:1	22	22 → 23	22 → 23	21 → 22	no	22
4:1	19	19	21	19 → 20	yes[b]	21
4:2	17 → 16	17	19 → 17	17 → 16	no	17
Group 2						
5:1	ND[c]	ND	24	22	yes	22
6:1	ND	ND	25 → 23	25 → 20	yes[b]	20[d]
7:1	ND[e]	ND	12	11	yes	12
8:2	ND	ND	22	21	yes	22
Group 3						
6:2	20 → 18	ND → 18	21 → 18	18	no	19
6:3	19	ND → 18	20 → 18	19 → 18	no	17
8:1	ND	ND → 15	16	14	no	15
9:1	ND → 17[e]	ND → 17[e]	20	18	yes	17

IRMA = immunoradiometric assay; intact-IRMA detected primarily intact LH; combo-IRMA detected intact LH, LH β-subunit and β-subunit fragments; IFMA = immunofluorometric assay.
[a]Subject:cycles in groups 1–3 are represented in Figures 1–3, respectively; group 1: LH surges were identified by all immunoassays; group 2: surges were identified by the IFMA, but by neither IRMA; group 3: surges were identified by the IFMA, but inconsistently by both IRMA.
[b]Pregnancy ended in early loss (Wilcox *et al*., 1987).
[c]ND = no LH surge detected.
[d]Indeterminate DOST was imputed.
[e]Critical specimen not assayed for LH: day 12 for subject:cycle 7:1 (intact-IRMA); day 20 for subject:cycle 9:1 (intact-IRMA); and day 18 for subject:cycle 9:1 (combo-IRMA).

began, and either 7–18 days before the next menses (non-conceptive cycles) or 6–12 days before the HCG rise (conceptive cycles). The LH surge peak occurred 0–3 days after an LH surge onset, which was defined as the first rise >3 SD above the mean of the previous 7 days. The LH surge peak values were greater than or equal to those on the day of the surge onset or the first and second days following the peak and exceeded an assay-dependent threshold (per ml and per mg creatinine): 8.5 mIU for the IFMA, 4.2 mIU for the intact-IRMA, and 7.6 mIU for the combo-IRMA.

The basis for the detection interval for LH surges in conceptual cycles was that the first day of HCG rise follows the DOST by 6–12 days in pregnancies lasting for >6 weeks (unpublished data). The HCG rise was defined as the first elevation above 0.015 ng/ml in urine that was sustained for at least 3 days. The DOST was an estimate of the day of ovulation and reflected the changes in oestrogen and progesterone production by the ovary around the time of ovulation (Baird *et al*., 1991, 1995).

The variances among the intervals between the selected indices of the IFMA LH surge and the DOST were calculated. Tests for differences between variances were performed as described by Snedecor and Cochran (1967). This method took into account the correlation between variances and involved taking the sum and difference of two correlated variables, and then using the correlation between the sum and the difference as a statistic for testing the variances. The method assumed bivariate normality. Different variances could reflect a difference in the variances of the attributes themselves, a difference in the measurement errors of the attributes, or, assuming measurement errors are not correlated, a difference in the covariances of the attributes with the DOST.

Results

The IFMA revealed unambiguous pre-ovulatory LH surges in urine specimens from all 14 ovulatory cycles. In contrast, LH surges were detected by both IRMA in only six of these cycles (Figure 1; Table I, group 1) and were not detected by either IRMA in four other cycles (Figure 2; Table I, group 2). In the remaining four cycles, the combo-IRMA detected LH surges only after creatinine adjustment, whereas the ability of the intact-IRMA to detect LH surges was variable (Figure 3; Table I, group 3). The IRMA LH surges that were revealed after creatinine adjustment aligned within 1 day of both the IFMA LH surge onset and the DOST (Table I).

Creatinine adjustment also shifted the timing of the LH surge onset and peak by 1–5 days in 11 of 14 cycles, irrespective of the immunoassay (Table I). The timing of various indices of the LH surge (i.e. onset, peak, before and after creatinine adjustment, different immunoassays) and the DOST differed within a cycle by 1–5 days (Table I). The variation among intervals between the IFMA LH surge and the DOST was smallest (SD = 0.80; $P \leqslant 0.03$) when the LH surge was defined by its onset after creatinine adjustment, compared to its peak (adjusted or unadjusted) or the unadjusted onset (SD range = 1.38–1.77).

The menstrual cycle depicted in Figure 4 exemplified the prominence of the LH surges detected by the IFMA for cycles where surge detection was problematic using other assays.

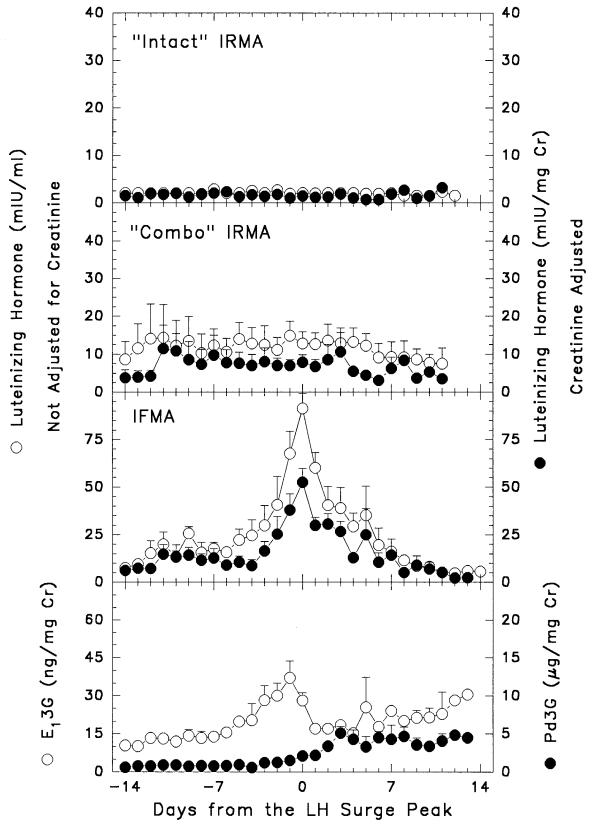

Figure 2. LH profiles for four menstrual cycles (group 2, Table I) during which surges were identified by the IFMA, but by neither IRMA. Each IFMA LH profile is centred graphically about its own surge peak, i.e. within each cycle the day of the LH surge peak may vary as a function of creatinine adjustment. (See Table I for the timings of LH surge peaks and DOST.) IRMA LH profiles are centred about the creatinine-adjusted IFMA LH surge peak for that cycle, as are the steroid values in the bottom panel. Profiles depict arithmetic means + SE for the four cycles. For abbreviations, see Figure 1.

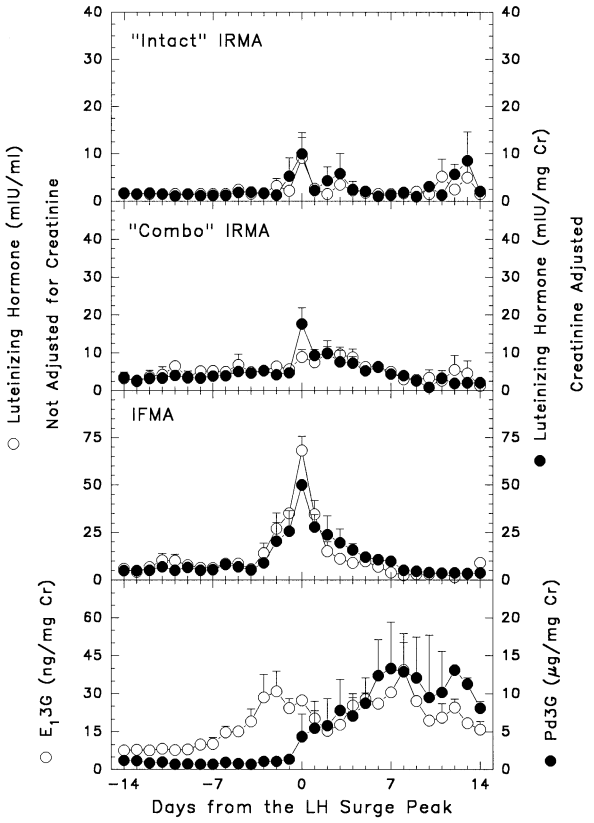

Figure 3. LH profiles for four menstrual cycles (group 3, Table I). Surges were detected in all cycles using the IFMA, but to varying extents by the IRMA. For one cycle (8:1), an LH surge was not detected by the intact-IRMA; for five IRMA profiles (one 'intact' and four 'combo'), surges were not identified until after creatinine adjustment. Each LH profile is centred graphically about its own surge peak, i.e. within each cycle the day of the LH surge peak may vary among immunoassays and after creatinine adjustment. (See Table I for the timings of LH surge peaks and DOST.) IRMA LH profiles that lack definable surges before creatinine adjustment are centred about the peak revealed after creatinine adjustment; for cycle 8:1, the intact-IRMA profiles are centred about the creatinine-adjusted combo-IRMA peak on day 15. Steroid values in the bottom panel are centred about the creatinine-adjusted IFMA LH surge peak. Profiles depict arithmetic means + SE for the four cycles. For abbreviations, see Figure 1.

Figure 4 also illustrates the impact of creatinine adjustment to reveal previously undetected LH surges (combo-IRMA), to shift the timing of LH surges (IFMA and intact-IRMA), and to improve the concurrence between the day of the IFMA LH surge onset and the DOST.

Discussion

Urine can be sampled conveniently, painlessly, and non-invasively, thereby providing an opportunity for the repetitive measurements needed to monitor and evaluate menstrual cycle functions for research or clinical purposes (Kesner *et al*., 1992b; Lasley and Shideler, 1994). However, Wilcox *et al.* (1987) reported that of three LH immunoassays, none was able to detect an LH surge in >75% of the menstrual cycles. The authors concluded that LH surges were being missed by their immunoassays, since many of the cycles without detectable LH surges exhibited patterns of oestrogen and progestin consistent with ovulation. Indeed, offspring were conceived during cycles in which there were no apparent urinary LH surges measured by a radioimmunoassay and two IRMA (Wilcox *et al*., 1987).

The present report demonstrates that an IFMA, adapted, characterized, and validated for measuring LH in urine specimens (Kesner *et al*., 1994a), reliably detects unambiguous LH surges in cycles from this study that were not detected by the two IRMA. To determine if the success of this IFMA was an aberration, we have since measured additional menstrual cycles from this same study in which LH surges had not been detected in ~30% of menstrual cycles using the two IRMA and a radioimmunoassay (Wilcox *et al*., 1987). Of the >450 cycles previously judged to be ovulatory by steroid secretion patterns, >99% yielded clear pre-ovulatory LH surges using the IFMA. Like the 14 cycles described herein, the samples from these additional cycles were 10–12 years old and had been stored frozen without preservative (unpublished data).

LH surge detection in urine has also been unreliable using some home diagnostic products (Nulsen *et al*., 1987; Kesner *et al*., 1994a). This is, at least in part, a function of the short

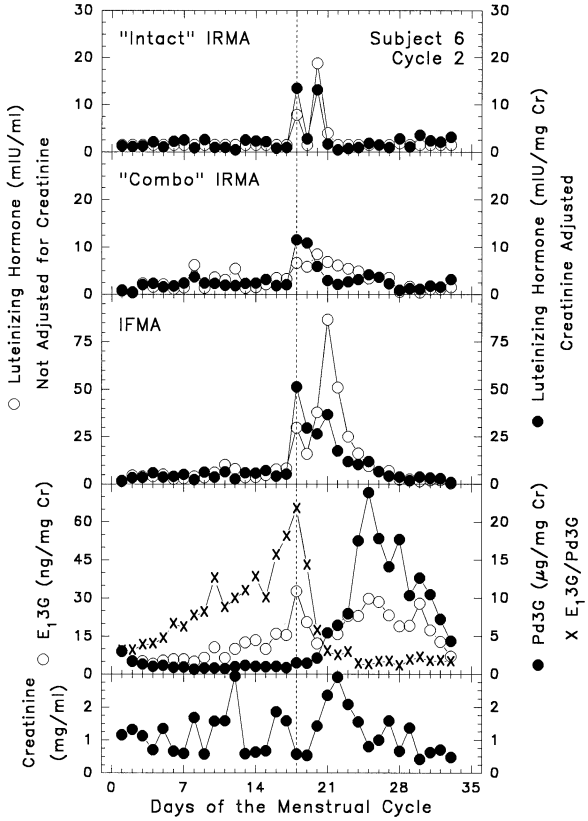

Figure 4. LH profiles measured by three immunoassays before and after creatinine adjustment for an individual menstrual cycle. Creatinine adjustment revealed the LH surge measured by the combo-IRMA and shifted the day of the LH surge peak for the intact-IRMA and IFMA. Creatinine adjustment improved the concurrence between the DOST on day 19 and the day of the IFMA LH surge onset. The dashed vertical line passes through the day of the creatinine-adjusted IFMA LH surge peak to help visualize the relative timing of the various endocrine events. Steroid and creatinine concentrations are presented in the bottom two panels.

time-frame during which the LH surge is detectable using these products, since increasing the frequency of sampling to two to eight times daily increases the rate of detection (Edwards, 1985; Nulsen *et al*., 1987; Corsan *et al*., 1990). And since the majority of pre-ovulatory LH surges seem to begin appearing in urine at 0700 h or later (Edwards, 1985), urine samples from the first morning void would be less likely to contain the highest concentrations of LH.

These do not appear to be problems with this IFMA described herein and elsewhere (Kesner *et al*., 1992a and 1994a). That is, the LH surges measured by this IFMA display a classically rapid onset, while the decay is unconventionally prolonged, lasting a few days (Kesner *et al*., 1994a). This slow decay may reflect compartmentalization of gonadotrophins by the kidney leading to a protracted clearance curve in the urine (Beitins *et al*., 1980; Nisula *et al*., 1989). Perhaps this IFMA recognizes components of this compartmentalized LH as it is cleared in the urine over several days, thereby maximizing the likelihood of detecting the urinary LH surge. Importantly, the urinary LH surges, especially the surge onset, measured by this IFMA align with serum LH surges from paired specimens (Kesner *et al*., 1992b and 1994a) and with the DOST.

Storing urine specimens frozen causes marked loss of LH immunoreactivity; adding glycerol to urine specimens abates this loss (Livesey *et al*., 1983; Saketos *et al*., 1994; Kesner *et al*., 1995). It is noteworthy, therefore, that the IFMA used in the present study detected clear LH surges in these urine specimens which had been stored frozen without preservative for 10–12 years. The nature of this IFMA to detect both intact LH and LH β-subunit may render it relatively resilient to freeze–thaw-induced LH dissociation. But while long-term frozen storage did not prevent detection of LH surges in these specimens, it may have altered the amplitude of the surge and the days identified as the LH surge onset and peak.

Others have also reported immunoassay configurations that reliably detect the LH surge in urine. Clough *et al*. (1992) have addressed this problem by developing an immunoassay that recognizes intact LH and the α-subunit shared by LH, FSH, TSH, and HCG. This assay facilitates measuring the pre-ovulatory 'gonadotrophin' surge when elevated concentrations of LH and FSH dwarf the other principal contributors of α-subunit, but does not permit LH to be measured reliably at other times when its concentrations are low and comparable to FSH and TSH. Cano and Aliaga (1995) have recently demonstrated that a commercial IRMA, directed against intact LH, reliably detects pre-ovulatory-like LH profiles concurrently in serum and untreated urine. Clearly, the ability to detect LH surges in urine is strongly dependent on the specific immunoassay.

Urinary concentrations of substances analysed are frequently divided by urinary creatinine concentrations to adjust for urine flow rate. The value of such an adjustment should be greatest when analysing quantitative data. The present report, however, reveals that qualitative attributes such as the timing or detection of the pre-ovulatory LH surge — an endocrine event of large proportions — can also be affected by creatinine adjustment. The magnitude of this impact reflects the large day-to-day variations in urine flow rates and creatinine concentrations within individuals.

Creatinine adjustment is a widely used and accepted approach to adjust for urine flow rate (Landy *et al*., 1990; Baird *et al*., 1991; Munro *et al*., 1991; Kesner *et al*., 1992a; Taylor *et al*., 1992; Keely and Faiman, 1994; Saketos *et al*., 1994) to improve the correlation between endocrine measurements in serum and urine (Seki *et al*., 1985). There are, however, reports that there is little or no advantage to its use (Chatterton *et al*., 1982; Boeniger *et al*., 1993; Demir *et al*., 1994; Hakim *et al*., 1994). The design of the present report does not directly address the validity of creatinine adjustment, and the small number of cycles examined limits the statistical confidence. Yet the qualitative effects described in this study are apparent and the statistical differences identified suggest that creatinine may improve the precision of the LH measurement.

For example, on the five occasions among four cycles when LH surges were detected by IRMA only after creatinine adjustment (group 3, Table I), the timing of these LH surges was similar to those measured in the same cycles by the other immunoassays, coinciding within 1 day of the creatinine-adjusted IFMA LH surge onset and the DOST. This indicates

that the surges revealed by creatinine adjustment are not random artefacts. A related adjustment, urinary density, has also been used to prevent the rising concentrations of the LH surge from being obscured by variations in urine concentration (Stenman *et al*., 1985).

It is not clear whether the altered timing of the onset and peak of the LH surge brought about by creatinine adjustment described here and elsewhere (Hedricks *et al*., 1994) represents an improved measurement. But in this study, variability between two indices intimately tied to ovulation, the IFMA LH surge onset and the DOST, was significantly reduced after the LH values were adjusted for creatinine (the same was not true for the peak of the LH surge). This is in keeping with the suggestion that the timing of ovulation is more precisely associated with onset of the LH surge than with its peak (Testart *et al*., 1981; Testart and Frydman, 1982; Taymor *et al*., 1983; Kesner *et al*., 1992b).

The small differences in timing between the various indices of ovulation described in our report may be critical for evaluating menstrual cycle functions. These indices include the LH surge measured by different immunoassays, the LH surge expressed before and after creatinine adjustment, the onset versus the peak of the LH surge, and the DOST (an index independent of creatinine concentrations and urinary density). Work is in progress to clarify which of these parameters is most precisely and accurately associated with a gold standard of ovulation (e.g. the LH surge in serum), and to determine if certain markers (e.g. the LH surge onset and the DOST) are redundant or whether they complement each other towards interpreting salient characteristics of the menstrual cycle.

Acknowledgements

The authors gratefully acknowledge the following: Dr Donna Baird (NIEHS) provided background information and invaluable scientific feedback; Joy Pierce (Survey Research Associates), Bob McConnaughey (Westat, Inc.), and Karen Catoe (CODA, Inc.) transferred specimens and data files between laboratories; John Clark (NIOSH) performed creatinine determinations for previously unanalysed cycles; Alma McLemore (NIOSH) provided secretarial support; and Drs Elizabeth Whelan, Shanna Swan, and Bill Lasley reviewed an earlier draft of the manuscript. The authors are also grateful for the generous gifts of $E_1$3G monoclonal antibody from Dr Fortüne Kohen (Weizmann Institute of Science, Israel), Pd3G monoclonal antibody from Dr Mohamed Gani (Unilever Research Colworth Laboratory, UK), and europium-labelled $E_1$3G and Pd3G from Dr Heikki Mikola (Wallac Oy, Finland). Mention of a trade name, proprietary product, or specific equipment in this manuscript does not constitute an endorsement, guarantee, or warranty by the National Institute for Occupational Safety and Health.

References

Baird, D.D., Weinberg, C.R., Wilcox, A.J. *et al*. (1991) Using the ratio of urinary estrogen and progesterone metabolites to estimate day of ovulation. *Statist. Med*., **10**, 255–266.

Baird, D.D., McConnaughey, D.R., Weinberg, C.R. *et al*. (1995) Application of a method for estimating day of ovulation using urinary estrogens and progesterone metabolites. *Epidemiology*, **6**, 547–550.

Beitins, I.Z., Shah, A., O'Loughlin, K. *et al*. (1980) The effects of fasting on serum and urinary gonadotropins in obese postmenopausal women. *J. Clin. Endocrinol. Metab*., **51**, 26–34.

Boeniger, M.F., Lowery, L.K. and Rosenberg, J. (1993) Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am. Ind. Hyg. J*., **54**, 615–627.

Cano, A. and Aliaga, R. (1995) Characteristics of urinary luteinizing hormone (LH) during the induction of the LH surges of different magnitude in blood. *Hum. Reprod*., **10**, 63–67.

Chatterton, R.T., Jr., Haan, J.N., Jenco, J.M. *et al*. (1982) Radioimmunoassay of pregnanediol concentrations in early morning urine specimens for assessment of luteal function in women. *Fertil. Steril*., **37**, 361–366.

Clough, K.M., Cole, F.X., Seaver, S.S. *et al*. (1992) Enzyme immunoassay method for total alpha gonadotropin in human urine samples. *Fertil. Steril*., **57**, 1241–1246.

Corsan, G.H., Ghazi, D. and Kemmann, E. (1990) Home urinary luteinizing hormone immunoassays: clinical applications. *Fertil. Steril*., **53**, 591–601.

Demir, A., Alfthan, H., Stenman, U.-H. *et al*. (1994) A clinically useful method for detecting gonadotropins in children: assessment of luteinizing hormone and follicle-stimulating hormone from urine as an alternative to serum by ultrasensitive time-resolved immunofluorometric assays. *Pediatr. Res*., **36**, 221–226.

Edwards, R.G. (1985) Current status of human conception *in vitro*. *Proc. R. Soc. Lond*., **B223**, 417–448.

Hakim, R.B., Gray, R.H. and Zacur, H.A. (1994) Is there a need for creatinine adjustment of urinary steroid hormone levels in studies of early fetal loss? *Clin. Chim. Acta*, **230**, 209–214.

Hedricks, C.A., Schramm, W. and Udry, J.R. (1994) Effects of creatinine correction to urinary LH levels on the timing of the LH peak and the distribution of coitus within the human menstrual cycle. *Ann. NY Acad. Sci*., **709**, 204–206.

Jaakkola, T., Ding, Y.-Q. and Kellokumpu-Lehtinen, P. *et al*. (1990) The ratios of serum bioactive/immunoreactive luteinizing hormone and follicle-stimulating hormone in various clinical conditions with increased and decreased gonadotropin secretion: reevaluation by a highly sensitive immunometric assay. *J. Clin. Endocrinol. Metab*., **70**, 1496–1505.

Jaffe, M. (1886) Uber den niederschlag, welchen pikrinsaure im normalen harn erzeught und uber eine neue reaktion des kreatinins. *Z. Physiol. Chem*., **10**, 391–400.

Keely, E.J. and Faiman, C. (1994) Measurement of human urinary prolactin as a noninvasive study tool. *Clin. Chem*., **40**, 2017–2021.

Kesner, J.S., Krieg, E.F., Jr, Knecht, E.A. *et al*. (1992a) Power analyses and immunoassays for measuring reproductive hormones in urine to assess female reproductive potential in field studies. *Scand. J. Work Environ. Health*, **18** (Suppl. 2), 33–36.

Kesner, J.S., Wright, D.M., Schrader, S.M. *et al*. (1992b) Methods of monitoring menstrual function in field studies: efficacy of methods. *Reprod. Toxicol*., **6**, 385–400.

Kesner, J.S., Knecht, E.A. and Krieg, E.F., Jr (1994a) Time-resolved immunofluorometric assays for urinary luteinizing hormone and follicle stimulating hormone. *Anal. Chim. Acta*, **285**, 13–22.

Kesner, J.S., Knecht, E.A., Krieg, E.F., Jr *et al*. (1994b) Validation of time-resolved fluoroimmunoassays for urinary estrone 3-glucuronide and pregnanediol 3-glucuronide. *Steroids*, **58**, 205–211.

Kesner, J.S., Knecht, E.A. and Krieg, E.F., Jr (1995) Stability of urinary biomarkers of female reproductive hormones stored under various conditions. *Reprod. Toxicol*., **9**, 239–244.

Landy, H., Schneyer, A.L., Whitcomb, R.W. *et al*. (1990) Validation of highly specific and sensitive radioimmunoassays for lutropin, follitropin, and free alpha subunit in unextracted urine. *Clin. Chem*., **36**, 340–344.

Lasley, B.L. and Shideler, S.E. (1994) Methods for evaluating reproductive health in women. *Occupational Medicine: State of the Art Reviews*, **9**, 423–433.

Livesey, J.H., Roud, H.K., Metcalf, M.G. *et al*. (1983) Glycerol prevents loss of immunoreactive follicle-stimulating hormone and luteinizing hormone from frozen urine. *J. Endocrinol*., **98**, 381–384.

Munro, C.J., Stabenfeldt, G.H., Cragun, J.R. *et al*. (1991) Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major metabolites as measured by enzyme immunoassay and radioimmunoassay. *Clin. Chem*., **37**, 838–844.

Nisula, B.C., Blithe, D.L., Akar, A. *et al*. (1989) Metabolic fate of human choriogonadotropin. *J. Steroid Biochem*., **33**, 733–737.

Nulsen, J., Wheeler, C., Ausmanas, M. *et al*. (1987) Cervical mucus changes in relationship to urinary luteinizing hormone. *Fertil. Steril*., **48**, 783–786.

Pettersson, K.S.I. and Söderholm, J.R.-M. (1990) Ultrasensitive two-site immunometric assay of human lutropin by time-resolved fluorometry. *Clin. Chem*., **36**, 1928–1933.

Saketos, M., Sharma, N., Adel, T. *et al*. (1994) Evaluation of time-resolved immunofluorometric assay and specimen storage conditions for measuring gonadotropins. *Clin. Chem*., **40**, 749–753.

Seki, K., Seki, M. and Kato, K. (1985) Correlation between urinary oestrogen levels determined by haemagglutination reaction and serum oestradiol levels determined by radioimmunoassay. *Acta Endocrinol*., **110**, 130–134.

Snedecor, G.W. and Cochran, W.G. (eds) (1967) *Statistical Methods*, 6th edn. The Iowa State University Press, Ames, Iowa, pp. 195–197.

Stenman, U.-H., Alfthan, H., Koskimies, A. *et al*. (1985) Monitoring the LH surge by ultrarapid and highly sensitive immunofluorometric assay. *Ann. NY Acad. Sci*., **442**, 544–550.

Taussky, H.H. (1954) A microcolorimetric determination of creatine in urine by the Jaffe reaction. *J. Biol. Chem*., **208**, 853–861.

Taylor, C.A., Jr, Overstreet, J.W., Samuels, S.J. *et al*. (1992) Prospective assessment of early fetal loss using an immunoenzymometric screening assay for detection of urinary human chorionic gonadotropin. *Fertil. Steril*., **57**, 1220–1224.

Taymor, M.L., Seibel, M.M., Smith, D. *et al*. (1983) Ovulation timing by luteinizing hormone assay and follicle rupture. *Obstet. Gynecol*., **62**, 191–195.

Testart, J., Frydman, R., Feinstein, M.C. *et al*. (1981) Interpretation of plasma luteinizing hormone assay for the collection of mature oocytes from women: definition of a luteinizing hormone surge-initiating rise. *Fertil. Steril*., **36**, 50–54.

Testart, J. and Frydman, R. (1982) Minimum time lapse between luteinizing hormone surge or human chorionic gonadotropin administration and follicular rupture. *Fertil. Steril*., **37**, 50–53.

Wilcox, A.J., Baird, D.D., Weinberg, C.R. *et al*. (1987) The use of biochemical assays in epidemiologic studies of reproduction. *Environ. Health Perspect*., **75**, 29–35.

Wilcox A.J., Weinberg C.R., O'Connor J.F. *et al*. (1988) Incidence of early loss of pregnancy. *New Eng. J. Med*. **319**, 189–194.

Received on March 20, 1997; accepted on September 29, 1997