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Daily Luteal Serum and Urinary Hormone Profiles in the Menopausal Transition: Study of Women's Health Across the Nation

Nanette Santoro, MD^{1,*}, Samar R. El Khoudary, PhD², Alexis Nasr, MD², Ellen B Gold, PhD³, Gail Greendale, MD⁴, Dan McConnell, PhD⁵, Genevieve Neal-Perry, MD, PhD⁶, Jelena Pavlovic, MD, PhD⁷, Carol Derby, PhD⁸, Sybil Crawford, PhD⁹

¹Department of Obstetrics and Gynecology, University of Colorado School of Medicine, Aurora, Colorado

²Epidemiology Data Center, University of Pittsburgh, Pittsburgh, Pennsylvania

³Department of Public Health Sciences, University of California Davis

⁴Department of Medicine, University of California at Los Angeles/David Geffen School of Medicine, Los Angeles California

⁵Core Ligand Assay Satellite Services (CLASS) Laboratories, University of Michigan, Ann Arbor, Michigan

⁶Department of Obstetrics and Gynecology, University of Washington School of Medicine

⁷Department of Neurology, Albert Einstein College of Medicine, Bronx, New York

⁸Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY

⁹Department of Medicine, University of Massachusetts Medical Center, Worcester, Massachusetts

Abstract

Objective: To further characterize the endocrinology of the menopause transition, we sought to determine: 1. whether relationships between urine and serum hormones are maintained as women enter their sixth decade; 2. whether a single luteal phase serum progesterone (P) is reflective of integrated-luteal urinary pregnanediol glucuronide (uPdG), and 3. whether serum P, like luteal uPdG, declines as women approach their final menses (FMP).

Methods: The Study of Women's Health Across the Nation (SWAN) Daily Hormone Study's (DHS) is a community-based observational study. A subset of participants underwent a timed, luteal blood draw planned for cycle days 16-24 during the same month of DHS collection. Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2) and P, and urine LH, FSH, estrone conjugates (E1c) and daily and integrated luteal urinary pregnanediol glucuronide

*corresponding author FAX: 303-724-2061, Phone: 303-724-2041, Nanette.Santoro@ucdenver.edu.

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(uPdG) were measured in 268 samples from 170 women. Serum/urine hormone associations were determined using Pearson's correlation and linear regression, adjusted for concurrent age, body mass index, smoking status and race/ethnicity.

Results: Pearson's r ranged from 0.573 (for LH) to 0.843 (for FSH) for serum/urine correlations. Integrated luteal uPdG weakly correlated with serum P (Pearson's $r = 0.26$, $p=0.004$) and explained 7% of the variability in serum P in adjusted linear regression (total R^2 0.09, $p=0.002$). Serum P demonstrated a marginally significant decline with approaching FMP in adjusted analysis ($P=0.04$).

Conclusions: Urine and serum hormones maintain a close relationship in women into their 6th decade of life. Serum luteal progesterone was weakly reflective of luteal PdG excretion.

Precis:

Women in the latter stages of the menopausal transition demonstrate good correlation between serum and urine reproductive hormones in the luteal phase of the menstrual cycle.

Keywords

menstrual cycle; reproductive aging; LH; FSH estradiol progesterone

Introduction

Urinary hormones have been used as proxy measures for circulating serum hormones in a number of studies¹⁻³. However, the women studied have typically been in their teens or twenties and thirties, and no studies have examined whether urine and serum reproductive hormones maintain the same relationship into midlife. Yet, processing of circulating sex steroids and gonadotropins might be dependent upon alterations in body composition, with an increase of fat deposition, and reductions in muscle mass with midlife aging^{4,5}. Increased serum creatinine, attributed to a lower glomerular filtration rate, could affect apparent urinary hormone concentrations because hormones are often indexed to creatinine⁶. SWAN, the Study of Women's Health Across the Nation, performed an initial validation of urine and serum hormones on a small sample ($n<30$) of women aged 43-53 years as part of the initial Daily Hormone Study (DHS)⁷.

Studies examining midluteal progesterone as a marker for overall luteal function in women have indicated that it serves as a marker for fertility and pregnancy outcome^{8,9} as well as cardiovascular health and endothelial function¹⁰. These findings make it of interest to examine the serum progesterone (P) and urinary pregnanediol glucuronide (uPdG) patterns in cycling perimenopausal woman. The SWAN DHS collected first-morning voided urine samples for an entire menstrual cycle in a subcohort of 848 women at the DHS baseline, and annually thereafter until women experienced their final menstrual period (FMP)¹¹. This sample is an ideal data set with which to examine whether serum P and uPdG retain the same relationship to each other that they have demonstrated in younger women and to see if serum progesterone is related to luteal PdG and demonstrates the same relationship to the FMP as does luteal PdG: a decline with proximity to the FMP¹².

The SWAN Luteal Pilot Study, a subset of the DHS, was designed to address questions about the relevance of luteal phase hormones to the menopausal transition. A selected subsample of women participating in the DHS were asked to come in for a blood draw at the anticipated time of their midluteal phase and concurrent urine and serum hormones: were assessed for concordance. The association of a timed luteal serum progesterone (P) level with integrated luteal uPd_g was also assessed. Finally, we determined whether luteal serum P demonstrated a progressive decline with proximity to the final menstrual period (FMP). We hypothesized that the relationship of urine to serum hormones would be maintained as women aged and approached their final menstrual periods (FMP), and that a single serum P would provide an estimate of luteal phase Pd_g production and would also demonstrate a decline as women approached their FMP.

Methods

Cohort.

SWAN is a community based, multiethnic cohort study of middle-aged women from seven communities in the United States¹³. The SWAN DHS has been previously described in detail^{7,11,12}. Briefly, the DHS was initiated two years after the SWAN inception cohort was enrolled; women eligible for the DHS had to meet the following criteria: (1) intact uterus and at least one ovary, (2) at least one menstrual period in the previous three months (*i.e.*, either premenopausal or early perimenopausal at the time of the first collection), (3) no sex steroid hormone use in the previous three months, and (4) not pregnant or lactating. Women collected their first morning voided urine samples daily for one complete menstrual cycle or 50 days (whichever came first). Collections were repeated once per year until the FMP or for up to 10 collections). Menopausal status was defined as: (1) premenopausal, bleeding in previous three months with no past-year change in cycle predictability; (2) early perimenopausal, bleeding in the previous three months with a decrease in cycle regularity in the past year; (3) late perimenopause, between 3 and 12 months of amenorrhea; and (4) postmenopause, at least 12 months of amenorrhea. DHS collections were initiated with the onset of menses in women who were pre- or early peri-menopausal; however, with progress toward the FMP, random, 50-day (or sooner, if menses occurred) collections could be initiated after two months of an unsuccessful attempt to initiate with a menstrual period. Hispanic and White women from the New Jersey site were not included in the luteal pilot study. The study was approved by the Institutional Review Boards affiliated with all participating sites. Written informed consent was obtained from each participant.

Luteal pilot protocol.

Women who were already committed to the SWAN DHS and who provided additional informed consent were part of the luteal pilot study. Participants were asked to come in for a blood draw between days 16-24 since their last menstrual period to attempt to capture a luteal phase sample. The planned day for the luteal blood sampling was based upon the participant's average menstrual cycle length over the past year and timed to coincide to 7 days prior to the anticipated subsequent menstrual period. Blood samples were promptly centrifuged per SWAN protocol¹³ and frozen at -80° C until assays were performed. Urine samples were stored at -20° C in non-frost-free freezers as previously described⁷. Urinary

hormone measurements were completed for all analytes reported in this study within one year of collection and were not previously thawed.

Hormone Measurements.

Serum was measured for LH, FSH, estradiol (E₂) and progesterone, and urine was measured for LH, FSH, estrone conjugates (E1c), and uPd_g using established, previously described methods^{7,14}. Both serum and urine hormones were measured in singlicate using an ACS-180 automated analyzer (Bayer Corp., Norwood, MA). Serum E₂ concentrations were measured with an ACS-180 platform-based immunoassay in which the software was adapted to accommodate the E2-6 antibody used. For this adapted assay using the unique E2-6 antibody, inter- and intraassay coefficients of variation averaged 10.6% and 6.4%, respectively, over the assay range, and the lower limit of detection was 1 pg/ml¹⁵. Serum FSH, LH, and progesterone (P) concentrations were measured with a two-site chemiluminometric immunoassay using constant amounts of two monoclonal antibodies provided by Bayer Corp. Inter- and intra-assay coefficients of variation and lower limits of detection (LLD) were as follows: for FSH 10.9% and 3.9% with an LLD of 1.18 IU/L¹⁴; for LH 10.7% and 4.8% with an LLD of 0.1 IU/L; for P 7.3% and 3.0% with an LLD of 0.1 ng/mL.

As previously described, urine samples were collected in 7% glycerol to preserve the integrity of the molecules and make the specimens suitable for long-term storage and all urinary hormone levels were normalized to creatinine prior to any analysis⁷. Inter- and intraassay coefficients of variation and LLDs were as follows: for FSH: 11.4% and 3.8%, LLD 0.3 mIU/ml; for LH 10.9% and 4.6%, LLD 0.1 mIU/ml; for E1c 11.5% and 8.1%, LLD 0.1 ng/ml; and for Pd_g 17.8% and 7.7%, LLD 0.1 pg/ml⁷.

Cycle evaluation.

Objective, validated algorithms were applied to detect a sustained, robust rise in Pd_g consistent with ovulation¹⁶, and the timing of this luteal shift was determined by a shift in the ratio of E1c to Pd_g, and designated as the day of luteal transition (DLT); data were centered to this date, which was set to 0¹⁷. Cycles in this study were divided into those that qualified as having robust luteal function (evidence of luteal activity: ELA) vs. those that did not qualify as meeting this criterion (non-ELA). Area-under-the curve methods, as previously described¹¹, were applied to Pd_g excretion curves to evaluate luteal function using urinary Pd_g (integrated uPd_g)¹². For ELA cycles, hormones were organized around the DLT, which was designated Day 0. Integrated uPd_g was measured from Day 0 through the end of the collection for ELA cycles.

Data analysis.

Because each of the study outcomes involved slightly variable sample availability, Supplemental Table 1 provides details on the exact sample sizes for each outcome. All cycles in the luteal pilot study that had a serum collection within the 16-24-day window after the last menstrual period were used to assess whether the relationship between urine and serum hormones are maintained as women enter the sixth decade of life. However, for hypotheses examining whether a single luteal phase serum P level is reflective of integrated-

luteal urinary Pd_g, only ELA cycles in which the serum sample was correctly drawn during the luteal phase were used (N=125); these cycles were retrospectively defined. To test the association between a single luteal phase serum P and time relative to the FMP, analytical sample only included women for whom we had FMP date and luteal phase serum P available. Hormone values were log transformed due to skewness in the distributions of values; after log transformation, the data were normally distributed. Correlations between concurrent serum and urine hormones were assessed using unadjusted Pearson's correlation. Linear regression was used to determine adjusted proportion of variability in serum hormones that were explained by their urinary metabolites while accounting for race/ethnicity¹² age and log-BMI,¹⁴ and smoking status at urine collection. Linear regression was also used to assess association between single luteal phase serum P and time relative to the FMP. Time to the FMP was calculated as the difference in years between date of serum collection and date of FMP. The significance level was adjusted to account for multiple testing per evaluated hormone as denoted in footnotes to tables 2 and 3a and b. Most of the women were sampled only once in the luteal pilot; however 77 women provided a concurrent serum and urine sample in more than one year, and those additional time points were not included in primary analysis. Secondary analyses using linear mixed model to adjust for correlated data provided same findings (data not shown).

Results

Analytical sample.

A total of 274 serum samples were collected concurrent with the DHS urine collection within a post-menstrual window of 16-24 days (see Supplemental Table 1 for flow chart). Six of these samples were excluded due to either missing serum or urine hormones for the date of collection, or missing creatinine values, which precluded accurate reporting of urinary hormone concentrations. Thus a total of 268 concurrently collected urine and serum samples were available across multiple time points. Serum and urine hormone data from first available collection were used for analysis from 170 women for E1c/E and for Pd_g/P pairs, but because two urine samples were missing for FSH and 11 serum samples were missing for LH, a total of 168 and 159 women had FSH and LH paired for serum and urine hormone assessments. Out of the 170 women with first available matching samples, 125 women had ELA cycles in which the serum sample was correctly drawn during the luteal phase and thus included in integrated luteal uPd_g analysis, of those, 77 women additionally had FMP date available and were thus included in analysis relative to time since FMP (Supplemental Table 1). Participants were distributed across the racial and ethnic groups represented by SWAN, with the exception of Hispanic women who were under-represented (Table 1a). Overall, participants were slightly younger and earlier in their menopausal transition than the rest of the SWAN cohort, consistent with their eligibility for the DHS. Most were in the early perimenopause, and premenopausal women and those in the early perimenopause were similar in characteristics (Table 1a). Serum and urine LH and FSH were significantly higher in early peri-menopausal women compared to premenopausal women (Table 1b).

Associations between serum and urine hormones.

For LH, FSH, estrogen and progesterone, the relationship between serum and urine levels was statistically significantly positive (Figure 1, Table 2).

- A. Pearson correlation. Pearson's r varied from a low for LH of 0.573 to 0.845 for FSH.
- B. Multivariable linear regression. After adjustment for race/ethnicity, BMI, and smoking status, β -coefficients ranged from a low of 0.44 to 1.1 for LH and P/uPdg, respectively. Corresponding adjusted R^2 values ranged from 0.37 for E/E1c, to 0.42 for LH, 0.74 for P/Pdg and 0.76 for FSH (Table 2).

Relationship between luteal P and urinary Pdg in luteal-phase-only samples.

Although blood sample collection was timed to coincide with the midluteal window (days 5-9 post-DLT), this level of precision was only achieved in 66 or 52.8% of first available cycles with evidence of luteal activity in the luteal pilot ($n=125$). Early luteal (days 0-4 post-day of luteal transition (DLT) serum samples constituted 29.6% ($n=37$) and late luteal (10 days post-DLT) samples 17.6% ($n=22$). The correlation of serum P to urinary Pdg was unchanged when only luteal samples were assessed (data not shown).

Relationship between luteal P and integrated luteal uPdg, and luteal serum P with approach of the FMP.

Adjusted linear regression for log-transformed serum P and integrated luteal uPdg indicated a statistically significant β -coefficient of 0.4 and R^2 of 0.09 (Table 3a). Pearson's r was also significant at 0.26 (Table 3a). When examined by the stage of the luteal phase when the blood sample was drawn, the mid-luteal phase (Days 5-9 after the DLT) and late luteal phase (10 days after the DLT) relationships remained significant, but the early luteal phase (Days 0-4 after the DLT) did not demonstrate a significant relationship between serum P and integrated luteal uPdg (Table 3b).

Finally, concurrent serum P, urinary Pdg, and integrated luteal uPdg were examined in relationship to the FMP. While both same-day urinary Pdg and integrated luteal uPdg were related to the time to the FMP, luteal serum P was not related to the timing of the FMP in the unadjusted analysis. Results were similar after adjusting for age, race/ethnicity, body mass-index and smoking status, although luteal serum P became marginally significantly associated with time to FMP (Table 4).

Discussion

Herein we have demonstrated that the robust relationships between urinary and serum hormones that have been previously reported in mid-to-late reproductive aged women^{1,2,7} are maintained in women as they approached their FMP and entered the sixth decade of life. These data provide reassurance for future research using urinary hormone analyses in populations of aging women and help further validate the analyses of the SWAN DHS. Moreover, we demonstrated the feasibility of a planned luteal phase blood sampling paradigm, despite the relative irregularity of menstrual cycles during that time. However,

unlike in midreproductive life, luteal serum progesterone seems less associated with overall progesterone output across the luteal phase.

Urinary hormone assays have demonstrated great usefulness for epidemiologic and animal studies, as they can provide a great deal of information over long periods of time, are non-invasive, and require minimal preparation and handling to obtain a reliable sample. The ability to follow women over months to years has been useful in elucidating the reproductive endocrine processes surrounding the onset of menarche³, the processes of premature menopause¹⁸ and diminished ovarian reserve¹⁹, in addition to a number of studies of the menopausal transition²⁰. Urinary hormones are assumed to be reflective of serum hormones, as urine is an ultrafiltrate of plasma, and, absent degradation in the circulation, urinary hormones should be an effective proxy for serum. It is therefore important to assure the integrity of urinary hormone assays, especially so in a study such as SWAN, which has 10 years' worth of longitudinal urinary hormone data. We found that, overall, the strongest relationships were seen between serum and urine for FSH and P/Pdg, and less strong, although highly statistically significant relationships were observed for LH and for E/E1c. These findings are somewhat expected, because both urine and serum FSH and P/Pdg represent the same molecular species, with Pdg, or pregnanediol glucuronide being the principal metabolite of serum P. However, the less strong relationship and greater overall variation between serum and urine LH was less expected, because correlations between serum and urine LH in younger women have been more robust^{2,7}. Finally, E1c, which represents a mixture of estradiol, estrone, and both glucuronide and sulfated conjugates, undergoes the most metabolism of all four reproductive hormones measured herein, and would therefore be more likely to have the weakest association with serum estradiol.

A single, prospectively timed luteal phase serum collection was successful in at least targeting some time point at or after the DLT for 52% of the evaluated women. This finding indicates that targeting the luteal phase for blood sampling may be feasible for women in the early menopausal transition, i.e., before cycle irregularity becomes too great. This is of interest, because luteal progesterone production may be a predictor of cycles that are more likely to be fertile and to result in pregnancy, which is true for midreproductive aged women⁸. Luteal sampling may also help identify women with anovulatory bleeding, which has been linked to endometrial hyperplasia risk in perimenopausal women²¹. It may also be desirable to time a luteal blood hormone collection to measure corpus luteum hormones that are not secreted into urine, such as inhibin A or relaxin.

A single, midluteal serum progesterone level has been used to determine the probability of pregnancy⁸ in 2376 cycles of infertile women who were undergoing ovulation stimulation with clomiphene, letrozole, or gonadotropins. These investigators found that a progesterone level above the 10th centile for each treatment group was associated with more than twice the probability of pregnancy than lower midluteal progesterone. This finding implies that midluteal progesterone reflects the overall robustness of the menstrual cycle. Others have examined progesterone levels in unstimulated pregnancy cycles of 297 women and compared them to 406 non-pregnancy cycles to determine the lower limit of progesterone associated with a pregnancy. They identified a fifth percentile of progesterone of 5.6 ng/ml, and no pregnancies were observed in women with a midluteal progesterone below 2.3 ng/

ml⁹. Our findings imply that the reproductive competency of the menstrual cycle of perimenopausal women may be evaluable with a timed midluteal progesterone level. However, hormonal output of the menstrual cycle in reproductively aging women is only one part of predicting fertility; oocyte quality and quantity are also critical predictors of reproductive outcome. Early, mid and late luteal serum progesterone levels (7.6 [4.6,10.1], 14.7 [10.8, 18.9] and 6.6 [4.2, 10.4] ng/ml, respectively) indicate that an expected pattern of luteal progesterone secretion was likely achieved, an approximately bell-shaped curve rising one day after ovulation and peaking in the midluteal phase at or around 7 days postovulation.

Progesterone may be of importance in the menopausal transition, apart from its role in ovulation and fertility. Ambient progesterone has been associated with decreased arterial stiffness in 42 midreproductive aged women who were studied during the early and late follicular phase and again in the luteal phase during confirmed ovulatory cycles¹⁰.

We have previously shown that integrated luteal uPd_g declines with proximity to the FMP¹². In this study, we observed that Pd_g was related to timing of the FMP but observed only a marginally significant decrease in luteal serum P with proximity to the FMP. Larger studies are needed to confirm this observation. This finding stands to reason, inasmuch as uPd_g represents multiple measurements over the course of the entire luteal phase, and therefore likely reflects most accurately the totality of corpus luteum function. However, urinary measurements are generally believed to be more subject to within- and between-woman variation and possibly less reflective of hormone production than are circulating serum hormones. The current study highlights the value of more comprehensive luteal phase sampling in detecting change over time and justifies the use of urinary measurements for this purpose.

This study had some strengths and weaknesses. We know of no other study that has been performed to assess luteal function in perimenopausal women. Because our serum hormones were collected in the context of a menstrual cycle in which women were collecting daily urine samples, it was possible to place each sample within a definite point in the menstrual cycle, with strong confirmation that we observed truly luteal blood samples. On the other hand, our ability to collect a sample within the midluteal window was only successful in about 50% of women, which likely limited our sample to the more regularly cycling women in the sample. Thus, this group may be less representative of the entire pool of women within the luteal pilot sample. Moreover, in breaking down the luteal samples to early, mid, and late luteal phases, sample sizes became small, with cell sizes as small as 22, which may have provided inadequate statistical power to detect some modest but meaningful associations (eg, the relation of serum P to timing of the FMP) as statistically significant. The multiple statistical testing performed in this study may also have led to some spurious findings of statistical significance. Adjusting the P level of significance for some of the statistical comparisons hopefully minimized this type of potential error.

Conclusion

In summary, we have demonstrated that the excellent correspondence between urine and serum hormones is maintained among women who are undergoing the menopausal transition, and that urinary hormone tracking of menstrual cycles remains a valid strategy for elucidating the reproductive endocrinology of this time period of a woman's life. Moreover, we have observed that a timed, midluteal progesterone level is reflective of the PdG output of the entire luteal phase, although the overall strength of the correlation was weak. These findings make it possible to study the reproductive endocrinology of the menopausal transition in greater detail, with an ability to focus on the corpus luteum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix:

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Steering Committee: Susan Johnson, Current Chair

Chris Gallagher, Former Chair

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Figure 1:

Fit plots for log-transformed serum and urine hormones: LH, FSH, E/E1c and P/Pdg. For each pair, the serum hormone is shown on the Y axis and the urinary hormone is on the X axis. 95% confidence limits are shown in gray.

Table 1a:Characteristics of DHS participants^a by menopausal status

Characteristics	Total (n=170)	Premenopausal (n=26)	Early Peri- menopausal (n=141)	p-value
Age, years, mean (SD)	49.4 (2.2)	48.8 (2.2)	49.5 (2.1)	0.18
Ethnicity, n (%)				0.90
White	55 (32.4%)	8 (30.8%)	46 (32.6%)	
Black	32 (18.8%)	6 (23.1%)	25 (17.7%)	
Chinese	31 (18.2%)	5 (19.2%)	25 (17.7%)	
Japanese	52 (30.6%)	7 (26.9%)	45 (31.9%)	
BMI, kg/m ² median (Q1, Q3)	24.70 (22.0, 29.2)	26.3 (22.8, 30.3)	24.6 (22.0, 29.2)	0.37
Smoking Status, n (%)				0.16
Never Smokers	117 (68.8%)	15 (57.7%)	101 (71.6%)	
Ever Smokers	53 (31.2%)	11 (42.3%)	40 (28.4%)	

Table 1b.

Paired serum and urine hormones from the same date for all participants and stratified by menopausal status

Hormone	Total	Premenopausal	Early Peri-menopausal	p-value
Serum				
LH, median (Q1, Q3)	5.16 (2.95, 11.71)	3.23 (1.58, 6.20)	5.47 (3.06, 11.88)	0.006
FSH, median (Q1, Q3)	6.80 (4.60, 16.40)	4.65 (3.30, 9.20)	7.1 (4.9, 17.2)	0.005
E2, median (Q1, Q3)	122.68 (79.95, 178.15)	145.83 (87.25, 221.70)	121.45 (78.10, 168.45)	0.12
Progesterone, median (Q1, Q3)	8.71(1.78, 14.37)	9.77 (4.33, 14.37)	8.72 (1.44, 14.52)	0.49
Urine				
LH, median (Q1, Q3)	1.18 (0.55, 2.76)	0.70 (0.45, 1.26)	1.28 (0.57, 3.16)	0.008
FSH, median (Q1, Q3)	8.43 (4.82, 21.89)	6.30 (3.27, 11.42)	8.66 (5.10, 25.44)	0.01
E1C, median (Q1, Q3)	48.51(35.00, 64.17)	51.15 (39.82, 51.15)	47.29 (34.06, 63.78)	0.22
Pdg, median (Q1, Q3)	2.91 (0.82, 5.23)	3.14 (1.61, 8.27)	2.91 (0.82, 5.04)	0.21

^aIncludes all women who had a paired serum and urine sample taken on the same date, timed to 16-24 days after a menstrual period. When comparing characteristics by menopausal groups, 3 participants (late perimenopausal (n=1) and indeterminate menopausal status (n=2) were excluded due to small sample size.

Table 2:

Pearson's correlation coefficients and multivariable linear regression for the association between log-transformed serum hormones and log-transformed urine hormones for participants with matching serum and urine collection dates within 16-24 days of the menstrual cycle

	Pearson's Correlation			Multivariable Linear Regression ^a				
Hormone	N	Pearson's r	p-value	N ^b	β (SE)	95%CI	R ²	p-value ^c
LH	159	0.573	<0.0001	156	0.44 (0.05)	(0.34, 0.53)	0.42	<0.0001
FSH	168	0.845	<0.0001	164	0.76 (0.03)	(0.66, 0.79)	0.76	<0.0001
E2/E1c	170	0.574	<0.0001	167	0.84 (0.09)	(0.65, 1.02)	0.37	<0.0001
P/uPdg	170	0.837	<0.0001	167	1.10 (0.05)	(0.99, 1.20)	0.74	<0.0001

^aModel adjusted for ethnicity, age, log-body mass index at urine collection and smoking status

^bLH: 2 observations with missing body mass index and 1 outlier excluded; follicle stimulating hormone (FSH): 2 observations with missing body mass index and 2 outliers excluded; estradiol/estrone conjugates (E2/E1c): 2 observations with missing body mass index and 1 outlier excluded; progesterone/urine pregnanediol glucuronide (P/uPdg): 2 observations with missing body mass index and 1 outlier excluded

^cSignificance level adjusted for multiple testing of serum LH, FSH, E2 vs urine LH, FSH, E1C=0.0125; and of P as related to uPdg= 0.01

Table 3a.

Linear regression models and Pearson's correlation coefficients for the association between log-transformed serum progesterone and integrated luteal uPd_g for all ELA participants who had a known DLT and serum collected post-DLT (n=122)

	Pearson's Correlation			Multivariable Linear Regression ^a				
Hormone	N	Pearson's r	p- value	N	β (SE)	95% CI	R ²	p-value ^b
Integrated Luteal uPd_g	122	0.260	0.004	122	0.40 (0.13)	(0.15, 0.65)	0.09	0.002

^aModel adjusted for ethnicity, age, log-body mass index at urine collection and smoking status

^bSignificance level adjusted for multiple testing of P as related to uPd_g =0.01

Table 3b.

Pearson's correlation coefficients for log-transformed serum hormones and urine hormones for all participants with cycles with evidence of luteal activity (ELA) who had a known day of luteal transition (DLT) and serum collected post-DLT (n=125) by group of DLT collection

	0-4 days post DLT		5-9 days post DLT		10 days post DLT	
	N	r (p-value ^a)	N	r (p-value ^a)	N	r (p-value ^a)
LH	36	0.691 (<0.0001)	61	0.146 (0.26)	20	0.299 (0.20)
FSH	36	0.767 (<0.0001)	65	0.655 (<0.0010)	22	0.659(0.0008)
E2/E1c	37	0.530 (0.0007)	66	0.143 (0.25)	22	0.543 (0.009)
P/Pdg	37	0.365 (0.03)	66	0.427 (0.0003)	22	0.713 (0.0002)

^aSignificance level adjusted for multiple testing of serum LH, FSH, E2 vs urine LH, FSH, E1C=0.0125; and of P as related to uPdg= 0.01

Table 4:

Linear regression for the association between log-transformed serum progesterone with time relative to the final menstrual period (FMP) in participants with cycles with evidence of luteal activity (ELA) with serum/urine measurements during the luteal phase

Hormones	Models ^a	N	β (SE)	95% CI	R ²	p-value
Serum Progesterone	Model 1	77	-0.07 (0.04)	(-0.15, 0.01)	0.04	0.09
	Model 2	77	-0.10 (0.05)	(-0.19, -0.003)	0.10	0.04
Urine pregnanediol glucuronide (PdG)	Model 1	77	-0.13 (0.05)	(-0.22, -0.04)	0.10	0.006
	Model 2	77	-0.15 (0.05)	(-0.25, -0.04)	0.17	0.007
Integrated Luteal uPdG	Model 1	75	-0.12 (0.03)	(-0.18, -0.06)	0.17	0.0002
	Model 2	75	-0.12 (0.03)	(-0.19, -0.06)	0.27	0.0005

^aModel 1: Unadjusted, Model 2: Adjusted for race/ethnicity, age, log-body mass index at urine collection and smoking status