A comparison of blood spot vs. plasma analysis of gonadotropin and ovarian steroid hormone levels in reproductive-age women

Alison Edelman, M.D.,a Richard Stouffer, Ph.D.,a,b David T. Zava, Ph.D.,c and Jeffrey T. Jensen, M.D.a,b

a Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, Oregon; and b Division of Reproductive Sciences, Oregon National Primate Research Center and c ZRT Laboratories, Beaverton, Oregon

Objective: To compare LH, FSH, P, and E2 levels obtained from blood spot vs. plasma (single-visit study) and to determine whether blood spots can document circulating hormone levels during ovulatory cycles (menstrual-cycle study).

Design: Cross-sectional study.

Setting: Academic center.

Patient(s): Women 18–35 years of age with regular menstrual cycles and no recent use of hormonal contraception.

Intervention(s): Women contributed both a blood spot sample from a finger-stick and a plasma sample through venipuncture on a random day within their menstrual cycle (n = 100, single study visit). Five additional women were followed for an entire menstrual cycle with biweekly venipuncture and daily self-collected blood spot sampling. Samples were analyzed for FSH, LH, P, and E2.

Main Outcome Measure(s): Correlation between blood spot and plasma levels.

Result(s): Significant positive correlations were found between the blood spot and plasma samples in the single-visit study (r²: FSH, 0.91; LH, 0.93; P, 0.83; and E2, 0.70). Two of the 5 menstrual-cycle study women had ovulatory cycles based on P levels (>3 ng/mL) and an LH surge. Daily blood spot sampling was better able to document hormonal changes than biweekly venipuncture.

Conclusion(s): Blood spot monitoring of FSH, LH, P, and, to a lesser extent, E2, appears to be as valid as traditional plasma assays for clinical research and care. (Fertil Steril 2007;88:1404–7. ©2007 by American Society for Reproductive Medicine.)

Key Words: Blood spot, gonadotropins, FSH, LH, progesterone, estradiol, hormone monitoring, ovulation, enzyme immunoassay

Plasma or serum samples obtained via repetitive venipuncture represent the accepted criterion standard for monitoring circulating levels of FSH, LH, E2, and P in published reproductive studies. Unfortunately for research subjects, the burden of frequent venipuncture is high (e.g., uncomfortable and time-consuming), and for researchers, venipuncture samples require immediate processing and storage facilities with freezers. Although less invasive techniques for measuring hormone levels currently exist, their reliability in reproductive research remains unproven.

One promising method involves self-collection of small blood samples through finger sticks (blood spot testing). The samples (collected on special paper and dried) do not require immediate processing and can be stored at room temperature at home by a research subject for several weeks. Frequent office visits can therefore be avoided, but frequent sampling still can be performed. Blood spots successfully have been used to screen newborns for metabolic diseases and hypothalamic function and for anthropologic research, but neither of these situations requires the assay range and specificity that are crucial for reproductive research (1–4).

Despite the apparent advantages, it remains unknown whether the results obtained from blood spot samples are reliable, specific, and sensitive enough to measure small changes in pituitary gonadotropins and ovarian steroid hormones that are important in studies of menstrual cyclicity and ovulation detection.

Further validation of blood spot assays is necessary before this approach is adopted for reproductive research. This study was designed to compare LH, FSH, P, and E2 levels obtained.
from blood spot vs. plasma samples, to determine whether blood spot assays can document peptide and steroid hormone changes seen in ovulatory cycles and to determine the feasibility of relying on self-collected samples for reproductive studies.

MATERIALS AND METHODS

The Institutional Review Board at Oregon Health and Sciences University (OHSU) and the General Clinical Research Center Scientific Advisory Committee approved the study protocol, and all patients provided informed written consent. Enrollment took place at OHSU between December 2004 and February 2005. Women 18 to 35 years of age and with a history of regular menstrual cycles and no recent use of hormonal contraception were eligible for enrollment (n = 100). Women contributed both a blood spot sample from a finger-stick and a plasma sample through venipuncture during a single-visit study during a random day within their menstrual cycles. Five additional women were followed for an entire menstrual cycle (menstrual-cycle study), with biweekly venipuncture, biopsy, and daily self-collected blood spot sampling. Demographic data was collected on all patients at study entry.

Venipuncture samples (approximately 15 mL) were collected and processed by centrifuge at 1,500 x g for 10 minutes. The plasma samples were then stored at −80°C until analyzed. Plasma samples were analyzed separately by both the OHSU General Clinical Research Center laboratory and a commercial laboratory specializing in blood spot testing (ZRT Laboratory, Beaverton, OR) by using commercially available kits (at OHSU, for LH, FSH, and P, an automated chemiluminescent assay [Diagnostic Products Corporation, Los Angeles, CA, http://www.dpcweb.com], and for E2 an RIA [Diagnostic Systems Laboratories, Webster, TX, http://www.dslabs.com]; and at ZRT, for E2 and P, an enzyme immunoassay [DRG Diagnostics, Marburg, Germany, http://www.drg-diagnostics.de], and for FSH and LH, a fluoroimmunoassay [Perkin Elmer-Wallac, Wellesley, MA, http://las.perkinelmer.com]).

Blood spot samples (whole blood) were obtained via finger-sticks (with lancets) and dropped onto specialized filter paper (Schleicher and Schuell 903; Bioscience, Keene, NH). Three to six spots, each of approximately 1 cm in diameter, onto one card, equalled one sample. Samples were dried for ≥1 hour and then were stored at room temperature for ≤1 month before being analyzed for FSH and LH. After processing the FSH and LH samples (described 3 paragraphs below), the blood spot specimens were desiccated, frozen at −70°C, and stored for approximately 10 months before being thawed for P and E2 analysis. Several random E2 blood spot samples were initially tested to try to improve the sensitivity of the assay, thereby leaving fewer samples for the final analysis.

Standard, control, and participant disks (6.4 mm) were punched out with the Wallac Multipuncher Dried Bloodspot Puncher (Perkin Elmer-Wallac) into 96 deep-well (2 mL per well) plates and rehydrated in 200 μL per disk of assay buffer containing phosphate-buffered saline (Diamedix, Miami, FL), 0.025% Tween 20, and 0.01% ProClin 950 antimicrobial (Sigma-Aldrich, St. Louis, MO). Blood spot assays were analyzed by using modified immunoassays at the ZRT laboratory (for E2 and P, with an enzyme immunoassay [DRG]; and for FSH and LH, with a fluoroimmunoassay [Perkin Elmer-Wallac]).

Standards for blood spot assays were prepared by mixing E2 or P standards (DRG) or LH and FSH standards (Perkin-Elmer) 1:1 with washed human red blood cells prepared by the Red Cross (Pacific Northwest Regional Blood Services, Portland, OR). Control blood spots containing low (BioRad1), medium (BioRad2), and high (BioRad3) levels of E2, P, LH, and FSH are prepared by mixing the reconstituted BioRad samples 1:1 with washed red blood cells (Bio-Rad Laboratories, Anaheim, CA). Large lots of standards and controls were prepared by spotting multiple 50-μL (near equivalent to volume of a finger-stick blood drop) aliquots onto large filter cards, desiccated, and then stored at −30°C to −70°C until brought to room temperature for analysis.

For blood spot FSH and LH, intra- and interassay coefficients of variance (CVs) were as follows: for FSH, 8.6%–16.0% (BioRad1: 7.1 U/L), 8.0%–15.8% (BioRad2: 15.3 U/L), and 5.9%–9.2% (BioRad3: 41.7 U/L); and for LH, 16.3%–17.7% (BioRad1: 1.6 U/L), 7.4%–9.6% (BioRad2: 16.8 U/L), and 7.9%–10.5% (BioRad3: 50.5 U/L). Blood spot intra-assay CVs for E2 and P were <10%. Interassay CVs for E2 were 22% (BioRad1: 113 pg/mL), 11.7% (BioRad2: 233 pg/mL), and 10.3% (BioRad3: 389 pg/mL). Interassay CVs for P were 19% (BioRad1: 0.94 ng/mL), 12.5% (BioRad2: 7.6 ng/mL), and 10% (BioRad3: 17 ng/mL). The limits of detection (sensitivity) based on blank average ±2 SD for FSH, LH, E2, and P were, respectively, 0.078 U/L, 0.065 U/L, 17 pg/mL, and 0.18 ng/mL. Assay linearity for FSH, LH, E2, and P were, respectively, throughout the ranges 0.2–250 U/L, 0.2–256 U/L, 30–1,000 pg/mL, and 0.3–40 ng/mL.

Blood spot and plasma samples were assigned unique identifying numbers, such that the analysis was blind to the relationship between the samples. Only the principal investigator, who was not involved in sample analysis, had access to the key. Descriptive statistics were used to summarize demographic data (means, frequencies). Two replicates of each sample were performed and then averaged. Plasma results from ZRT and OHSU were compared by using Pearson correlation testing. Plasma results from ZRT and OHSU were separately compared with blood spot results for each paired sample from the single-visit study by using a paired t-test and Pearson correlation testing. For the menstrual-cycle study, the same statistics were used. All analysis was performed on the basis of intent to treat. Statistical analyses were performed by using the Statistical Program for Social Sciences (version 10.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

Of the 100 women enrolled in the single-visit study, paired plasma and blood spot samples were available for analysis.
from 90 for LH and FSH (1 protocol violation with age > 35 years, 1 unsuccessful venipuncture, and 8 insufficient plasma samples), 66 E₂ samples (24 insufficient blood spot samples), and 59 P samples (31 insufficient blood spot samples). A total of five women were enrolled in the menstrual-cycle study. Overall for the entire study cohort, the average subject was a 28-year-old (SD, 3.9 y), Caucasian (82%), nulliparous (61%) woman with a body mass index of 25 kg/m² (SD, 5.8).

Plasma levels for gonadotropin and steroid levels were comparable when analyzed by the ZRT and OHSU General Clinical Research Center laboratories ($r^2$: FSH, 0.97; LH, 0.97; P, 0.92; and E₂, 0.86); therefore, the plasma samples used for comparison to the blood spot samples are those from ZRT Laboratory. Significant positive correlations were found between the blood spot and plasma samples for the single-visit study (Fig. 1; $r^2$: FSH, 0.91; LH, 0.93; P, 0.83; and E₂, 0.70). Excluding E₂ data points of <50 or <100 pg/mL did not improve the correlation between plasma and blood spot samples. The mean hormone values obtained from blood spots appeared modestly but significantly different ($P < .001$) than did those derived from plasma samples (FSH, 4.0 ± 1.8 mIU/mL vs. 4.5 ± 1.9 mIU/mL; LH, 5.3 ± 3.4 mIU/mL vs. 6.1 ± 4.1 mIU/mL; P; 6.1 ± 8.0 ng/mL vs. 4.2 ± 6.2 ng/mL; and E₂, 117.2 ± 57.4 pg/mL vs. 54.7 ± 32.1 pg/mL; Fig. 1).

Two of the five women providing daily blood spots and biweekly plasma samples (menstrual-cycle study) had ovulatory cycles based on P levels of ≥ 3 ng/mL, with an LH surge by either blood spot or plasma sampling. Figure 2 demonstrates the FSH, LH, P, and E₂ variation during one subject’s ovulatory cycle with biweekly plasma sampling and daily, self-collected, blood spot sampling. Although comparable in appearance, the LH peak was missed with biweekly venipuncture sampling.
**DISCUSSION**

Blood spot sampling appears to be an effective and accurate alternative to venipuncture for FSH, LH, and P monitoring. Although promising, E₂ results are more varied. These results are not surprising because even RIAs for E₂ on plasma samples experience this problem because of the low levels of E₂ (pg/mL) in blood.

The mean hormone values for the single-visit study were significantly different when comparing blood spot with venipuncture sampling. The magnitude of these differences is clinically insignificant given that these hormones normally have a much larger range over the course of a menstrual cycle and that a commercial assay’s CV can be ≤10%. However, two paired P samples were extremely incongruous (blood spot, 24 ng/mL, vs. plasma, 0.28 ng/mL; blood spot, 12 ng/mL, vs. plasma, 25 ng/mL). The technique for both of these blood spot samples was not optimal (e.g., supersaturated or overlapping blood drops), and this may have affected the results, although exclusion of imperfect blood spot samples from the overall analysis did not affect correlation of blood spots and plasma samples.

The main advantage of blood spots over venipuncture is the opportunity to capture a greater number of data points without significantly increasing the burden to patients and/or research subjects. The menstrual-cycle study participants successfully performed daily self-sampling and stored samples at home. As demonstrated by our results, biweekly venipuncture sampling missed the LH surge, whereas daily blood spot sampling was able to document the peptide and steroid hormone changes seen in a typical ovulatory cycle (Fig. 2). Although diabetics perform serial self-sampling as a necessity, ours is the first study that has proven the feasibility of this method for research subjects in fertility studies. Blood spot testing should be given greater consideration for clinical and/or research scenarios when frequent testing of gonadotropins and ovarian hormones are needed in longitudinal studies.

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**REFERENCES**