

Measuring endocrine profiles of women in field studies

by James S Kesner, PhD,¹ Edwin A Knecht, MSc,¹ Edward F Krieg Jr, PhD²

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Improved methods are needed to evaluate the effects of occupational and environmental hazards on the reproductive health of human female populations. This communication describes highly specific, sensitive, and reliable time-resolved fluorescence immunoassays for measuring luteinizing hormone (LH) and follicle-stimulating hormone (FSH), estrone 3-glucuronide (E₃G), and pregnanediol 3-glucuronide (Pd3G) in urine, a fluid that is convenient and painless to collect serially from large populations. Furthermore, some of the technical issues relevant to the successful application of these measurements to field studies are discussed.

Key terms estrogens, follicle-stimulating hormone, luteinizing hormone, menstrual cycle, progestins, urine.

Heightened awareness towards occupational safety and health has improved conditions in the workplace. However, workers remain at risk, with the vast majority of workplace chemicals, physical agents, and other potential hazards still untested and with new ones continually being introduced. The reproductive health of women is especially vulnerable as the number of women continues to grow in the work force, as they postpone their procreation until later in life, as they work longer into their pregnancies, and as they continue to enter jobs traditionally reserved for men. Furthermore, the imbalance of the female sex hormones that can result from reproductive toxicity can reduce the general health of women and increase their risk of cancer, heart disease, and osteoporosis.

Despite the risks posed by occupational hazards on women's reproductive health, most research on human reproductive toxicity has focused on the male and on pregnancy. Most studies that have addressed the reproductive potential of women have used the overt, insensitive descriptors of menses as end points.

Accordingly, improved biological monitoring methods are needed to evaluate the reproductive potential of women in population studies. These methods should afford good participant tolerance and compliance, while yielding sensitive, specific, and reliable information

about women's reproductive status. This report describes research conducted in our laboratory to develop methods to measure the principal female hormones in urine for application in field studies of occupational safety and health.

To evaluate efficacy and participant compliance, we previously compared several methods for detecting ovulation or assessing menstrual cycle function (1, 2). Urinary levels of the primary female reproductive hormones correlated well with those in serum (1) and revealed various perturbations of menstrual cycle function (figure 1). Transvaginal ultrasonography contributed structural insights into the ovarian activity for the atypical cycles (1). In contrast, a semiquantitative dipstick failed to detect a large proportion of preovulatory luteinizing hormone surges in these frozen urine samples (1). Basal body temperature, salivary and vaginal mucous electrical resistance, and cervical mucus self-examinations afforded less reliable indications of ovulation and menstrual cycle status (1). While working women judged transvaginal ultrasonography to be uncomfortable and were reluctant to conduct cervical mucus self-examinations, they found collecting first-morning urine to be convenient, painless, and acceptable (2). In this and subsequent field studies, participants have collected more than 97% of all scheduled urine samples (2, unpublished observations).

¹ Experimental Toxicology Branch, Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, Ohio, United States.

² Statistics Activity, Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, Ohio, United States.

Reprint requests to: Dr James S Kesner, 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. [E-mail: JSK4@cdc.gov]

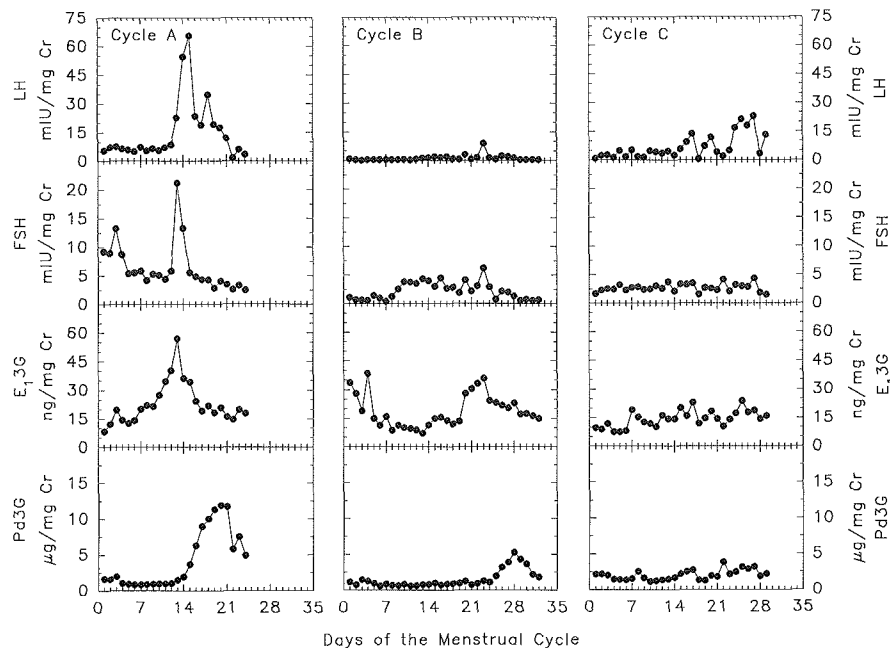


Figure 1. Profiles of urinary luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrone 3-glucuronide (E_13G), and pregnanediol 3-glucuronide (Pd3G), measured by time-resolved fluorescence immunoassays and adjusted for creatinine (Cr) levels, during 3 menstrual cycles. Although all 3 cycles have normal intermenstrual intervals, only the endocrine profile for cycle A conforms to a normal ovulatory menstrual cycle. The low Pd3G levels and short 9-day luteal phase for cycle B suggest luteal phase deficiency, while the absence of typical endocrine peaks during cycle C is consistent with anovulation.

Our laboratory developed 2 types of time-resolved fluorescence immunoassays to measure the principal female hormones in urine. Both types use the long-lived fluorophore europium as the label to minimize background noise and maximize sensitivity. For the glycoprotein hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), we modified and characterized noncompetitive 2-site immunofluorometric assays (3). The LH assay measures intact hormone, and its free beta subunit using monoclonal antibodies that bind distinct epitopes on the beta subunit. The FSH assay primarily detects the intact hormone with antibodies that bind the alpha and beta subunits. To measure the principal urinary metabolites of estradiol and progesterone, estrone 3-glucuronide (E_13G) and pregnanediol 3-glucuronide (Pd3G), we configured and characterized competitive fluoroimmunoassays (4). The following list presents the characterizations we conducted to validate these urinary assays (3, 4): sensitivity; precision (within and among assays); specificity (cross-reactivity); sample matrix interference; parallelism of dilution curves for standards versus samples; effects of urine components, eg, pH and osmolality; quantitative recovery of added mass; comparison of pituitary and urinary gonadotropin reference curves in terms of potency and parallelism; analyte stability at various storage conditions; correlation between urinary and serum hormone concentrations; correlation between urinary hormone levels and physiological response; participation in proficiency surveys and or inter-laboratory comparisons.

The stability of urinary analytes was determined as a function of storage duration, temperature, and preservative (5). While all 4 endocrine analytes were stable

without preservative for 1–2 weeks at 4°C, 7% glycerol protected the gonadotropins from damage caused by frozen storage.

To be practical, end points of population studies should afford statistical power. A rather modest difference, 20% effect, can be detected at $\alpha = 0.05$ and $\beta = 0.20$ with 25–100 participants per study group, depending on the specific urinary endocrine measure. Statistical power is increased more effectively by increasing the number of participants versus the number of cycles per participant. Greater statistical power is available from a single follicular-phase endocrine measurement in urine than in serum (6).

Immunoassays detect given analytes (eg, LH) with varying specificity, yielding values that can differ quantitatively and qualitatively among assays. A dramatic example is the ability to invariably detect clear LH surges, using the LH IRMA, in urine samples from ovulatory menstrual cycles in which LH surges had not been detected using 1–3 other immunoassays (figure 2) (7). Specificity, sensitivity, and reagent costs are among the variables to be considered when immunoassays are selected.

While urinary measurements are often divided by urinary creatinine levels to standardize for urinary flow rate, some reports suggest that creatinine adjustment neither reduces the variability of serial hormone levels nor improves the correlation between paired urinary and serum values. We showed that creatinine adjustments can have profound effects, revealing previously undetected LH surges and shifting the timing of the LH surge by as many as 5 days, in a manner consistent with improving the validity of the LH measure (7).

In conclusion, biological monitoring methods, which are highly specific, sensitive, reliable, and acceptable to participants, are available to evaluate the reproductive potential of female populations. Our laboratory is currently applying these methods in collaborative studies to evaluate the effects of occupational hazards on the reproductive health of female nurses, airline flight attendants, and US Air Force personnel. However, these methods also have value in many other research and clinical applications when serial sampling is needed. At this point, our laboratory is collaborating to identify which aspects of these complex endocrine profiles (ie, which analytes and days) best reflect variations in the fecundity of an individual person and the population.

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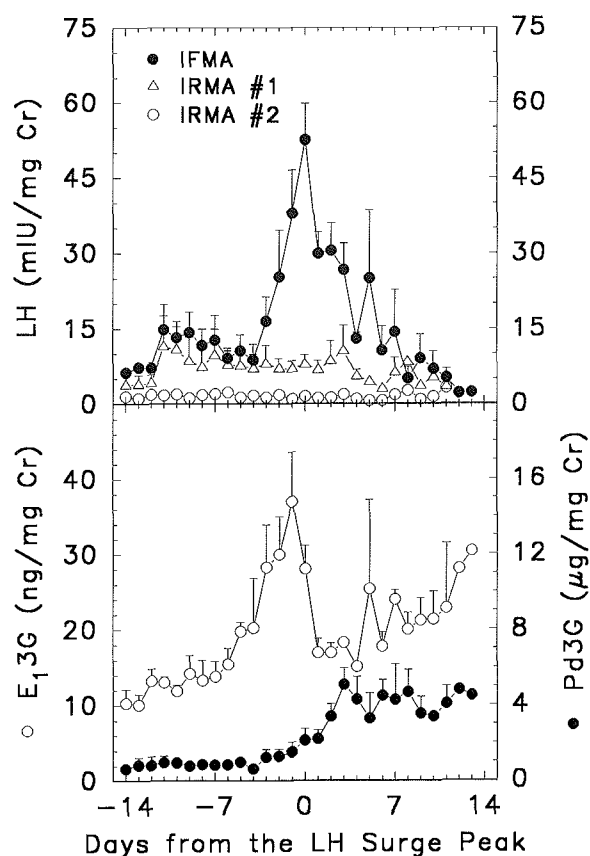


Figure 2. Detection of preovulatory luteinizing hormone (LH) surges in urine by different immunoassays. While LH surges were not detected by 2 immunoradiometric assays (IRMA), they were unambiguously and invariably detected by the immunofluorometric assay (IFMA). Endocrine profiles are centered about the IFMA LH surge. The values were adjusted for creatinine (Cr) and are arithmetic means plus standard errors for 4 cycles.

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