Detecting pre-ovulatory luteinizing hormone surges in urine

James S. Kesner¹, Edwin A. Knecht¹, Edward F. Krieg Jr², Allen J. Wilcox³ and John F. O’Connor⁴

¹Experimental Toxicology Branch and ²Statistics Activity, Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, OH 45226, USA
²Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and ³Irving Center for Clinical Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA
⁴To 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...
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., 1987). Subjects and specimens

Materials and methods

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 125I (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 (E13G; the primary urinary metabolite of oestradiol) and Pd3G were measured by competitive fluoroimmunoassays (Kesner et al., 1994b). LH, E13G 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

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 peaks and day of ovarian steroidogenesis transition (DOST; described below) results.
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 concep- tual cycles was that the first day of the previous menstruation. An arrow (→) indicates a change in timing after creatinine adjustment.

**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.

<table>
<thead>
<tr>
<th>Subject:cycle</th>
<th>Day of the LH surge peak or onset</th>
<th>Conceived</th>
<th>DOST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak</td>
<td>Peak</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>15</td>
<td>17 → 18</td>
<td>17</td>
</tr>
<tr>
<td>1:2</td>
<td>12</td>
<td>13 → 16</td>
<td>12</td>
</tr>
<tr>
<td>2:1</td>
<td>14 → 16</td>
<td>12 → 16</td>
<td>14</td>
</tr>
<tr>
<td>3:1</td>
<td>22</td>
<td>22 → 23</td>
<td>22</td>
</tr>
<tr>
<td>4:1</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>4:2</td>
<td>17 → 16</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5:1</td>
<td>ND</td>
<td>ND</td>
<td>24</td>
</tr>
<tr>
<td>6:1</td>
<td>ND</td>
<td>ND</td>
<td>25</td>
</tr>
<tr>
<td>7:1</td>
<td>ND</td>
<td>ND</td>
<td>12</td>
</tr>
<tr>
<td>8:2</td>
<td>ND</td>
<td>ND</td>
<td>22</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:2</td>
<td>ND</td>
<td>ND</td>
<td>21</td>
</tr>
<tr>
<td>6:3</td>
<td>ND</td>
<td>ND</td>
<td>20</td>
</tr>
<tr>
<td>8:1</td>
<td>ND</td>
<td>ND → 15</td>
<td>16</td>
</tr>
<tr>
<td>9:1</td>
<td>ND → 17c</td>
<td>ND → 17c</td>
<td>20</td>
</tr>
</tbody>
</table>

IRMA = immunoradiometric assay; intact-IRMA detected primarily intact LH; combo-IRMA detected intact LH, LH β-subunit and β-subunit fragments; IFMA = immunofluorometric assay.

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.

Pregnancy ended in early loss (Wilcox et al., 1987).

ND = no LH surge detected.

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).

**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 = 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 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 progesterin 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
Measuring LH surges in urine

![Figure 4. LH profiles measured by three immunoassays before and after creatinine adjustment for an individual menstrual cycle.](image)

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 unconditionally 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 feedback; Joy Pierce (Survey Research Associates), Bob McCon-

References


Measuring LH surges in urine


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